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MEMOIRS:

The Structure of certain Chromosomes and the Mechanism of their Division.

By

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With Plates 1 and 2.

Part I. Structure.

(*a*) *Historical.*

The first suggestion of any structure at all observable in chromosomes seems to be due to Pfitzner ('Morph. Jahrb.', vii, 1882), who suggested that a chromosome is made up of a row of granules of chromatin embedded in an achromatic or less chromatic thread. Belief in these granules—later dignified by the names of 'chromomeres', 'chromioles', and the like—long held sway, and still lingers in many minds. I do not think it necessary to enter into a detailed discussion of this view; for I think it is now indubitable that the supposed granules are nothing but the misinterpreted images of twists of the chromosome, or of bulges in it. The figures illustrating this paper afford abundant instances of bulges caused by twists of the chromosomes; and those illustrating my paper on the chromosomes of Paris quadrifolia ('La Cellule', xxviii, 2, 1912, p. 265) of bulges caused by alveoles in them; either of which, if indistinctly seen, may lend themselves to an erroneous interpretation as granules.[1]

At the present time two other theories are in the field: the chromonema theory, and the alveolation theory.

[1] The chromomere theory seems to have been given up even by Flemming, who at one time accepted it. For in his paper, "Neue Beiträge zur Kenntniss der Zelle", II. Th. ('Arch. mikr. Anat.', xxxvii, 1891), whilst discussing the division of chromosomes, no mention is made of the granules, which he had formerly taken to be active agents of the division; and his figures no longer show any such granules, but in many places show instead more than hints of the bulges of a twisted thread.

The chromonema theory conceives of the chromosome as composed (at least at a certain stage) of a continuous filiform chromatic element—often spirally coiled—supported on an achromatic core, or contained in an achromatic cylindrical matrix.

This notion is due to Baranetzky, who in 1880 ('Bot. Zeitung', p. 241) described and figured, in the pollen mother-cells of Tradescantia virginica, a fine chromatic fibre spirally coiled, at the surface of the chromosomes, round an achromatic core.

In 1901 Janssens ('La Cellule', t. xix, pp. 55 and 58) described similar chromatic spirals uncoiling themselves from the chromatin clumps of the resting spermatogonia of the newt, and even figured similar filaments coiled within the chromosomes of the telophase, closely applied to an enveloping membrane. Later ('La Cellule', t. xxii, 1905, p. 413 and figs. 42 to 50 and 52 to 55) he figured achromatic membranes clearly existing around the 'pachytæne' chromosomes of the auxocytes of Batrachoseps attenuatus, and concluded that in the stages of the bouquet and the strepsinema all the chromosomes are in contact with their neighbours by means of these membranes—'les chromosomes se touchent tous'.

Bonnevie ('Arch. Zellforsch.', i, 1908, p. 450, and particularly pp. 471, 473, 477, 479, 509; ii, 1908, p. 201, and particularly pp. 266–70; ix, 1913, p. 433) from a study of chromosomes of Ascaris, Allium, and Amphiuma, deduces the following conclusions: A prophasic chromosome consists of an achromatic core on the surface of which is spread a continuous mantle of chromatin (I find no mention of a membrane). In the telophase this mantle becomes differentiated into a spirally coiled thread, whilst the achromatin is cast out into the new nucleus. The spiral threads of chromatin then put forth lateral processes which anastomose with those of neighbouring threads, and so form a nuclear network. At the next prophase the anastomoses are withdrawn, the chromatin threads shorten and thicken, and differentiate into chromosomes showing a newly formed achromatic core with a continuous mantle of

chromatin derived from the persisting chromatin of the telophasic spirals. These spirals are therefore the rudiments of a new generation of chromosomes.

K. C. Schneider ('Festschr. f. R. Hertwig', i, 1910, p. 215) also describes the chromosomes of the anaphase as consisting of a chromatic spiral enveloping an achromatic core; but finds this spiral become double in the telophase. He does not find in the quiescent nucleus a network formed by anastomosing processes of the spirals, but only a tangle formed by the attenuated and elongated spirals themselves. But these spirals are differentiated into chromatic granules united by an (apparently) achromatic thread. The prophasic chromosomes are formed by the condensation of the granules into (two) new chromatic spirals enveloping this thread.

Vejdovsky ('Zum Problem der Vererbungsträger', Prag, 1912) also finds that a 'ripe' chromosome consists of an achromatic core round which is wound a chromatic fibre. To this fibre he gives the name of 'chromonema'. He finds no membrane. At the telophase, the achromatic core is cast out, and, swelling, forms the nuclear enchylema. But the chromonema differentiates into a new achromatic thread with chromatic granules ('chromioles') imbedded in it. The threads thus constituted anastomose into the network of the quiescent nucleus. At the prophase the anastomoses are withdrawn, and the chromioles fuse into a new continuous chromonema, spirally coiled round the persisting threads. In the later prophase the chromonema segments into 'chromomeres' which undergo bipartition, and so bring about the division of the chromosomes. So that Vejdovsky, though a supporter of the chromonema theory in so far as he recognizes the chromatic thread as a chief constituent of the chromosome, does not entirely discard the granule theory of Balbiani and Pfitzner. Like Bonnevie, he conceives of the chromonemas as the rudiments (Anlagen) of a new generation of chromosomes (op. cit., p. 171, et passim).

The alveolation theory was foreshadowed by some observations of van Beneden's, but has only been worked up

into a theory of the quiescent nucleus lately, by Grégoire and his pupils (Grégoire et Wygaerts, "La Reconstitution du Noyau et la Formation des Chromosomes", 'La Cellule', xxi, 1903, p. 7; Grégoire, "La structure de l'élément chromosomique au repos et en division", ibid., xxiii, 1905, p. 311; and other papers by himself and his pupils). According to this, the homogeneous chromosomes of the prophase become during the telophase honeycombed with numerous vacuoles or alveoles, which end by splitting each of them up into a mere network of chromatin. These networks then anastomose by lateral processes, and there is thus formed a network of networks, the reticulum of the quiescent nucleus. At the next prophase the anastomoses are drawn in, and homogeneous chromosomes are formed anew from the remaining reticular tracks by the obliteration of their alveoles and condensation of their honeycombed chromatin into a homogeneous thread.

I have already ('La Cellule', xxviii, 1913, p. 265) published a study of the essential points at issue between Grégoire and Bonnevie, as exemplified in the pollen grains of Paris quadrifolia. I there found the chromosomes to be alveolated as described by Grégoire; but I did not find their alveolation to progress in the telophasic chromosomes to the point of breaking them up into networks. On the contrary, I found their alveoles to disappear, and the chromosomes to condense into thin spiral threads. But I did not find these threads to anastomose into a network in the resting nucleus, as described by Bonnevie. I found nothing worthy of the name of a network, but only a tangle of the much elongated and attenuated spiral chromosomes. I found these persisting throughout the interphase, and at the next prophase forming typical chromosomes by shortening and thickening and at the same time again becoming alveolated. Fig. 1[1] represents a typical group of

[1] This is a drawing of the anaphase shown in fig. 6 of my paper, amended by the addition of the sheath and lateral processes round the axis of the chromosomes, which had escaped me when the original drawing was made. I think it quite likely that there may be also a very fine periaxial spiral, in correspondence with the lateral processes, round the axis of the chromo-

chromosomes honeycombed by easily perceptible alveoles, of the existence of which there can be no doubt. For a detailed description of the characters of these alveoles, the reader will do well to refer to the paper quoted. Fig. 2, which is a slightly corrected copy of fig. 13[bis] of the same paper, shows the solid spiral threads into which these alveolated chromosomes become transformed during the telophase.

Later, I have extended this study to the chromosomes of the nuclei of the pollen cells and of some tissues of Lilium croceum and L. martagon, and obtained exactly the same results. Combining these results with those of Grégoire and Wygaerts for Trillium grandiflorum and T. cernuum, of Grégoire for Allium cepa, A. ascalonicum, and A. porrum, and of Sharp ('La Cellule', xxix, 1913, p. 297) for Vicia faba, and rejecting as erroneous the statements of those writers who have described in plant chromosomes a spiral fibre instead of alveoles,[1] we find that all the plant chromosomes that have been successfully studied hitherto possess an alveolated structure in the prophases, equatorial phases, and anaphases.

The present paper deals with certain animal chromosomes. Only one recent writer, Kowalski, has described any of these as alveolated. Kowalski ('La Cellule', xxi, 1904, p. 349), studying divers nuclei of the larval Salamander, arrived at the conclusion that their chromosomes all conform to the alveolation theory. I have carefully examined all the chromosomes studied by Kowalski, and many other of the Salamander larva,

somes; and that if this spiral cannot be made out with certainty (I think I sometimes catch glimpses of it), it is because the image of it is obscured by that of the walls of the alveoles. But this, if it exists, is certainly not the spirally coiled thread described by Bonnevie. I intend to return to this point in another paper.

[1] Baranecki's observations may safely be rejected, because they have been controlled by Carnoy and by Strasburger, who did not find the alleged fibre; and those of Bonnevie on Allium, because they are contradicted by the everyday experience of botanical cytologists. Both these writers have apparently misinterpreted images of walls of alveoles, or of torsions of the whole chromosome, as images of a spiral fibre.

and find that neither these nor any other of the animal chromosomes that I have studied do so ; but that on the contrary, at one period of their existence, they all do possess a certain spiral differentiation answering, to some extent, to Vejdovsky's 'chromonema'. The following pages set forth the evidence for this, but will, as I think, also show that the advocates of the chromonema theory have pushed it too far; for the spiral differentiation in question does not constitute an independent fibre, and does not form the germ of a new chromosome.

The chromosomes described are chiefly those of prophases, equatorial phases, anaphases, and telophases ; but I have touched on those of some interphases in which certain of their characters are demonstrable. I do not attempt in this paper to describe the nuclein elements of completely 'resting' nuclei. The results set forth are based on the study of chromosomes of the Amphibia (chiefly Urodela). Careful investigation of the nuclei of the other classes of the Vertebrata has shown that their chromosomes, though conforming apparently in all respects with those of the Amphibia, are mostly too small to afford trustworthy images of the details in question. The same is the case with most of the Invertebrata, only certain nuclei of the Orthoptera being found to possess chromosomes which, though smaller than most of those of the Amphibia, yet afford images which are often clearer. The majority of the figures are of chromosomes of spermatogonia, the most favourable kind for study. Those of spermatocytes and oöcytes are excluded from the survey, because in them the details are obscured by the complications due to the processes of conjugation. Most of the images described are from paraffin sections : surface preparations show nothing more than these. The most trustworthy fixing agent has been found to be picro-formol (Bouin's formula). Iron haematoxylin has been found to be incomparably the best stain ; but it should not be used quite as laid down in the books, which give excessive times and strengths. You should mordant (sections of 7·5 microns, or less) for not more than $2\frac{1}{2}$ minutes in a solution of iron alum of 4 per cent. or

weaker; and stain in a half per cent. (or weaker) solution of haematoxylin till the sections appear dark grey, not black (about twenty-five minutes in a virgin solution, or not more than four in one which has already had several slides passed through it); and differentiate in the iron solution for at least a couple of minutes after the sections, examined in water, seem sufficiently extracted. For the stain always appears much lighter in water than in balsam. For the study of the sheath, mount in Gilson's camsal balsam or euparal, rather than in balsam.

(*b*) *Descriptive.*

It will be best to begin with the study of some chromosomes taken at the anaphase, the most favourable moment, figs. 3 to 18.[1] The chromosome of fig. 6, which may be taken as typical, is from a spermatogonium of Salamandra maculosa. It shows the following two (not three) constituents, namely a chromatic (basophilous) axis, and an 'achromatic' (i.e. acidophilous) sheath enveloping this. The chromatic axis is by far the more conspicuous of the two; so much so that, as the sheath is seldom conspicuous enough to compel attention, the axis alone is all that is usually seen, and is therefore generally taken as the whole of the chromosome. But the sheath (which is none other than the achromatic membrane described by Janssens, 'La Cellule', xxii, 1905, p. 413 and figs. 42 to 50 and 52 to 55, as found in the auxocytes of Batrachoseps attenuatus), though it is a difficult object on account of its great tenuity, can generally be made out in well fixed specimens.

The axis has approximately the form of a cylinder, showing a circular section. But it is not a cylinder of regular calibre, for it is generally somewhat dilated at the ends, as seen in figs. 6, 7, 14 (and to a slighter degree in figs. 3 and 4), thus becoming somewhat claviform. And it is generally notably narrower at the polar bend than elsewhere, figs. 3, 4, and

[1] For the objects from which these figs. are taken, see the Explanation of the Plates.

especially 14 ; and at this point is generally somewhat flattened. At its ends (where not sectioned by the knife) it terminates in a smooth dome-shaped surface, from the summit of which there can frequently be seen to emerge a tiny tag, the vestige of its union with its late sister chromosome, figs. 6, 7, 14, 5, 12, all of which show the tag ; and 3 and 4. It is undoubtedly solid, not hollow. Surface views (see the figs. quoted) show no lumen, nor any trace of the alveoles found in plant chromosomes ; but they may seem to show a border darker than the innermost part, as in one or two of the chromosomes of figs. 3, 4, and 5. But in these cases it is generally possible to see that this border is not continuous, but consists of a series of elongated dots. Transverse sections frequently show as disks with a dark border and lighter centre, fig. 15, which may give rise to the impression that there exists an axial lumen. But I have satisfied myself that the axis is in reality solid, and that the dark border is due, for the most part at least, to the periaxial spiral, about to be described, showing there. It is frequently possible, by very careful focusing, to see that this border is darker at one side of the disk than the other, which I take to be due to a sector of the spiral being in sharpest focus there. Thus in fig. 15 *a*, at the top left it is darker to the right ; at the top right, darker at the bottom ; and in the lowest disk darker at the top. And the darker sector can be seen to turn round the disk with every change of focus ; which is just as a spiral viewed end-wise must behave.[1] Similar images are shown, more clearly, by three of the less darkly stained chromosomes of fig. 15 *c*. Those of fig. 15 *b* show the darker border as an apparently entire ring, not a mere sector ; and the fourth chromosome of 15 *c* shows as a disk with a mere hint of a darker border.

Further, in the lighter-coloured centre of the disk there can sometimes be seen a darker comma-shaped dot. One of these is seen as a mere dot in the two upper disks of fig. 15 *a*, and as

[1] For this spiral to be demonstrated it is imperative that the chromosome be not overstained, for if it is the axis will appear as dark as the spiral, and the spiral will not be seen. Vejdovsky's figures grossly exaggerate the distinctness of the spiral at the best of times.

a comma in the lower one. This I have no doubt is nothing but an out-of-focus portion of the periaxial spiral coming into view from a lower depth, in a somewhat tilted chromosome.

I think the utmost that can be admitted in the way of any hollowness of the axis is that this may possibly possess a cortical layer somewhat denser than the rest. But I think the appearances are sufficiently accounted for by the periaxial spiral.

On the surface of this otherwise homogeneous cylinder there runs a spiral of somewhat denser substance than the rest, figs. 3 to 14. This periaxial spiral is evidently somewhat denser than the rest, because it resists decoloration in regressive staining more strongly; but it is evidently of the same composition, for its affinities for stains are the same. It is not something separate from the rest of the cylinder, but is continuous with it. It is not fittingly described as a fibre wound round a core: for there is no space between the spiral and the rest of the axis; there is no hint of a discontinuity between the two either in surface views or in section. Nor should it be described as a fibre countersunk or partially embedded in the axis: for if it were a fibre its section would show as a small circle (or other figure) having a definite limit all round; but these spirals only show a definite limit outside the general surface of the core; inside, they merge in its substance indistinguishably. Vejdovsky's term of 'chromonema' is a misnomer: the thing is not a fibre, but a rib or ridge. It must therefore be taken to be a mere spiral condensation of the cylinder substance.

It is true that cases such as that shown in the left-hand chromosome of fig. 3 are not very infrequent. At the middle of the longer limb of this chromosome there is a break; and the spiral is seen to bridge over the gap between the two parts. But I take it that that is only because its toughness has enabled it to resist where the rest yielded; just as when you break a twig you frequently get the two parts hanging together by a strip of bark.

The periaxial spiral sometimes seems to course uninterruptedly

the whole length of the chromosome (with the exception of the extreme tips). But often, as shown in fig. 14, it seems to be interrupted at the polar bend, the bend only showing an attenuated tract of the core without any perceptible ridge on it. At the tips, the spiral ceases at the base of the dome-shaped surface, and is not continued up to its summit, figs. 6, 7, 14.

It seldom shows a regular pitch throughout, for its turns are sometimes very widely spaced, as in figs. 6 and 7, but often so closely approximated that they almost touch one another, as shown at the tip of the right-hand limb of fig. 14. The drawings, in which the spacing between each turn has been reproduced with scrupulous care, will give a better idea of this than any description.

It has been said that the spiral shows no definite limit *inside* the general surface of the axis; but *outside* this it does. Its optical section there shows as a series of minute conical elevations, giving, in inferior images, the appearance of a row of minute thorns. These elevations are figured in several of the drawings of recent observers, and are by their authors considered to be in effect minute thorn-like processes. But careful observation of well-preserved specimens (with good objectives and a first-class condenser) shows that the two outlines of each of these apparent cones do not terminate at the apparent apex shown under inferior definition, but merge there into a single line which is continued outwards, generally in a perceptible curve, till it reaches the membranous sheath. And it can often be seen to insert on this by means of a delicate conical enlargement. All the drawings, figs. 2 to 18, show some of these lines, and the enlargement is shown very clearly in figs. 7 and 23, and less clearly, but still recognizably, in several parts of the remaining figures. These enlargements, then, show as a row of minute cones having their bases applied to the inner surface of the sheath, and their apices continuous with the line which springs from the cones on the axis. There is always one of these cones on the sheath for each one on the core. Those on the sheath can often be seen to be situate, not diametrically opposite to those on the core, but a little higher

up or lower down, at the extremity of a line which prolongs the course taken by the spiral across the axis. This is shown in fig. 14 ; but in the remainder of the figures is not shown clearly on account of the frequent derangement of the symmetry of the disposition caused by stretching or other displacement of the sheath. But there can be no doubt that the relations of the two sets of cones are as described.

The line that joins the elevations on the axis to the sheath, including its aponeurosis thereon, is very faint, but it can sometimes be seen to be stained. In that case, it stains in the same tone as the axis ; for instance, I have obtained it unmistakably red with safranin. This ligament, then, is a prolongation of the substance of the spiral. And, taking all these facts together, we must come to the conclusion that each of these apparently filiform ligaments is nothing but the optical section of a flange-like or pterygoid membranous expansion of the spiral. This cannot be seen as a membrane, full face, because it winds round the axis in such a way as always to present its edge to the observer ; and also because it is so thin (I should think anything under a twentieth of a micron) that if ever a portion of it should come to lie full face it would still be invisible through its thinness.[1]

We may, if we like, call the optical sections of this membrane lateral processes of the axis ; which well describes the optical image. But then we must bear in mind that there is in reality only one of them, which courses continuously round the axis like the lamina spiralis cochleae round the modiolus. And we can make a rough model of a chromosome of this type by taking a carpenter's screw and inserting it into a quill into which it will just fit.

The whole of the chromatic axis, the innermost part as well as the spiral and the lateral processes, is most decidedly basophilous: no part of it is achromatic nor acidophilous (which is what the authors quoted in the Introduction mean when

[1] The aponeurosis of this membrane on the sheath can sometimes be seen as a spiral line running along the sheath. I have abstained from drawing it on account of the difficulty of showing it clearly.

they say 'achromatic'). It stains energetically in the fresh state with acid methyl green; and in the fixed state it stains energetically and selectively with safranin, gentian violet, and the other usual basic stains. The only ground that I can discover for the belief in an 'achromatic' core in it is the fact discussed above, that the periaxial spiral generally seems more darkly stained than the rest of the cylinder round which it winds. But that does not in the least point to a difference of chromatophily between the two. The inner part of the axis stains (generally) less darkly than the spiral because it is less dense. And that is all; for the two stain, qualitatively, with exactly the same selectivity for stains.

The sheath is a continuous tubular membrane, of a thickness of the order of about one-twentieth of a micron. It is of irregular calibre, but roughly of a diameter of about three times that of the axis (see figs. 2 to 18 and others). It is very frequently seen to be indented where the lateral processes insert on it, as though it were held down at these points, but blown up between them. It is sometimes seen to be continued round the tip, as in most of the figures given; but sometimes seems only to reach to the base of the dome-like surface, as in fig. 14. It is absolutely structureless. It is decidedly acidophilous, staining readily though somewhat feebly (to about the same degree as spindle fibres, for instance) with Säurefuchsin, Säureviolett, or Lichtgrün; and not staining with basic dyes. The space between this membrane and the axis is filled with a substance of glassy clearness, which is free from all trace of granules or other differentiations, and entirely achromatic, not staining in any way. If it appear to be tinted, as it sometimes may, that is due to the staining of the membrane. This substance may be liquid, or may be gelatinous.

I find the sheath on all anaphase chromosomes of which I can obtain sufficiently good images; and have concluded that it is as universal an attribute of all chromosomes of this stage as the axis and the periaxial spiral.

These, then, are the features which can be detected on favourable specimens of animal chromosomes at the anaphase.

We have now to inquire to what extent they are present in other phases ; and this with special reference to the assertion of Bonnevie and Vejdovsky that at the telophase one part of the chromosome axis is cast out into the new karyoplasm, whilst another persists as a spirally coiled thread which forms the rudiment of the new chromosome.

At the end of the anaphase the ' daughter-star ' of chromosomes contracts into a figure which is called by some the ' tassement polaire ', a term which we may translate by polar clump. In this clump (figs. 29 to 34) the chromosomes become so densely crowded, and even agglutinated together, that it is impossible to follow out their minute details with accuracy throughout (in the Amphibia : in some other groups the case may be different). Still, enough can be seen in suitably fixed clumps, such as those of figs. 30 and 31, to warrant the assertion that the essential features of the chromosomes persist. In fig. 30, for instance, the chromosome axes can in many places be made out, appearing as thin threads (therefore considerably shrunken) collocated in pairs (an important detail, the discussion of which is best reserved for Part II). The periaxial spirals can just be detected on some of them ; and on others, where they cannot be seen as lines wound round the shaft, their presence is made probable by the lateral processes which can be seen on their edges. And towards the ends of the chromosomes, wherever they stand clear, the sheath membrane can generally be made out as a fine line bridging over the tips of the processes. The sheath can indeed generally be seen round the edges of even highly-agglutinated clumps, figs. 32, 33, 34. In fig. 31 (Bombinator) these details can only just be glimpsed here and there, on account of the smaller size of the elements ; but indubitably exist there as described for fig. 30. We may conclude that at the height of the clump stage the chromosomes—though generally much shrunken, compressed, crumpled, and otherwise distorted—have more or less retained all their essential features.

This stage is of short duration, the clump soon passing by a process of expansion (to be explained in Part II) into the

telophase. This next stage will be most conveniently studied in the spermatogonia and oogonia of the Amphibia. For here, as the clump passes into the telophase, it expands into a wide ring, on the surface of which the chromosomes are set on widely spaced meridians, figs. 43, 44, 45, 48, 49, 50, and others. Owing to this arrangement they show only a minimal amount of overlapping, and, standing out on a clear background, can be studied with sufficient accuracy.

In the earliest stages of this process of expansion (figs. 35 to 38) we find much the same state of things as in the denser clump. The paired chromosome axes can be more clearly distinguished : periaxial spirals can be just detected on some of them, and on others their existence is placed beyond all reasonable doubt by the lateral processes visible on the edges of the axes. And the sheath can be made out on many of them (same figs.). In later stages such as figs. 39 to 47, the demonstration of these details becomes more difficult, mainly on account of two complications which here ensue. One of these is the formation of trabeculae (' anastomoses ' of some authors) between the chromosomes. These trabeculae obscure the lateral processes, with which they are easily confused, and so deprive us of an important guide for the detection of the periaxial spirals. The other is, that as the clump expands, the chromosomes elongate ; and as they elongate their duplicate axes twine round one another, figs. 35, 39 to 47.[1] This involves

[1] This gives us the key to Kowalski's assertion (op. cit.) that the chromosomes of the salamander larva are at certain periods alveolated. Thirteen of his figures purport to show the alveoles in question. Eight of these are of telophases. On comparing them with my figs. 39 to 51 it becomes evident at once that Kowalski has interpreted images of doubled and entwined chromosome axes as borders of alveoles—which is very natural, for a thus doubled chromosome easily gives the impression of an alveolated cylinder if you are not able to obtain a sufficiently sharp focusing of its entwined axes. The remaining five of Kowalski's figures of 'alveolated' chromosomes are of spiremes, such as my figs 25 to 27, and manifestly only show that the chromosomes he had before him were double, transverse trabeculae uniting their two moieties being taken for transverse walls of axial cavities in an undivided cylinder or riband.

a continual displacement of the direction of the axes, making it extremely difficult to follow them accurately for more than very short distances, and thus making it next to impossible to distinguish the periaxial spirals running across them. Still, at this stage, it can be inferred with certainty that these exist at least to some extent ; for indubitable lateral processes can be made out in some places ; and the sheath can be observed with certainty in favourable places, as shown in figs. 39 to 45 (in some places of these, where not sufficiently evident in the drawings, I have marked it with a cross).

When the expansion of the clump has attained its greatest extent, we have t h e t e l o p h a s i c r i n g , figs. 48 to 51, and others. The chromosome axes are here about as distinct as before ; but the periaxial spirals, lateral processes, and sheath seem to be w a n i n g . The spirals can no longer be seen as lines running across the shaft : and the lateral processes can only be distinguished from the interchromosomal trabeculae here and there. But this does not necessarily imply that they have diminished in number. For at this stage the chromosomes have elongated considerably ; and since by their elongation the periaxial spirals and their processes must be pulled away from one another, we naturally find far fewer processes than before on any given length of an axis. But this is probably not all that happens. The chromosome of the anaphase and early polar clump is a very t i g h t l y t w i s t e d cylinder ; and there is nothing forced in the supposition that the spirals on its surface, and their lateral processes, are mere effects of the torsion it has undergone. And it appears natural that as the axis elongates at the telophase, it should u n t w i s t ; and that in consequence of this untwisting the spirals come to subside into the shaft, carrying their processes down with them. Not that the substance of the spirals and processes degenerates or dissolves ; but that it undergoes a change of configuration : as when I extend a finger, wrinkles start up on its surface ; and when I flex it these wrinkles are smoothed down. But be this as it may, it is certain that in the telophase the periaxial spirals and processes begin to wane out of sight, till in the

interphase it is seldom possible to detect even a vestige of them with certainty.

As to the sheath at this stage, the appearances are similar. In the nucleus of fig. 47 (Bombinator) (which shows one half of a ring such as that of fig. 50), I am not able to see it, except (possibly) on the chromosome at the extreme left. In the nucleus of fig. 48, a later stage, also Bombinator, I have not been able to detect it. In that of fig. 49 (Triton) I think I can see it in the two places marked with a cross, and glimpse it in one or two others. In that of fig. 50 (Salamandra) I have been able to see it in a fragmentary way in half a dozen places, as marked. In that of fig. 51 (Triton, follicle nucleus of testis) I have been able to detect it in only three places (also marked). It is certainly less abundantly evident in these nuclei than in the earlier stages. And this can hardly be accounted for by greater difficulties in the way of observation; for the chromosomes are now more widely spaced than before, and observation of their edges should therefore be easier. Add to this that the sheath when detected can only be made out in a fragmentary way; can only be followed for very short distances; is less regular than in earlier stages, being frequently distinctly dilated; and can in some places be seen distinctly to be ruptured (details which it is not possible to render satisfactorily in a drawing). It may be stated as certain that towards the end of the telophase the sheath has generally to a great extent disappeared. And this disappearance seems to be due to a process of real disintegration ending in destruction, rather than to a mere change of configuration or relation of parts. For in completely 'resting' nuclei, even if these are such as to offer every facility for observation, not a trace of it can be detected.

The periaxial spirals and sheath thus lost to view at the telophase come into view again gradually at the next prophase. In the earliest stages in which the spireme is recognizable as being indubitably such (figs. 24 and 25) it seems to consist merely of tortuous naked threads (often clearly double, same figs., and especially fig. 25). These may be united by inter-

chromosomal trabeculae, but show no other lateral processes nor sheath, though they may show in considerable abundance minute nodes or varicosities. And the appearances suggest that these are nothing but nodes of contraction and torsion which may well be the first visible stage of the formation of periaxial spirals and processes. In more advanced stages of the spireme, such as that of fig. 26, lateral processes and a sheath can often be made out with certainty, though with extreme difficulty. At this time (when the loops of the chromosomes are still so closely crowded together that almost all the sheaths are in contact with their neighbours) the lateral processes are sometimes so abundant that when fairly well visible they give the image of a dense network spread over the whole of the ground of the nucleus, as shown in fig. 26. Periaxial spirals cannot be made out on the axes at this time ; but since we have found that lateral processes are signs of the existence of the spirals—being in fact only lateral expansions of these outwards—we must admit that by this time the spirals are in course of formation, if not completely formed, even when we cannot so much as glimpse them.

As the chromosomes contract, they become more widely spaced, and by the time they have contracted into the state known as the ' segmented ' spireme the lateral processes and sheath have come into evidence as clearly as in the anaphase, figs. 27 and 28. In fig. 27 the periaxial spirals cannot be made out, the moieties of the chromosomes being here especially thin (as I invariably find to be the case in endothelium nuclei). In fig. 28 they can just be glimpsed in some places. But not till we come to the chromosomes of the equatorial plate, figs. 19 to 23, do we find the axis clearly differentiated into a shaft with regular spirals on its surface. In equatorial plates whose chromosomes have not entirely assumed the form which they show when definitively arranged on the spindle, the aspect of the axes is still rather that of a structureless though twisted thread than that of a shaft with spirals on it (fig. 19). In the entirely completed and regularized plate the spirals certainly exist throughout, see figs. 20 to 23. If they do not

at this time show with all the vigour and distinctness with which they show at the anaphase, this may be sufficiently accounted for by the greater difficulty of observing them in the closely collocated moieties of the equatorial chromosomes. But it may equally well be that they only attain their complete development at the anaphase. We find, then, that the periaxial spirals are only temporary formations. The assertion of Bonnevie and Vejdovsky that they persist after the telophase as rudiments of a new generation of chromosomes is contrary to the facts. For we have found that the chromosomes of the late telophase are for the most part without periaxial spirals and sheath; and that that which persists and passes into the interphase is nothing but the thus simplified axes of the chromosomes. These, on passing into the interphase, frequently become coiled into very regular spirals, such as have been described and figured by many observers (for instance, Bonnevie for Ascaris and Allium, Vejdovsky for Ascaris and other objects, Schneider for Salamandra, and myself for Paris quadrifolia); but these do not consist of periaxial spirals set free from the shaft of the axis, but of the entire axis in a simplified state. The chromonema theory is a mare's nest.

We may now sum up. There are two types of chromosomes: one (hitherto only found in plants) which is alveolated from the prophase to the telophase; and one (hitherto only found in animals) which is not alveolated at those stages or any other. This last consists (at those stages) of a solid basophilous axis, possessing a certain spiral sculpturing of its surface, which we have called the periaxial spiral, and enclosed in an acidophilous sheath. But this sheath is perhaps common to both types; and if the suggestion thrown out in the note on p. 4 should prove correct the periaxial spiral would also be common to both. Then the only important difference between the two would be that the plant chromosomes have an alveolated, i. e. more or less hollow, axis, whilst the animal chromosomes have an entirely solid one.

Part II. Division.

(*a*) *Historical.*

It was made out by Flemming in 1880 that the chromosomes of the equatorial plate are double, that is, composed of two similar longitudinal halves, closely approximated. The parallelism and close approximation of these halves naturally suggested that they arise by a longitudinal splitting of a previously undivided mother chromosome; and this suggested inquiry as to the means by which the supposed splitting could be brought about.

In 1881 Pfitzner[1] put forth a schema of this splitting which seemed plausible and met with general acceptance. According to this, the mother chromosomes are composed either of a single row of globular granules of chromatin, of a diameter exactly equal to that of the chromosome and embedded in an achromatic matrix; or of a double row of such granules, of only half the size of those of the single row. These double rows are sometimes very closely approximated, sometimes less so; and finally separate from one another as daughter chromosomes. The 'splitting' of the mother chromosome would thus seem to be brought about by the binary division of each of its constituent 'granules'.

This theory won ready acceptance; and the supposed 'granules', under the names of 'Pfitzner's granules', 'microsomes', 'chromomeres', 'chromioles', and the like, are still described and believed in and made the basis of much fanciful explanation.

According to my own very extended observations, this notion of the 'splitting' of chromosomes being brought about by the splitting of their component 'chromomeres' is baseless. For no such granules exist at any time. It is abundantly clear to me that all the appearances that have been described as

[1] "Über den feineren Bau der bei der Zelltheilung auftretenden fadenförmigen Differenzirungen des Zellkerns", in 'Morpholog. Jahrbuch', vii, p. 289—a much quoted but rather wretched performance.

'Pfitzner's granules', 'chromomeres', and the like, are, as already explained, nothing but ill-seen and faultily interpreted images of bulges and twists of the axis of the chromosomes (figs. 3 to 23 and many others of this paper should make this sufficiently clear). It therefore only remains to be seen whether any other mode of division can be made out.

To settle this point, the first step must be to make out at what stage chromosomes can first be seen to be double. According to Flemming ("Neue Beiträge zur Kenntniss der Zelle", ii, in 'Arch. mikr. Anat.', xxxvii, 1891, pp. 737, 744, and 745) the supposed splitting takes place in the spireme stage. And this is apparently the view still taken by the great majority of cytologists.

I am not aware that any observer has asserted a division of chromosomes during the interphase. A longitudinal splitting at the telophase has been asserted by several writers, and with especial insistence by Dehorne. This writer even maintains (in his "Recherches sur la division de la cellule", in 'Arch. f. Zellforschung', vi, 1911, p. 613) that it may take place as far back as the anaphase. This is indubitably erroneous. For beyond all doubt at this stage the chromosomes show no hint of duplicity. But as regards the telophase I find that—in some cases at least—at that stage the chromosomes are certainly double—in a sense; and I acknowledge the essential correctness of Dehorne's clever figs. 7, 9, 10, 11, 12, and 18 (his fig. 6, which corresponds to my fig. 43, I think has been imperfectly understood by him). But I find no trace of any evidence that this duplicity is brought about by a longitudinal splitting.

A division of the chromosomes at the telophase has also been maintained by K. C. Schneider. In his 'Lehrbuch der vergleichenden Histologie', 1902, pp. 10, 118, 848, and 939, he states it as a probable inference. He suggests that at this stage the chromosomes segment transversely at the polar bends; and that the two moieties thus formed grow past one another so as to become parallelly approximated throughout their lengths. I have duly investigated this point, and find no

signs of such a process. I need not enter into further details, as Schneider himself seems to have abandoned his supposition. For in a later work (his "Histologische Mittheilungen", iii, "Chromosomengenese", in 'Festschr. f. R. Hertwig', i, 1910, pp. 218, 219, 221) he maintains his view that a division of the chromosomes probably takes place at the telophase (or anaphase), but now supposes it to be a longitudinal one.[1]

Of this also I find no evidence. But I do find evidence of another and simpler process by which the observed images of duplicity are brought about. To the consideration of this we may now proceed.

(*b*) *Descriptive.*

We have already seen incidentally, in Part I, that in the Amphibia the chromosomes of the later telophase are double structures, that is, that they consist of two chromatic threads, longitudinally collocated and more or less entwined.

This is by no means peculiar to the Amphibia. In smaller chromosomes than theirs the images are more difficult; and in much smaller ones it may be impossible to obtain satisfactory resolution. But enough can be made out to leave no doubt that it is a very widespread phenomenon. In the Mammalia I have found it fairly clear in Homo, fig. 54. In some of the Insecta (notably the Orthoptera) it is as certain as in the Amphibia, see figs. 62, 66, 67. I think we may take it as the invariable rule that in animals all the telophase chromosomes are thus doubled, that is, possess already the duplicity observed in the chromosomes of the prophase. This relieves us from the necessity of looking for any process of splitting in the phases between the telophase and the prophase; and it only remains for us to make out in what way the telophasic doubling is brought about.

[1] The reason he gives for this is a strange one. He admits (p. 218) that the daughter chromosomes of the metaphase only show one spiral; but thinks (without asserting it positively) that in the anaphase and telophase they contain two, because 'the coils they show are so closely set that they could hardly be the expression of a single spiral'. How about a reel of cotton?

To ascertain this we must return to the study of the earlier telophase, or polar clump. In the daughter-star of the anaphase (figs. 3, 4, 5, 61) we have a loose assemblage of chromosomes, radially arranged in a ring. These contract into short staves; and as they contract the whole figure shrinks (figs. 29 to 34), so that the staves become closely huddled together and come into contact by their margins. They generally seem to agglutinate there, and their outlines become hardly distinguishable, indeed very often quite indistinguishable. The clump then appears (figs. 30, 31, 33) as an almost homogeneous ribbed disk, with a central pore, generally obturated by a perforated membrane or web formed (as shown by profile views) by the confluent remains of the polar spindle fibres. The mutual contact or agglutination of the chromosome staves takes place first in the region of the clump that is nearest to the pole, their more distal portions remaining longer free: so that at this stage we get the image of a compact ring with digitiform processes depending from it—the 'figures pectiniformes' of Henneguy (figs. 32 and 34). In badly fixed cells the clumping results in a formless mass, in which the chromosomes seem to have become completely fused together. This state is shown in fig. 34. But, as I gather from the study of my most favourably fixed specimens, this is an artefact; and there is not at any time a real fusion of the chromosomes, but only intimate contact to the point of indistinctness, or possibly superficial agglutination.[1] Fig. 33 seems to me to show the utmost degree of agglutination that should be taken to be normal; and the real state of things to be fairly well represented by fig. 30 or 31.

Careful examination of the staves of the clump at this stage seems to show that they are always in reality double structures; for in favourable cases they show unmistakable indications of a longitudinal duplicity. In fig. 29 there are four staves, marked with a cross, which show this. In the left-hand one (near the top) the tip is distinctly bifid; and this is

[1] Cf. Janssens, 'La Cellule', xix. 2, 1901, p. 86, and Janssens et Dumez, ibid., xx. 2, 1908, p. 450 and fig. 15, who have arrived at the same conclusion.

also the case with the one at the bottom. In the two right-hand ones the tips are distinctly double ; and by careful focusing it can be made out that each of these staves is composed of two longitudinal moieties, superposed and to a slight extent twisted round one another. And in three or four of the short dark staves of the inner tier there can be seen a light longitudinal dividing line (not sufficiently clear in the drawing).

In fig. 33 nearly one-half of the twenty-one staves drawn are seen to be notched at the periphery, and two of them show a longitudinal dividing line continuing the notch inwards. In fig. 30 I find three cases similar to these, and in fig. 32 two. I have no doubt that with better fixation these nuclei would have shown several more such cases. In the clump of fig. 31 I think I can detect three or four similar cases, though doubtfully.

The clump does not long remain in this state of dense agglomeration, but soon begins to expand into the telophasic ring. The manner of this expansion is as follows. Amongst the staves of the clump—but never on their outer surfaces—there appear certain hyaline globules which, growing, push the staves apart and so loosen the clump. In fig. 38 are shown two such globules, one to the right, and one to the left ; in fig. 35 three (on the left ; one very indistinct) ; in fig. 37 five ; in the nucleus of fig. 36 there are a dozen or so, of which only a portion of one (at the left) could be shown in the drawing, the rest being too much masked by the sheaths. In fig. 62, to the right, are seen three ; in fig. 67 two can just be glimpsed (at the left and middle). These globules are entirely hyaline and uncolourable. Their outlines are generally quite smooth. They are, as I think, ovoid in shape, not spherical : they may show a circular outline, as in the left-hand ones of figs. 38 and 43, and other places ; but that is the expression of a transverse section of them. I suspect that there is formed at first one of them for each chromosome. If that be the case it is a likely hypothesis that they consist of the clear contents of the sheaths of the chromosomes, expressed from them by the pressure of the clump. But it is difficult to ascertain the number formed, because they soon fuse with one another into a small number of large globules, see figs. 43, 44, 45.

They ultimately all fuse, apparently, into a single homogeneous ring, as shown in figs. 49, 50, and others.

As soon as these globules have attained a certain size, figs. 43, 44, 45, 49, and less clearly yet still indubitably in figs. 36, 37, 38, the chromosomes, which in the clump appear as straight staves, now appear as more or less sharply curved staves, set on the surface of the globules or ring, that is, outside them and not embedded in them, see particularly the profile views figs. 43, 44, 45. Their outer surface is irregularly convex; but their inner surface is flattened on to the curvature of the globule or ring. They are—at the stage we are considering—of a length equal to about that of one of the limbs of the V-shaped chromosomes of the anaphase (see figs. 3, 4, 17, 61). They do not form complete hoops round the ring, but arcs that embrace about half a meridian of it. They thus show two ends, a polar end and an antipolar end. The polar ends, abutting on the lumen of the ring, are generally closely huddled together and sharply curved downwards, so that it is impossible to get clear images of them. But their antipolar ends are generally widely spaced (figs. 43, 44, 45), and here their two component threads may frequently be seen, with certainty, to be widely divaricated, figs. 43 (in the middle), 44, 45, which is not the case with the polar ends.

As soon as the process of expansion has set in, the images of the clump become less indistinct, and the chromosome staves appear as shown in figs. 30, 33, 35, 36, 37; that is, they are seen with certainty to contain or consist of the thin chromatic threads running in pairs, which in our study of the clump in Part I we recognized by their structure as shrunken chromosome axes, without discussing the fact of their collocation in pairs. The members of these pairs run very close together and in the main parallel to one another, as shown in figs. 30 to 35. Images such as these may suggest, strongly, that during the earlier stages of the clump the chromosomes have contracted into short staves, each of which has undergone a longitudinal division; so that the threads would be the cleavage products of such a division. Now there is no sign of any such division

taking place at any time ; but there is evidence that each of these threads represents an entire limb of the anaphase V from which it is derived ; and that their parallelism in pairs is brought about by the folding together of the two limbs of that V. This evidence is contained in the following considerations.

In the daughter-star of the anaphase the chromosomes are indubitably V-shaped, with equal limbs diverging to an angle of some 45 degrees,[1] figs. 3 and 5 (the apparent shortness of some of the limbs in these figures, and the apparent hook shape, is due partly to unequal degrees of contraction, partly to foreshortening). But as the star passes into the clump stage this divergence becomes less pronounced, and in the completed clump we find no such open V's, but in their place a bundle of short straight staves, figs, 29 to 33, each of which shows the two thin chromatic threads mentioned above. The observer's first impression naturally is that each of these staves represents one limb of a V, the relation of this one to the other being masked by the crowding of the elements. But consideration shows that this can hardly be. For the staves are only present in a far smaller number than the limbs of the anaphase V's—in the completed clump in only half that of the limbs. Take for instance fig. 29. This clump, a very early one, contains, as I make it, thirty-two seeming staves, of which twenty-nine are shown in the drawing. Now the anaphases of Salamandra atra, from which this is taken, have twenty-four V's, therefore forty-eight limbs. Manifestly, therefore, not all the staves of the clump can represent single limbs ; but some of them must represent entire chromosomes. Let us suppose that sixteen of them are in this case ; then these will account for thirty-two limbs ; and the remaining sixteen staves will represent sixteen single limbs, thus making up the required tale of forty-eight. Now take fig. 30, a completed clump. I make out twenty staves shown fairly distinctly (not all drawn), and the unanalysable portions of the clump may account for a very

[1] This for the nuclei of the Amphibia. As we shall see, it is not the case for those of all groups of animals.

few more. So here we have about twenty-four staves, representing forty-eight limbs. Or take fig. 33, also a completed clump. It shows twenty-one staves, and may contain a very few more. Therefore here again about twenty-four staves for forty-eight original limbs. Now take fig. 31, a nearly completed clump from Bombinator igneus. The diploid number of chromosomes in this species is sixteen, showing therefore thirty-two limbs at the anaphase. The clump contains twenty staves. Therefore not all of these can represent limbs of V's; but twelve of them probably represent twelve whole V's, and the remaining eight represent single limbs of such; total, thirty-two.

It is therefore certain that in any polar clump some of the staves—and highly probable that in the completed clump all of the staves—must represent each of them two limbs of a V. And the conclusion follows, that each of those of the completed clump is in fact a V whose limbs have folded together. So that the observed duplicity of the staves is not due to the chromosomes having undergone a cleavage after having in some other way assumed the shape of staves, but to their consisting of the two limbs of an anaphase V—or what remains of these. For the folding fully accounts for the duplicity.

In the Amphibia the postulated folding of the V's takes place as a rule only during the formation of the polar clump, not before. But exceptionally it may take place during the early anaphase. Fig. 4 is a case in point. In this anaphase the limbs of the V's are in several instances closed in to a distance of only about half a micron (as measured by the drumhead of the fine-adjustment), and so accurately superposed on radii of the figure that it is only by the most careful attention that the elements can be seen to consist of two superposed moieties.

But this, which in the Amphibia seems to be the exception, is in some other animal groups the invariable rule. For instance, in the spermatogonia of the Acridian Oedipoda cothurna (Arcyoptera variegata) I invariably find the state of things represented in fig. 61. This is a sagittal section of a mid-anaphase, the chromosomes being not yet half-way to the

pole. They consist, all of them, of tightly-folded V's, appearing as short staves with the spindle-fibre insertion at the end. But they are certainly folded V's with the insertion at the apex: the two limbs can be made out with certainty at the tips of four of them; and a longitudinal duplicity can be at least glimpsed in all of them.[1] I find the same state of things exactly in Oedipoda germanica, Oe. coerulescens, Oe. (Mecostethus) parapleura, Gomphocerus rufus, Stenobothrus morio, St. biguttulus, and some other species of Stenobothrus which could not be determined with certainty. So that in all the Acrididae I have examined the folding takes place not later than the early anaphase. And as at this stage the images are not obscured by the crowding of the chromosomes which takes place in the polar clump, there can be no doubt about the folding actually occurring.

So also in the Locustidae. Fig. 64 shows an anaphase of a spermatogonium of Decticus verrucivorus. The chromosomes are here smaller than in the Acrididae, and appear for the most part as short rods with the spindle-insertion at the end. But it can be made out in favourable instances that they are in reality folded V's; and where this cannot be done, the analogy with those of the Acrididae puts it out of doubt that they are in the same case. Similar images are afforded by Decticus griseus, Locusta viridissima, L. cantans, and Pterolepis aptera. In Gryllotalpa vulgaris and Gryllus campestris I find apparently the same state of things, the anaphase chromosomes (with the exception of the monosome in Gryllus) appearing as short rods inserted by one end on the spindle. These apparent rods are too small to be analysed with certainty; but judging by the analogy of those of the other Orthoptera mentioned there can be no doubt that they are in reality

[1] The drawings figs. 12 and 13 (Dissosteira carolina), and 18 (Steiroxys), of the paper of Davis, "Spermatogenesis in Acrididae", in 'Bull. Mus. Comp. Zool. Harvard', with the interpretations given, pp. 69, 70, 71 of the text, should, as I conceive, be corrected in the sense indicated above.

tightly-folded V's.[1] And this is also doubtless the case with the very short thick chromosomes of the Hemipteron Pentatoma (Carpocoris) nigricornis.

We find, then, that in the nuclei we have been studying the chromosomes become doubled at the telophase, or before, through a folding-in of their limbs. This brings those limbs into a state of parasyndesis or close juxtaposition throughout their length, so that little change (other than the elongation due to their growth during the interphase) is required in order to bring them into the state in which they are found at the commencement of the spireme stage. This is illustrated in figs. 55 to 59. But this process is perhaps not followed exactly in all nuclei. I have evidence that the folding, or at all events the definitive parasyndesis, of the limbs may be deferred, and

[1] In the Orthoptera the folding takes place not only as early as the early anaphase, but sometimes as early as the equatorial phase. In the equatorial figures shown in figs. 60 (Oedipoda cothurna) and 63 (Decticus verrucivorus) all the chromosomes are tightly folded into the stave shape. The same is the case in Oedipoda germanica, Oe. coerulescens, and Oe. (Mecostethus) parapleura. In Gomphocerus rufus the majority of the chromosomes appear in the stave form; but there may be some open V's. In Stenobothrus biguttulus I suspect that the equatorials have always exactly two large chromosomes of the open V shape, all the others being tightly folded into the stave shape. It is perhaps not rash to conclude that all the cases of chromosomes described by authors as straight rods with a terminal spindle insertion are in reality cases of tightly-folded V's with an apical spindle insertion.

Fig. 63 (Decticus verrucivorus) shows sixteen large autosomes, fourteen small ones, and a monosome, therefore thirty-one in all. This is as it should be: for in this species I find in all unobjectionable images either sixteen large autosomes and fourteen small, or fifteen large and fifteen small, and a monosome; the difference resulting from the fact that it is sometimes difficult to decide whether a chromosome is an unusually small 'large' one or an unusually large 'small' one. Buchner ('Arch. Zellforsch.', iii, p. 342, and fig. 82 of Taf. xix) correctly gives the number as thirty-one in all. Vejdovsky (op. cit., pp. 33 and 44), notwithstanding that he had this description before him, insists that there are only twenty-three in all. Reference to his figs. 65 to 69 shows that he has mistaken entire chromosomes tightly folded into the stave shape, and fortuitously approximated at their apices, for mere limbs of open V's.

take place only at the moment of the formation of the spireme, or even at an advanced period of its evolution. In this case, the limbs pass through the interphase in a more or less widely divaricated state, which gives to the interphase a facies very dissimilar to that of the interphase of nuclei in which the parasyndesis has taken place at the telophase. A description of this is reserved for a future paper. But in either case the mechanism of the division of the chromosomes is the same in principle. There is no longitudinal splitting. The division is a transverse one, brought about by the folding of the chromosomes at their middle, and their ultimate segmentation at the bend there formed. The moieties which separate at the metaphase are the two limbs of the chromosome thus folded, therefore metameric, not antimeric, moieties

EXPLANATION OF PLATES 1 AND 2.

Illustrating Mr. Arthur Bolles Lee's paper on 'The Structure of certain Chromosomes, and the Mechanism of their Division'.

Magnification 1,500 diameters throughout.

Plate 1.

Fig. 1.—Anaphase of pollen grain of Paris quadrifolia. Chromosomes alveolated, with sheath.

Fig. 2.—Early interphase of pollen grain of P. quadrifolia. Chromosomes without sheath, not alveolated, elongated into spirals.

Fig. 3.—Triton alpestris. Anaphase of spermatogonium. The chromosomes as open V's, showing the chromatic axis and periaxial spirals and sheath.

Fig. 4.—The same, a somewhat later stage, showing the chromosomes folded into very narrow V's.

Fig. 5.—Bombinator igneus, spermatogonium. Portion of anaphase, showing the chromosome axes and periaxial spirals, but not the sheath.

Fig. 6.—Salamandra maculosa. One limb of an anaphase chromosome, spermatogonium. Chromatic axis, periaxial spirals (very widely spaced), lateral processes, and sheath.

Fig. 7.—Salamandra atra, do., do. Shows same details; also the terminal tag on the dome-shaped end of the axis.

Fig. 8.—Salamandra maculosa, oogonium. Anaphase chromosome, entire. Same details.

Fig. 9.—Do., epiderm. Anaphase chromosome, one limb. Same details.

Fig. 10.—Do., epidermal gland; anaphase; one limb of a chromosome. Spiral with very wide pitch.

Fig. 11.—Do., kidney cell. Same details.

Fig. 12.—Do., cornea. Spirals much flattened on to axis.

Fig. 13.—Do., retina of larva, rod and cone layer. Details as last.

Fig. 14.—Triton alpestris, larva, pulmonary epithelium. Entire anaphase chromosome. Note the spiral very closely coiled at tip of right-hand limb, and not continued round the polar bend.

Fig. 15.—*a*, Triton palmatus, spermatogonium; *b*, Salamandra maculosa, spermatogonium; *c*, do., epiderm. Transverse sections of anaphase spermatogonia. See text.

Fig. 16.—Homo, pus corpuscle from ulcerated skin. Two chromosomes from an equatorial division figure. Sheath and lateral processes shown, periaxial spirals invisible, though doubtless existent.

Fig. 17.—Gallus domesticus, embryonic cartilage. Portion of an anaphase. Periaxial spirals just visible, sheath strong.

Fig. 18.—Ancylus lacustris, buccal epithelium. Tangential section of anaphase. Spirals, lateral processes, and sheath just visible.

Fig. 19.—Salamandra maculosa, epiderm. Chromosome from a not completely regularized equatorial figure. Spirals indistinct, giving an impression of 'granules'.

Fig. 20.—Do., from a completed equatorial figure of a spermatogonium. Details as last.

Fig. 21.—Do., portion of equatorial chromosome of an oogonium. Details as last two figs.

Fig. 22.—Do., renal epithelium. One limb of an equatorial chromosome. Spirals distinct on each of the two moieties.

Fig. 23.—Oedipoda cothurna. Equatorial chromosome of secondary spermatogonium. Details as for fig. 19, but sheath stronger.

Fig. 24.—Triton palmatus, spermatogonium. Spireme, early stage. Chromosomes double, no sheath or other detail.

Fig. 25.—Salamandra maculosa, larva, epithelium. Spireme somewhat more advanced than last. Moieties of chromosomes varicose (dawn of periaxial spirals).

Fig. 26.—Do., pulmonary epithelium. Spireme, later stage. Moieties very varicose, with abundant lateral processes and sheath.

Fig. 27.—Do., pleural endothelium. 'Segmented' spireme. Moieties with large varicosities (Pfitzner's 'granules'), and lateral processes and sheath.

Fig. 28.—Triton palmatus, spermatogonium. Later spireme. Periaxial spirals can just be glimpsed.

Fig. 29.—Salamandra atra. Spermatogonium. End of anaphase. Chromosome V's folded into the stave form.

Fig. 30.—Triton palmatus. Spermatogonium. Polar clump. Chromosomes tightly folded, much contracted.

Fig. 31.—Bombinator igneus. Spermatogonium. Polar clump. As last.

Fig. 32.—Triton palmatus. Spermatogonium. Clump showing chromosomes coalesced. Wholly or in part an artefact.

Fig. 33.—Do., do., do. Clump in polar view.

Fig. 34.—Triton alpestris. Do., do., do. Profile view.

Fig. 35.—Triton palmatus. Do. Clump expanding, early stage.

Fig. 36.—Do., do., do. Later stage.

Fig. 37.—Do., do., do. Later stage of expansion, clump passing into telophase.

Fig. 38.—Do., do., do. Same stage, profile view.

Fig. 39.—Salamandra maculosa. Oogonium. Same stage, or early telophase. Axes of limbs of chromosomes closely entwined round one another.

Fig. 40.—Do., do., do. Somewhat later stage, chromosomes elongating.

Fig. 41.—Bombinator igneus, spermatogonium. Clump in stage of figs. 38 and 39.

Plate 2.

Fig. 42.—Salamandra maculosa, spermatogonium. Telophase, early, showing telophasic ring in profile (section).

Fig. 43.—Triton palmatus. Do., do., do. Note the chromosomes flattened on to the outside of the hyaline globules, which are in course of fusing into a ring.

Fig. 44.—Do., do., do. Tangential section of ring. As last. Two large hyaline globules shown in the middle. Note the ends of the chromosome axes showing divaricated at the antipolar ends.

Fig. 45.—Do., do. Profile view of a ring at a slightly later stage. Chromosome moieties looser; chromosomes longer.

Fig. 46.—Salamandra maculosa. Renal epithelium. Telophasic ring, same stage as last, same details.

Fig. 47.—Bombinator igneus. Spermatogonium. Section of ring, same stage as last, and same details.

Fig. 48.—Do., do. Later stage of telophasic ring, polar view.

Fig. 49.—Triton palmatus. Polymorph spermatogonium. Mid-telophase, ring beginning to close. Chromosomes elongated.

Fig. 50.—Salamandra maculosa, oogonium (primary). Telophasic ring, about same stage as last, chromosomes more elongated and taking on an erratic course.

Fig. 51.—Triton palmatus. Large endothelium nucleus from follicle of testis. Late telophase, ring almost closed. Nucleus very flat; almost all the chromosomes drawn; chromosome axes distinctly doubled and entwined.

Fig. 52.—Do., do., a smaller nucleus, somewhat later stage.

Fig. 53.—Bombinator igneus. Endothelium nucleus, entire, testicular peritoneum. Polar view (not a section) of telophase of same stage as last. All the chromosomes have been drawn, though not throughout all their length.

Fig. 54.—Homo. Endothelium of vein of cutis. Section of telophase, about the stage of fig. 51 or 53.

Fig. 55.—Triton palmatus. Spermatogonium, early interphase.

Fig. 56.—Do. Late interphase, or dawn of spireme.

Fig. 57.—Do., do. Early spireme. Karyoplasm browned by osmium.

Fig. 58.—Bombinator igneus. Peritoneal endothelium. Early rest stage.

Fig. 59.—Do., do. Later rest stage.

Fig. 60.—Oedipoda cothurna. Spermatogonium. One half of an equatorial figure. Chromosomes all of them as tightly-folded V's.

Fig. 61.—Do., do. Sagittal section of anaphase. Chromosomes so tightly folded that they appear as stout curved staves.

Fig. 62.—Do., do. Early telophase, tangential section of ring. Shows three hyaline globules (to the right).

Fig. 63.—Decticus verrucivorus. Spermatogonium. Equatorial figure. All the chromosomes drawn. All are tightly folded into the stave shape; *m* is the monosome.

Fig. 64.—Do., do, anaphase, polar view. Chromosomes folded into the shape of wedges; *m*, monosome.

Fig. 65.—Do., do. End of anaphase. Chromosomes as before.

Fig. 66.—Do. Primary spermatogonium. Mid-telophase · *m*, the monosome. Some of the chromosomes seem to have their moieties divaricated at both ends, as if a transverse segmentation had taken place at the polar ends.

Fig. 67.—Do. Nucleus of connective tissue enclosing cyst of testis. Early telophase.

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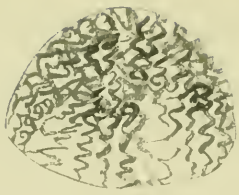

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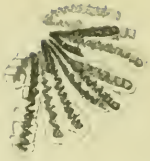

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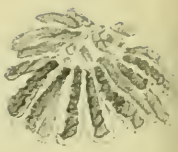

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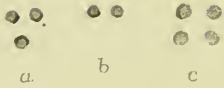

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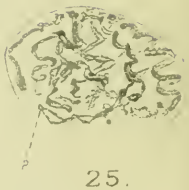

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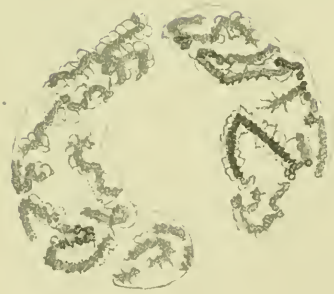

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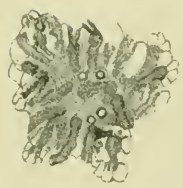

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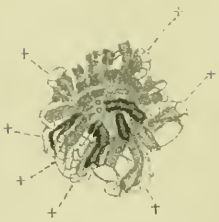

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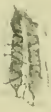

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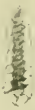

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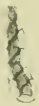

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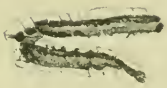

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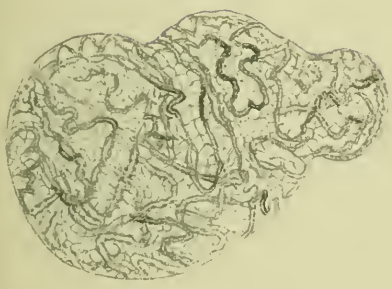

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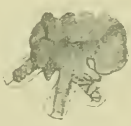

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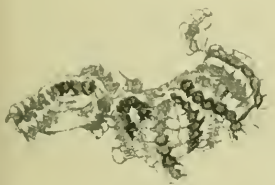

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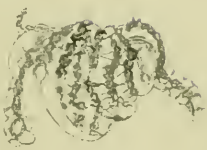

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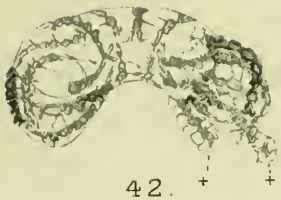

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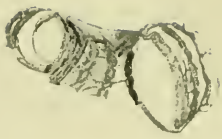

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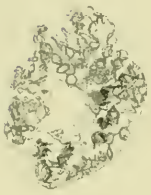

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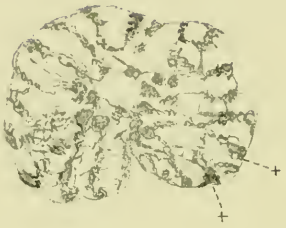

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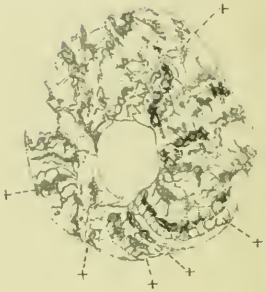

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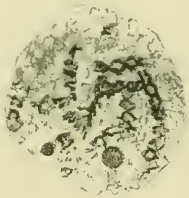

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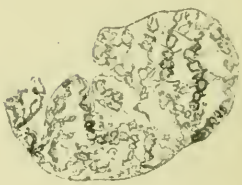

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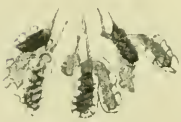

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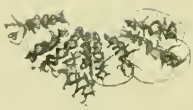

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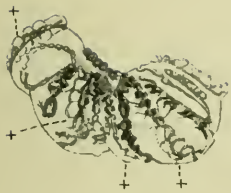

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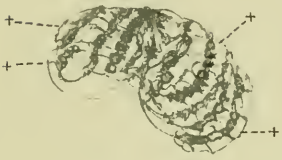

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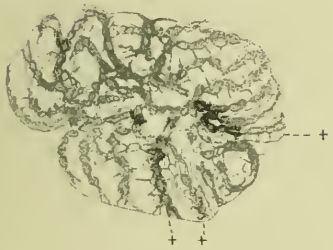

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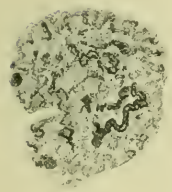

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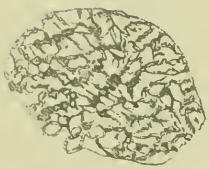

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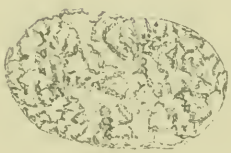

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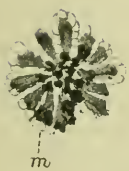

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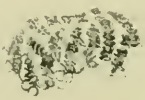

67.

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On the Pharyngeal or Salivary Gland of the Earthworm.

By

D. Keilin, Sc.D.

Beit Memorial Research Fellow.
(From the Quick Laboratory, University of Cambridge.)

With Plate 3 and 7 Text-figures.

Contents.

1. Previous Work on the Pharyngeal Bulb.

It is well known that the dorsal wall of the pharynx in all earthworms is much thickened, and forms a real pharyngeal bulb, which bulges prominently into the coelomic cavity. By dissection from the dorsal surface of the earthworm, this pharyngeal bulb can be easily seen with an ordinary lens and, in the

larger specimens, even with the naked eye. It is richly vascularized and its surface is irregular and lobulated. In longitudinal median section the pharyngeal bulb is seen to be composed of the three following portions: (1) an external epithelial sheath, (2) a median mass of musculo-vascular tissue, and (3) an internal portion composed of aggregates of deeply-staining cells.

Almost all zoologists who have dealt with the anatomy of earthworms have given more or less attention to this organ, but, unfortunately, their opinions as to the nature and function of the deeply-staining cellular aggregates are either unsupported by observations or contradictory. I do not intend to give here a complete account of the previous work on this subject, as this has already been done by Vejdovsky (1884, pp. 101–6) and Stephenson (1917, pp. 253–60). I shall therefore confine myself to a brief indication of the main views held on this subject by previous authors, classifying them under the four following groups:

(1) Several authors, without paying special attention to the structure of the pharyngeal bulb, accorded to it the function of a salivary gland; in this category come the observations of Leo (1820) and Clarke (1856) (cited by Vejdovsky), Lankester (1864, p. 264), Vogt and Yung (1888, pp. 461–3), and Beddard (1895).

(2) Vejdovsky (1884, pp. 101–6), Willem and Minn (1899), de Ribeaucourt (1900, pp. 246–7), and others, succeeded in tracing ducts which led from the deeply-staining cellular aggregates, through the muscular portion, but, although they could not detect any continuity of these ducts with the pharyngeal lumen, they nevertheless accorded to these cells a secretory function similar to that of a salivary gland.

(3) Michaelsen (1886, cited by Hesse), Hesse (1894, pp. 10–12 and Pl. 1, fig. 24), and especially Eisen (1894–6), found the ducts of the deeply-staining gland cells to pass through the muscular portion, penetrating between the cells of the pharyngeal epithelium and opening into the pharyngeal lumen.

(4) Finally, Stephenson (1917) completely denied the existence of any communication between the deeply-staining cells, which

he calls 'chromophile cells', and the pharyngeal lumen. The function of these cells, according to this author, remains unknown.

Of all the above-mentioned views, those of Eisen and Stephenson are specially interesting, as being diametrically opposed, though both based upon the study of the detailed structure of this organ. They deserve, therefore, to be examined in greater detail.

Eisen (1894–6), in his series of papers on the Oligochaetes of the Pacific Coast of America, describes and figures the pharyngeal or salivary glands of almost all the earthworms he studied, and especially those of the following five species: Phaenicodrilus taste (1894, pp. 66–7, Pl. xxx, figs. 1, 2, and Pl. xxxii, fig. 18), Pontodrilus Michaelseni (1894, pp. 77–8, Pl. xxxiv, fig. 36), Benhamia nana (1896, p. 129, Pl. xlvii, figs. 15–18), Sparganophilus Benhami (1896, pp. 104–5, Pl. liii, figs. 112–13), and Sparganophilus Smithi (1896, p. 157).

To demonstrate the views of this author, we shall quote from his paper the following descriptions which concern respectively the salivary glands of the first two species mentioned above.

Phaenicodrilus taste (pp. 66–7): 'The narrow ducts from the gland penetrate the pharyngeal epithelium and form, at its outer edge, small ovoid pockets for temporarily storing a small amount of the salivary secretion. These ducts end with the pharynx, the oesophagal epithelium neither being furnished with ducts nor storage pockets. . . .'

Pontodrilus Michaelseni: 'The ducts lead directly to the pharyngeal epithelium; arrived here they branch out, sending numerous discharge-tubes between the epithelial cells (fig 36, *gl. dt.*), discharging the salivary mucus in the pharyngeal cavity. These ductules are frequently, though not generally, branched while in the epithelial layer. Each ductule is furnished at the distal end with a small storage-chamber (36, A Pl. 34) of oblong form and considerably smaller than the nucleus of the epithelial cells.'

According to these observations, the pharyngeal cells, which

exist probably in all earthworms, form a salivary gland which pours its secretion into the pharynx. This has been denied, however, by Stephenson, in a paper specially devoted to this subject.

After a careful critical examination of the work of all the previous authors, Stephenson writes (loc. cit., p. 260): 'The authors who have seen ductules and their ending in the pharyngeal epithelium have, I believe, been misled by preconceived ideas due to the transformation of the deeper cells into connective tissue.' Earlier (p. 259) he says: 'It will save repetition to state that in none of my sections, which were taken in all the three planes, have I seen structures that could be interpreted as ductules.'

He passes then to the description of these cells and their gradual transformation into the 'fibrillar or reticular packing tissue ("Füllegewebe") between the muscles' in several species of earthworms belonging to the genera Pheretima and Helodrilus (Allolobophora). His study is concluded by the following statements: 'The "pharyngeal gland-cells" of earthworms are not gland-cells in the usual sense, and do not communicate with the pharynx; the term "chromophile cells" is proposed for them because of their intense coloration by haematoxylin and similar stains. The so-called "septal glands" of earthworms are aggregations of similar cells at a more posterior level.' . . . 'While most of the cells form a more or less compact aggregate on the surface of the pharyngeal mass, a number penetrate inwards towards the pharyngeal epithelium, and become progressively metamorphosed into fibrillar connective tissue.'

As to the function of the chromophile cells, he writes (p. 281): 'Though in the light of what has gone before we may reject the usual supposition that the cells pour a secretion into the pharynx (or oesophagus, in the case of the smaller more posteriorly-situated aggregates), it is not easy to propose another hypothesis to take its place.' . . . 'That the main function of the cells is metabolic is, though only a vague statement, perhaps as far as we are justified in going.'

During my research on Pollenia rudis, a Calliphorine fly, the larvae of which live as parasites in Allolobophora chlorotica, I often had occasion to study sections of the pharyngeal bulb of several species of earthworms, and I always believed that I was dealing with a salivary gland as described by Eisen. The recent paper of Stephenson came therefore as a surprise to me. It induced me to re-examine more closely my previous sections, and to prepare fresh ones, using this time special methods, which, as we shall see further on, enable us to solve finally the questions as to the nature, and, consequently, the functions of the deeply-staining cell-aggregates.

This seems to me to be very important, for two reasons: (1) the pharyngeal bulb is an organ of conspicuous size and appears to exist in all earthworms, and (2) the common earthworm being generally used as a type for the purpose of class dissection, it is very necessary that all observations concerning its anatomy should be accurate, in order to avoid a wide dissemination of erroneous information.

2. Material and Methods.

The earthworms used for this study comprise three species: Allolobophora chlorotica Sav., Allolobophora foetida Eisen, and Lumbricus sp. For the study of the general structure of the pharyngeal bulb I used as fixatives: Bouin and Schaudinn with 3 per cent. of acetic acid, followed by staining in P. Mayer's Haemalum or Glychaemalum with Eosin or Orange, or in Magenta-red and Picro-Indigo-carmine. For the more delicate structures of the gland and pharyngeal epithelium small pieces were fixed in Champy's chromo-osmic solution and stained with Iron Haematoxylin and Eosin. The protoplasmic inclusions were examined in sections prepared by Champy's (1911) method (fixation in Champy's solution, post-chromization with potassium bichromate, and staining in Iron Haematoxylin).

For the study of the glandular secretion, which I naturally supposed to be mucin, I had to apply several methods. Since Langley's important research on salivary glands and their

secretion (1889) a fairly large literature on mucin glands has accumulated, and several good methods now exist which enable us to detect the smallest amount of mucin in very fine ductules. For a critical account of these methods, the reader is referred to the papers of Hoyer (1890 and 1903) and Michaelis (1903).

The methods of staining which I have used in connexion with this study are of two kinds :

(*a*) A purely mucin stain : Mucihaematein of P. Mayer (1896).

(*b*) Metachromatic stains : Thionin and Toluidin blue.

(*a*) Mucin stain: Anterior portions of earthworms are fixed for twenty-four hours in Bouin's Picro-formol or in a modified solution of Bouin's Picro-sublimate formol (Corrosive sublimate, saturated sol. 20 c.c., Picric acid, saturated sol. 20 c.c., Formol, 20 c.c., Acetic acid, glac. 5 c.c.). After fixation they are well washed in Alcohol (70 per cent.) and embedded by the ordinary method. The sections (4–6 μ in thickness), having been freed from paraffin, are stained from two to five minutes in a 10 per cent. solution of Mucihaematein. They are then either mounted without any supplementary staining, or stained with the Magenta-red and Picro-Indigo-carmine. I have obtained good results by staining the sections with Iron Haematoxylin (twelve hours in Iron alum and twelve hours in 1 per cent. solution of Haematoxylin) and counterstaining for five minutes in Mucihaematein, and for a few seconds in Orange G.

(*b*) Metachromatic stain. Slightly modified methods of Hoyer (1890, 1903) and Hári (1901) give very good results. Portions of earthworms are fixed either in 5 per cent. solution of corrosive sublimate, or, with much better results, in the above-mentioned Picro-sublimate formol, from two to eight hours. The sections, freed from paraffin, are passed through the series of alcohols into the distilled water and then for ten minutes into 5 per cent. solution of corrosive sublimate. They are then washed rapidly in strong alcohol and distilled water and stained in an aqueous solution 0·1 per cent. of Thionin (Lauth's violet), or Toluidin-blue. In about one to two minutes

all the mucin appears red ; in two to seven minutes the mucin is stained red, while all the rest of the tissue is stained blue. It is better to examine the sections while they are still in the solution of Thionin, as it is very difficult to mount them without destroying the metachromasy. There are, however, several ways of mounting the slides in Canada balsam, by which the metachromatic effect may be retained for at least seven days. I shall mention only the following few methods which have given me very satisfactory results.

(1) Very rapid passage through absolute alcohol, xylol, and mounting in Canada balsam.

(2) Sections stained in Thionin, washed rapidly in distilled water, fixed in a 10 per cent. aqueous solution of Potassium ferrocyanide (Krause's method), rewashed in distilled water, and then passed rapidly through the graded alcohols, absolute alcohol, and xylol, into Canada balsam.

(3) The sections are stained by the previously described Thionin method, before freeing them from paraffin, washed rapidly in distilled water, dried thoroughly with filter paper, and then freed from paraffin and mounted in Canada balsam.

(4) Instead of alcohol, Acetone is used for dehydration, and xylol for clearing ; and the sections are then mounted in Canada balsam (method recommended to me by Dr. W. H. Harvey).

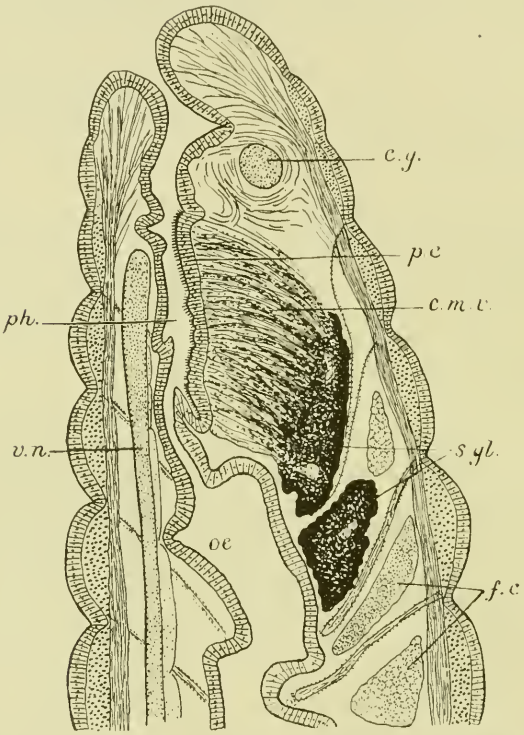
Mounting the sections in levulose syrup, or syrup of Apathy, is not advisable, for even when it preserves the metachromasy, sections thus prepared do not show clearly the cytological structure, particularly under examination with high magnifications. I did not succeed in differentiating the sections with Hári's mixture (1901). Finally, the use of artificial light for examination of the sections is strongly recommended, as it shows a more striking contrast between the red and the blue colours of the stained sections.

3. The Structure of the Pharyngeal or Salivary Bulb.

The pharyngeal bulb has been already morphologically described by several authors who have dealt with the anatomy of earthworms. In almost all species of earthworms, it has the

same general form and the same relations with the surrounding organs, varying only in the size and the number of the glandular lobules. The general structure of this organ is sufficiently

Text-fig. 1.



Longitudinal median section of All. foetida. *c. g.* = cerebral ganglion; *c. m. v.* = conductive or musculo-vascular portion of pharyngeal bulb; *f. c.* = mass of coelomic cells containing droplets of fat (cf. Text-fig. 7, p. 57 of this paper); *oe.* = oesophagus; *p. c.* = ciliated pharyngeal epithelium; *ph.* = pharyngeal lumen; *s. gl.* = deep or glandular portion of the pharyngeal bulb, composed of basophile, salivary cells; *v. n.* = ventral nerve cord. × 26.

clearly shown by Text-figures 1 and 2, which represent longitudinal median and submedian sections of the anterior portion of the earthworm.[1]

[1] For the morphological variation of this organ the reader is referred to the published papers on the anatomy of earthworms.

As to the histological structure of the pharyngeal bulb, we shall, for the sake of clearness, examine separately the structure of its three portions : (*a*) the deep glandular portion, (*b*) the conductive or musculo-vascular portion, and (*c*) the superficial or epithelial portion.

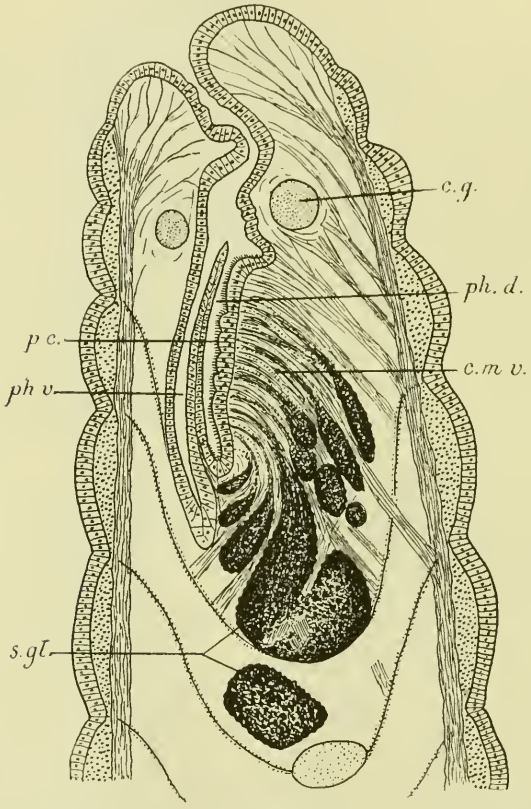
(*a*) *The deep or glandular portion.*

The deep or glandular portion of the pharyngeal bulb is composed of a certain number of lobules of various sizes, suspended in the coelomic cavity of the earthworm and extending backwards as far as the fifth or the sixth segment of the body (Text-figs. 1 and 2, *s. gl.*). These lobules, as well as the entire bulb, are surrounded by a thin peritoneal membrane (' capsule ' of Stephenson) composed of flattened cells with elongated nuclei. The peritoneal membrane penetrates between the lobules, and in some places into the lobules, especially where the latter are traversed by muscular bundles, or by the blood-vessels, which are directed forwards and ramify in, and form the main part of, the musculo-vascular portion of the bulb (Text-figs. 1 and 2, *c. m. v.*).

The cells which compose the glandular lobules are very polymorphic, being either spherical or elongated, or even semilunar. Sections derived from well-fixed material (in Champy's fixative, for instance) do not show clearly the boundaries between the cells, while on the other hand, a less perfect fixation, which slightly contracts the cells, defines their contours, and demonstrates that, in some places, the protoplasm of these cells is continuous. The size of these cells varies as much as their form ; in Allolobophora chlorotica, for instance, they are from 20 μ to 30 μ long and 18 μ wide. Each cell contains a large spherical nucleus of 7–8 μ in diameter which is provided with a large nucleolus of 3–4 μ in diameter (Pl. 3, fig. 4, *m. gl.*). The peripheral chromatin of the nucleus is generally much reduced, but its quantity seems to depend upon the activity of the cells. The protoplasm, as was shown by Stephenson, is very basophile, for which reason he called these cells ' chromophile '. When stained by Haemalum, Iron Haematoxylin, or

Magenta-red, the perinuclear protoplasm of these cells is often so deeply stained that it decolorizes more slowly even than the nucleus. Nearer the border of the cell the basophile protoplasm is very irregularly distributed, and this gives to the

Text-fig. 2.



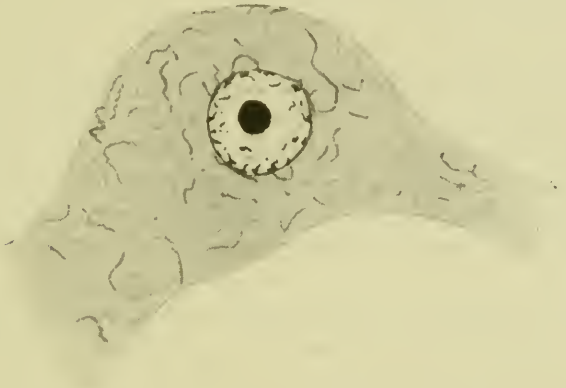
Longitudinal submedian section of All. foetida: *ph. d.* = dorsal or salivary chamber of pharynx; *ph. v.* = ventral chamber of pharynx. Other letters as in Text-fig. 1. × 26.

stained cells a very peculiar spotted appearance (Pl. 3, figs. 2 and 4).

The clear areas of the protoplasm have a very granular structure, the nature of which we shall examine later. The

basophile protoplasm does not show any special structure, and it appears to contain a diffused chromatic substance (extra-nuclear chromatin). In sections of the glandular cells of Lumbricus sp. prepared by Champy's method (fixation in Champy, postchromization followed by Iron Haematoxylin) the protoplasm is seen to contain a number of bodies which are probably mitochondria (Text-fig. 3). These protoplasmic bodies appear as irregular, curved and ramified filaments or

TEXT-FIG. 3.



Glandular or salivary cell of Lumbricus sp. showing a vesicular nucleus with large nucleolus and with numerous intraprotoplasmic mitochondrial bodies. × 2,200.

patches composed of small darkly-staining granules, and are distributed throughout the protoplasm, not being confined to its basophile portions. Their number and size varies in different cells, some of which are crowded with them, while in others they are more or less scattered.

As to the nature of the granular substance filling the clear parts of the protoplasm of these cells, from the sections prepared by an ordinary method (fixation in Bouin and staining in Haemalum), I had already ample evidence that it is ordinary mucin. On the other hand, as the supposition of a secretion of mucin by these cells was absolutely denied by Stephenson, I had to study these glands in sections prepared by special methods

(Mucihaematein or Thionin), which enable one to detect the most minute quantities of mucin. Moreover, to obtain a definite result by these methods, it was important to apply them simultaneously to the pharyngeal gland and to some other glandular cells which are known to contain mucin. The best control tissue of this kind is undoubtedly the external integument of the same earthworm. In sections, not only of an extracted pharyngeal gland, but of the whole anterior portion of the earthworm, it is always possible to make a comparison of the staining reactions of the pharyngeal gland with those of the mucin cells of the skin. We will now examine the longitudinal median sections of the anterior segments of Allolobophora chlorotica stained by the Mucihaematein method (see p. 38 of this paper). These sections, after thirty seconds to two minutes staining in 10 per cent. solution of Mucihaematein, show already a very clear picture of the distribution of mucin in the different tissues. These sections, when counterstained with Magenta-red and Picro-Indigo-carmine, become still more instructive ; the skin then shows clearly (Pl. 3, fig. 1), (1) the epidermal cells with greenish-yellow protoplasm and red nuclei, and (2) the mucin cells (*mu. c.*), in all stages of secretion of mucin, stained deep violet ; the small nuclei of these cells are displaced laterally or basally by the mucin (*mu.*), which in some cells is seen to issue from a small pore in the cuticle (*cu.*).

The same sections show also the salivary secretion of the pharyngeal gland cells (Pl. 3, figs. 2 and 4, *m. gl.*).

The basophile protoplasm of these cells is stained red, while the clear protoplasmic areas are now seen to be composed of granular mass (*mu.*) stained, like the mucin of the cutaneous gland, deep violet. This shows that the granular substance of the pharyngeal gland cells, which has been already mentioned by Stephenson, is composed of ordinary mucin. The results obtained by the Mucihaematein method were corroborated by the Thionin method. Sections of the anterior portion of Allolobophora foetida prepared by this method have also shown the pharyngeal gland cells filled (Pl. 3, fig. 9, *m. gl.*) with granules of mucin (*mu.*) similar to those of the mucin cells

of the skin (Pl. 3, fig. 10, *mu. c.*). In these sections the mucin is stained red, while the rest of the tissue stains in all shades of blue.

(*b*) *Conductive or musculo-vascular portion.*

As one follows them continuously from the deep glandular portion to the muscular or central region of the pharyngeal bulb, the glandular cells gradually change their structure (Pl. 3, fig. 5, *m. gl.*). They become smaller, their basophile protoplasm becomes more and more reduced, while the clear protoplasm, filled with granules of mucin, rapidly increases in quantity. These granular mucinous portions of the cells fuse together and form wide strands of mucin, the granules of which are regularly distributed in a multitude of sinuous rows (*mu.*). Nearer to the pharynx several small cells with basophile protoplasm may still be found embedded in this mucin, but usually one finds on the surface of these mucin ducts a few small nuclei (Pl. 3, fig. 6, *d. mu.*) filled with chromatic granules. These large mucin ducts subdivide and pass gradually into smaller ducts which are interlaced with the muscle fibres (*m.*) and blood-vessels (*v.*) This gradual passage of the glandular salivary cells into the salivary or mucin ducts was misinterpreted by Stephenson for a gradual transformation of his ' chromophile ' cells into fibrillar or reticular packing tissue (' Füllegewebe '). It is also evident that the connective tissue described by Stephenson is no other than the above-described salivary ducts containing precipitated and stained mucin. The musculo-vascular portion of the pharyngeal gland thus contains : (1) very strongly developed muscle fibres, (2) blood-vessels, and (3) salivary ducts filled with mucin.

To these we can now add : (4) nerve fibres, (5) nephrocytes or excretory cells similar to the yellow cells of the alimentary canal, and, finally, (6) cells with bacteroids or crystals of uric acid (Pl. 3, figs. 2 and 9, *ur.*). Concerning the nature of the last two elements I have more to say in the supplementary notes to this paper (p. 54).

(c) *Superficial or epithelial portion.*

It is a matter of surprise that, in spite of the fact that he absolutely condemns Eisen's observations as to the existence of ductules in the pharyngeal epithelium, Stephenson made no special study of this particular portion of the pharynx, although such study is all-essential for making a correct interpretation of the function of the pharyngeal gland cells.

The lumen of the pharynx (Text-figs. 1, 2, and 6, A) in all earthworms is divided by means of two longitudinal folds of the lateral walls into dorsal and ventral chambers. An elongated median slit, bordered by the free margin of these folds, establishes a communication between these portions of the pharyngeal lumen. The lateral folds meet posteriorly in the median line to form a posterior dorsal pharyngeal pocket which communicates with the two lateral pockets and forms the dorsal or salivary chamber of the pharynx (Text-fig. 1, *ph. d.*, and Text-fig. 6, A, *ph. d.*), while the ventral chamber (*ph. v.*) is continued into the oesophagus (*oe*).

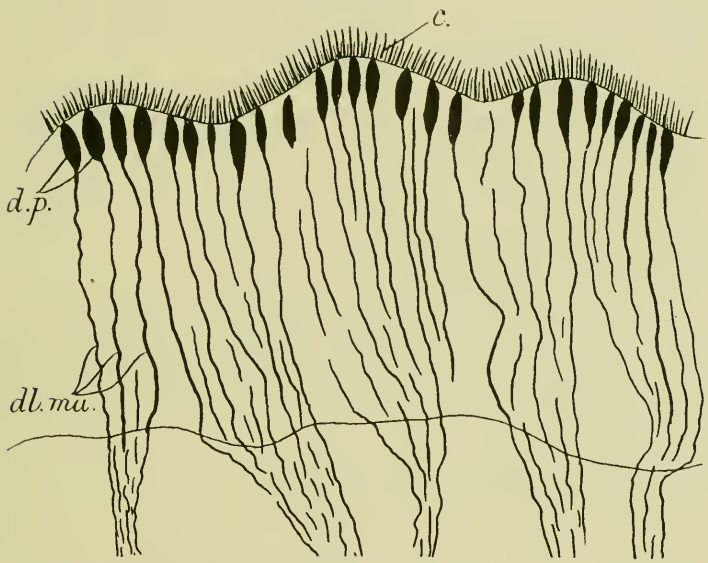
Of all the pharyngeal epithelium, the dorsal portion only, to which the pharyngeal bulb is attached, is composed of ciliated cells. The cells of the remaining portion of the pharyngeal epithelium are covered by a thin cuticular layer similar to that which lines the oesophagus.

The dorsal portion of the pharyngeal epithelium of Allolobophora chlorotica (Pl. 3, fig. 3) is composed of elongated cells, the oval nuclei of which are provided each with one or two nucleoli besides the chromatic granulation. These cells are usually so crowded that, in sections, their nuclei appear to lie at different levels. The free border of the cells bears the vibratile cilia (*cl.*).

The basal ends of the cells are very narrow and covered with a basal membrane. Near the free border of the epithelium one often sees the darkly-stained nuclei in all stages of the karyokinesis. As one follows their approach to the internal surface of the pharyngeal epithelium, the mucin ducts (Pl. 3, fig. 3, *d. mu.*), which, as we have previously seen, are interlaced with

the muscle fibres (*m.*) and blood-vessels, are seen to become parallel to each other and perpendicular to the epithelium. Reaching the basal membrane of the latter, these salivary ducts give off numerous small ductules (*dl. mu.*) which penetrate between the epithelial cells and terminate separately in a multitude of small pockets (*d. p.*) of mucin lying immediately

Text-fig. 4.



Section of the ciliated pharyngeal epithelium of All. foetida (stained with Mucihaematein only, showing the intra-epithelial mucin ductules = *dl. mu.*, ending in the discharge pockets = *d. p.*; *c.* = cilia). × 750.

beneath the free surface at the base of the cilia. These fine ductules, with the terminal discharge pockets, are very clearly seen in sections stained by Mucihaematein alone (Text-fig. 4), or combined with Magenta-red, Picro-Indigo-carmine, or by the Thionin method. In the first two cases they are all stained violet while the surrounding protoplasm is either unstained or greenish yellow in colour (Pl. 3, fig. 3), in the second case (ex. All. foetida) these ductules are red, while the rest of the

tissue is blue (Pl. 3, figs. 7 and 8). Some of the sections of All. foetida stained by the latter method showed the actual discharge of the mucin from the terminal or discharge pockets (*d. p.*) into the pharyngeal lumen (Pl. 3, fig. 8 *d. p.* and *mu.*). The latter in all sections is shown to be filled with mucin (*mu.*), which flows partly towards the buccal cavity and partly towards the oesophagus. It is very important to examine now a number of observations of certain histologists, who, treating of the minute structure of this organ from quite a different standpoint, and using a totally different technique, discovered nevertheless the ductules with their discharge pockets in the pharyngeal epithelium, but unfortunately completely misunderstood their nature and their function. I am alluding here to the papers dealing with the study of the peripheral nerve endings and sensory cells of earthworms.

In 1892 Retzius discovered in the pharyngeal epithelium special fibrils which he named clubbed fibrils—'Kolbenförmige fasern'—and which he supposed to be the gustatory sensory cells.

In 1894 Smirnow, to whom we owe the discovery of free nerve endings in the skin and the pharyngeal epithelium of the earthworm, using Golgi's method, detected in the pharyngeal epithelium the clubbed cells of Retzius.[1]

Smirnow's description of these cells closely resembles that of Retzius; he found in the pharyngeal epithelium an enormous number of these cells, which in their terminal dilated portion seem to contain nuclei. Their elongated portion he described as somewhat tubular with the lumen filled with a granular substance, and the whole structure of the club-shaped cells leaves, according to Smirnow, some doubt as to their nervous origin.

A year later (1895) Retzius confirmed Smirnow's discovery of the free nerve endings of the skin and the pharyngeal epithelium of the earthworm; and, returning to the subject of his clubbed fibrils, he now denied the existence of nuclei in the

[1] It may be mentioned that, under the name of oesophagus, Smirnow was actually dealing with the salivary portion of the pharynx.

dilated terminal portion of these fibrils ; he also disagreed with Smirnow as to their tubular structure and he described them once more in some detail. These fibrils in traversing the pharyngeal epithelium do not ramify and are completely devoid of the varicose nodules so characteristic of the nerve fibrils which are met with in the same pharyngeal epithelium. He failed again to detect the origin of these fibrils and still considered them to be nervous elements, but he added that further study, and especially the discovery of their central origin, would finally solve the problem as to their nature and their function.

The same year Langdon (1895), relying upon Smirnow's description, denied the nervous nature of the clubbed fibrils and considered them to be glandular or mucous cells.

More recently, Dechant (1906) demonstrated the same fibrils by a metallic impregnation method, and, in accordance with Retzius, described them as nervous elements.

I myself have recognized the structures described as clubbed fibrils by Retzius in the pharyngeal epithelium of L u m b r i c u s s p. fixed with Champy and stained with Iron Haematoxylin. The fibrils, in enormous numbers, run between the pharyngeal cells and are either straight or sinuous ; they all terminate in a very dilated portion filled with granular substance (Text-fig. 5, A and B).

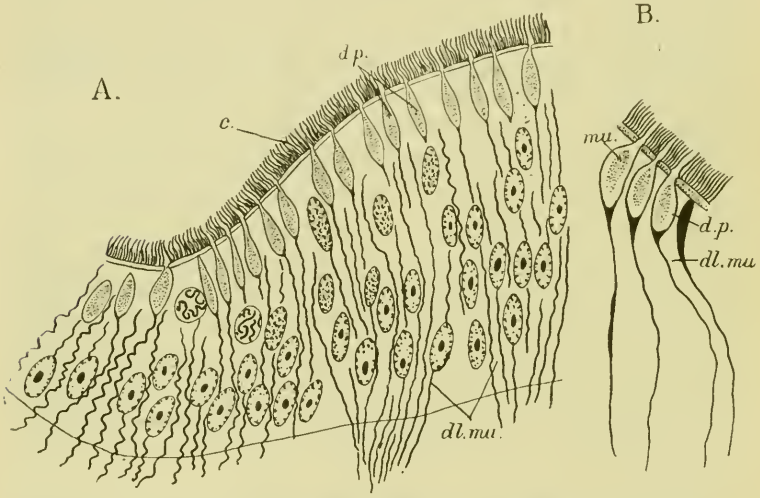
The merest glance at the structures convinced me that I was dealing with the same mucin ductules and their discharge pockets. The only difference between these structures and those previously described consists mainly in the fact that, while previously we stained only the mucin which fills the ductules and the pockets, now we stained the ductules and the pockets themselves. Moreover, the figures of the clubbed fibrils as shown in the papers of Retzius, Smirnow, and Dechant are similar in all respects to my figures of the intra-epithelial mucin ductules and their discharge pockets (Pl. 3, figs. 3, 7, and 8, and Text-figs. 4 and 5). On the other hand, the fact that these authors succeeded in detecting these salivary ductules by metallic impregnation methods is not surprising, as these

methods were already advocated by Müller (1895), Zimmermann (1898), and Retzius himself, for the detection of minute, or even intracellular, capillary ductules of secretion.

(*d*) *Septal glands.*

The salivary gland cells in all earthworms are intimately connected with some other cell aggregates which, being cyto-

Text-fig. 5.



A and B. Sections of the ciliated pharyngeal epithelium of Lumbricus sp. (fixed in Champy's solution and stained with Iron-haematoxylin) demonstrating that the clubbed nerve fibrillae of Retzius are the intra-epithelial mucin ductules (*dl. mu.*) with their discharge pockets (*d. p.*); *c.* = cilia; *mu.* = contracted mucin in some of the discharge pockets. A × 734; B × 734.

logically similar to the salivary cells, differ from the latter in the fact that they are completely devoid of mucin (Pl. 3, fig. 2, *e. gl.*).

Similar glandular aggregates, devoid of mucin, are found posteriorly in the coelomic cavity, surrounding the oesophagus. In places I believe that I have been able to trace a communication between these deeply-lying glandular elements (septal glands) and the pharyngeal bulb. In other places, although I

could not trace any communication between these cell aggregates and the pharyngeal or oesophageal walls, on account of the difficulty of following the course of these fine ductules in sections, I nevertheless believe that such communication exists. The function of these cells, as we shall see later, consists probably in elaborating a digestive enzyme which is discharged into the lumen of the pharynx or oesophagus.

4. Function of the Pharyngeal Gland Cells.

All the foregoing has proved, beyond doubt, that the pharyngeal bulb of the earthworm is a true salivary gland, which pours its secretion (mucin) into the lumen of the dorsal or salivary chamber of the pharynx. The mucinous salivary secretion accumulates in the pharyngeal cavity and oesophagus, and there it performs an important service during the operation of feeding. In view of the unusual diet of earthworms in general, it would be a matter of surprise to find that no special provision was made by which the relatively enormous quantities of earthy matter, composed, in great part, of hard and insoluble particles, could be conveniently passed through the alimentary tract.[1]

In addition to the function of the formation of the food bolus, the salivary secretion has also a digestive function. In connexion with this digestive function of the pharyngeal bulb, it is interesting to examine briefly the available information concerning the digestive ferments of earthworms.

Frédéricq (1878) was the first to discover in the alimentary canal of the earthworm the existence of two ferments : the one amylolytic, and the other proteolytic, the latter being active in either a slightly alkaline or a slightly acid medium.

Darwin (1881, pp. 35–43), in his classical observations on the habits of earthworms, stated that they emit from the mouth an alkaline secretion, containing a ferment similar to the pancreatic

[1] In several earthworms, according to Vejdovsky and Eisen, the salivary portion of the pharyngeal wall is very easily protruded or evaginated from the buccal cavity and serves a more or less prehensile function.

enzyme, which digests the leaves which are dragged into the burrows before they are taken into the alimentary canal. This mode of extra-stomachal digestion he compares to that of insectivorous plants, as Drosera or Dionaea.

The amylolytic and proteolytic ferments in earthworms were also described by Willem and Minne (1899), and more recently by Lesser and Taschenberg (1908). The last two authors found, in addition, the following enzymes: (1) an enzyme capable of hydrolysing glycogen, (2) Invertase, (3) Lipase, (4) Katalase, and (5) one which very probably was an Aldehydase.

Of the work cited above, that of Willem and Minne is of especial interest, inasmuch as they prepared extracts separately from the individual parts of the alimentary tract, while the other authors used extracts of the entire alimentary canal. Thus the extract which they obtained from the isolated pharynges of several earthworms digested fibrin in alkaline media and produced peptone. According to these authors this proteolytic ferment is derived only from the pharyngeal gland cells, although they failed to establish the existence of an actual communication between their ductules and the pharyngeal lumen.[1]

The pharyngeal bulb, with its accessory glandular aggregates, has, then, a double function: (1) secretion of mucin, and (2) secretion of a proteolytic enzyme. We have seen, on the other hand, that the glandular aggregates comprise two kinds of cells, the one containing the mucin, and the other devoid of it; it is then very probable that the cellular aggregates devoid of mucin are those which elaborate the proteolytic ferment. This is cor-

[1] The following is a quotation from the papers of Willem and Minne (pp. 2 and 3) relating to this question: 'Il est très pénible de suivre sur les coupes le trajet des conduits glandulaires; on en retrouve des tronçons au sein de la masse des fibres musculaires, et l'épithélium cylindrique du cul-de-sac pharyngien dorsal présente entre ses cellules des lumières qui nous paraissent correspondre aux extrémités de ces canaux. Les éléments dont nous parlons sont les seuls de la masse pharyngienne dont la structure soit compatible avec une fonction glandulaire, on doit leur attribuer la sécrétion du ferment peptonisant dont nous avons constaté l'existence dans les parois de l'organe.'

roborated by the fact that the extracts from the oesophageal portion, which, as we have seen, is surrounded only by the non-mucinous glandular cells, contains, according to Willem and Minne, a proteolytic ferment, although in smaller quantity than that of the pharyngeal bulb.

Having established the glandular nature of the pharyngeal bulb, and having shown its function, it seems to me quite superfluous to seek further proof in a study of the development of the pharyngeal glandular cells. As to the origin of these cells, Stephenson's statement that they are derived from the peritoneal layer appears to me to be doubtful. His description, and especially his figures, do not give the slightest support to this opinion, and I consider that the question of the development of the pharyngeal gland cells remains still open for further investigations.

5. Summary and Conclusions.

1. The pharyngeal dorsal bulb of the earthworm is a true salivary gland.

2. The function of the basophile cell-aggregates of this bulb is the production of mucin and a proteolytic enzyme.

3. These products of secretion are collected in a system of salivary ducts lying in the conductive musculo-vascular portion of the pharyngeal bulb. The salivary ducts, on reaching the pharyngeal ciliated epithelium, divide into innumerable fine ductules which penetrate between the epithelial cells and terminate near the free surface in the discharge pockets. The salivary secretion accumulates in these pockets before it is discharged into the dorsal or salivary chamber of the pharynx.

4. The club-shaped fibrillae of the pharyngeal epithelium discovered by Retzius are not of a nervous nature, as he supposed ; they are the ordinary salivary ductules with their discharge pockets.

5. The question as to the development of the pharyngeal bulb of the earthworms remains open for further investigations.

6. In addition to the glandular cells with their ducts, muscles, nerve fibres, and blood-vessels, the pharyngeal bulb contains

bacteroid or uric acid cells and amoebocytes, similar to the yellow cells of the alimentary canal.

6. Supplementary Notes.

According to Cuénot (1897) and Willem and Minne (1899) there are five different excretory organs in earthworms: (1) nephrida, (2) chloragogenous cells, which contain guanine, (3) cells with bacteroids or with crystals of uric acid, (4) yellow cells of the walls of alimentary canal, (5) amoebocytes of the blood. As the two latter elements are found in the pharyngeal bulb, we will examine them in greater detail.

(*a*) *Cells with bacteroids or crystals of uric acid.*

These cells are very common in earthworms, being found in enormous numbers on the peritoneum, the septa, between the muscle fibres, on the nerve ganglia, in the nephridia, &c. In the case of Allolobophora foetida, I found them in large numbers between the muscles of the pharyngeal bulb (cf. p. 45 of this paper). These cells, of various shapes and sizes, are filled with elongated crystalline bodies. In sections, or in stained smears, these bodies so closely resemble bacteria, that several authors have considered them to be such. Thus, according to Cuénot, Cerfontaine (1890) described them as bacilli; he also thinks that the tubercle bacilli, found in such numbers by Lortet and Despeignes (1892) in the bodies of earthworms which lived in soil mixed with the sputum of tuberculous patients, were also the bacteroids of these excretory cells, and, moreover, Cuénot believes that among the three kinds of commensal bacteria, found by Lim Boon Keng (1895) in the coelomic fluid of earthworms, there were undoubtedly some of the bacteroids which had become accidentally freed from the cells. The crystalline nature of these bacteroid bodies was demonstrated by Cuénot, while their chemical composition (i.e. that they are formed of uric acid) was proved by Willem and Minne.[1]

[1] It is important to mention here that Willem and Minne (1899, pp. 16–19) have completely misunderstood Cuénot, in ascribing to him the opinion

(*b*) *Yellow cells of the alimentary canal.*

In the wall of the alimentary canal of the earthworm, between the epithelial cells, there are often found special cells filled with yellow spherules. These cells vary in size and shape; they may be either spherical or oblong, or even irregular and amoeboid. The number of nuclei depends upon the size of the cell, and the cells occupy a variable position in the wall of the gut, being either very deeply placed in the epithelium, near the coelomic cavity, or extending themselves to the lumen of the gut. Cuénot, to whom we owe a very good description of these cells, considered them as belonging to the intestinal epithelium, and ascribed to them an excretory function. According to Willem and Minne these cells do not belong to the alimentary canal, but are amoebocytes which originate from the haematic system.

They make their way through the walls of the blood-vessels and the epithelial cells of the mid-gut, which they destroy on their way, and then, filled with the products of excretion, they leave the organism by way of the intestine.

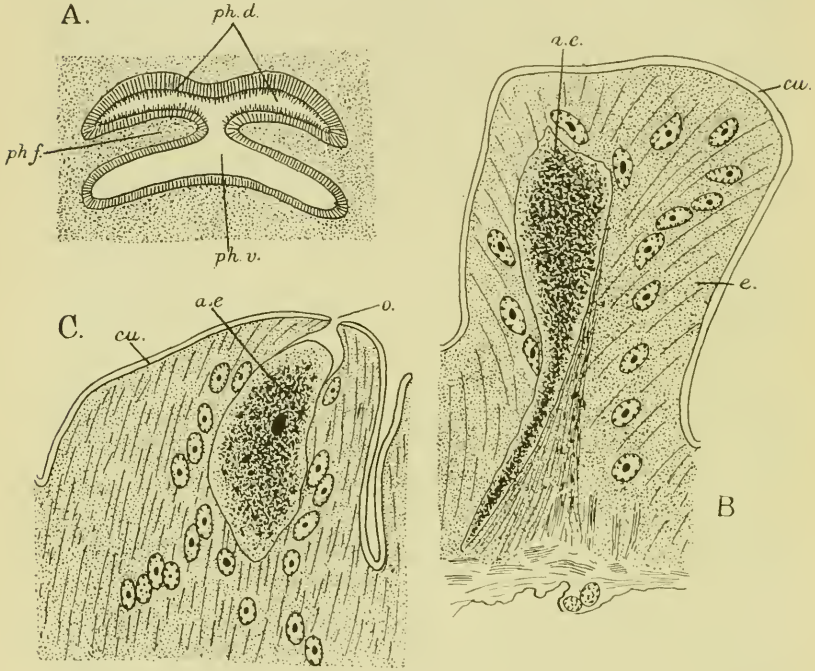
The distribution of these cells in different specimens is very irregular; in some specimens they are rare and difficult to find, while in others they are very numerous.

Up to the present these cells have only been mentioned as occurring in the wall of the alimentary canal between the crop and anus. During this study I frequently found them in the pharyngeal bulb and especially in the wall of the oesophagus, which they traverse in the same manner as they do the wall of the intestine. Text-figure 6, B and C, shows these cells lying in the wall of the oesophagus, their protoplasm being filled with corpuscles of excretion, fat spherules, and some albuminoid bodies. On several occasions I found the cuticle of the oesophagus perforated at the place of contact of the yellow cells, thus establishing a communication (Text-fig. 6, C, *o*.) between

that the bacteroid bodies are the real bacilli. Throughout his work Cuénot criticized this opinion, and described and figured these bodies as 'cristalloïdes' of excretion.

the latter and the oesophageal lumen. It is very easy to conceive that a violent contraction of the earthworm will expel these cells, with their contents, into the lumen of the alimentary

TEXT-FIG. 6.



A. Schematic figure representing a transverse section of the pharynx of the earthworm: *ph. d.* = dorsal or salivary chamber of pharynx; *ph. f.* = lateral folds of the pharyngeal wall; *ph. v.* = ventral chamber of pharynx.

B and C. Sections of the oesophageal wall of All. foetida, showing a yellow cell or excretory amoebocyte in the act of traversing it. × 500. *ae.* = amoebocyte; *cu.* = cuticle of oesophageal epithelium; *e.* = oesophageal epithelium; *o.* = opening in the oesophageal wall through which the amoebocyte will pass into the lumen of the alimentary canal.

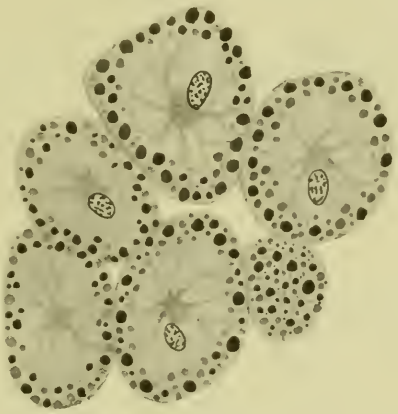
canal. The fact that these excretory cells are found indifferently in all the portions of the alimentary canal corroborates the supposition of Willem and Minne, that these cells do not

belong to the intestinal epithelium but are amoebocytes of the haematic system which fulfil an excretory function.

(*c*) *Reserve substance in Oligochaetes.*

From the work of Gegenbaur, Beddard, and Cuénot it is known that the usual nutrient reserve substance of Oligochaetes is glycogen, which is localized in the special peritoneal cells which surround the nephridia. These authors have also mentioned that in some earthworms the glycogen is replaced by fat.

Text-fig. 7.



Coelomic cells containing droplets of fat (cf. Text-fig. 1, *f. c.*, p. 40 of this paper). × 1,100.

More recently Willem and Minne (1899 a), who have made complete analyses of earthworms, found that their reserve substance is composed of fat and glycogen, the first being localized in the ciliated cells of the intestinal epithelium, while the second is found in the peritoneal cells.[1]

[1] The following is a quotation from the paper of these authors: 'On rencontre chez les lombrics, comme produits de réserve, de la graisse et du glycogène; la première, constituée surtout par de l'oléine, est localisée dans des cellules ciliées de l'épithélium intestinal; le glycogène s'observe dans des cellules péritonéales et fournit, comme dérivé, de la dextrine' (pp. 42–3).

In Allolophobora foetida, I found that the coelome of segments 5, 6 and 7 is often filled with a crowded mass of cells surrounding the glandular portion of the pharyngeal bulb (Text-fig. 1, *f. c.*). These cells, in sections fixed with Carnoy or Brazil, show a central nucleus lying in a condensed central portion of the protoplasm, while the remaining part of the latter is filled with vacuoles (Text-fig. 7).

Sections of specimens fixed with Champy's solution show that the external or vacuolar portion of these cells contains numerous globules stained in all shades, from dark brown to black. These globules are undoubtedly droplets of fat, which, in specimens fixed with Carnoy, are dissolved. It is quite possible that this accumulation of fat, not only in the cells of the alimentary canal or peritoneal cells, but in the free coelomic cells, is only seasonal, and is related to the period of sexual activity of the earthworm.

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EXPLANATION OF PLATE 3.

Illustrating Dr. Keilin's paper: 'On the Pharyngeal or Salivary Gland of the Earthworm.'

Key to Lettering on Plate.

cl. = cilia.
cu. = cuticle.
dl. mu. = intra-epithelial mucin ductules.
d. mu. = mucin ducts.
d. p. = mucin discharge pockets.
e. gl. = enzyme-secreting glandular cells.
m. = muscles.
m. gl. = mucin-secreting pharyngeal, or salivary cells.
mu. = mucin.
mu. c. = mucin cells of the skin.
v. = blood-vessels.
ur. = crystals of uric acid or bacteroids.

Figs. 1 to 6 concern Allolobophora chlorotica Sav. All the sections were stained with the Mucihaematein of P. Mayer, and Magenta-red and Picro-Indigo-carmine (see pp. 38–9 of this paper).

Figs. 7 to 10 represent sections of Allolobophora foetida stained by the Thionin method (see pp. 38–9 of this paper). The nuclei of the cells are of a dark-blue colour, not purple as shown in these figures.

Fig. 1.—Section of the skin of All. chlorotica, showing mucin cells (*mu. c.*) in different stages of activity. × 825.

Fig. 2.—Deep glandular portion of the pharyngeal bulb showing the mucin-secreting salivary cells (*m. gl.*) and the enzyme secreting-cells (*e. gl.*). × 825.

Fig. 3.—Epithelial and subepithelial portion of the pharyngeal bulb, showing the salivary or mucin ducts (*d. mu.*) dividing into a multitude of fine ductules (*dl. mu.*), which penetrate between the cells of the pharyngeal epithelium and terminate in the discharge pockets (*d. p.*) lying beneath the cilia (*cl.*) of the epithelial cells. × 562.

Fig. 4.—Glandular or salivary portion of the pharyngeal bulb, showing granules of mucin within the cells. × 825.

Fig. 5.—Portion of the pharyngeal bulb showing the transition between the glandular and the conductive regions. The mucin-secreting, basophile cells are widely separated by strands of mucin. × 825.

Fig. 6.—Conductive portion of the pharyngeal bulb, showing the mucin ducts (*d.mu.*), muscles (*m.*), and blood-vessels (*v.*). × 825.

Fig. 7.—Epithelial portion of the pharyngeal bulb of All. foetida stained by the Thionin method. Section similar to that of All. chlorotica represented by fig. 3, but with mucin stained red. × 562.

Fig. 8.—Portion of the pharyngeal epithelium of All. foetida showing the emission of mucin from the discharge pockets into the pharyngeal lumen. × 825.

Fig. 9.—Section of the glandular portion of the pharyngeal bulb of All. foetida showing the basophile cells filled with mucin. × 825.

Fig. 10.—Portion of the skin of All. foetida showing the mucin cells. × 825.

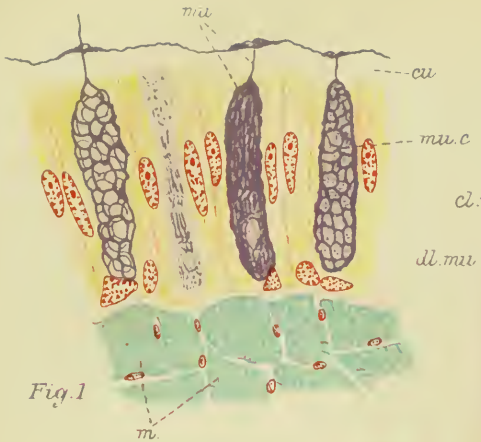


Fig.1

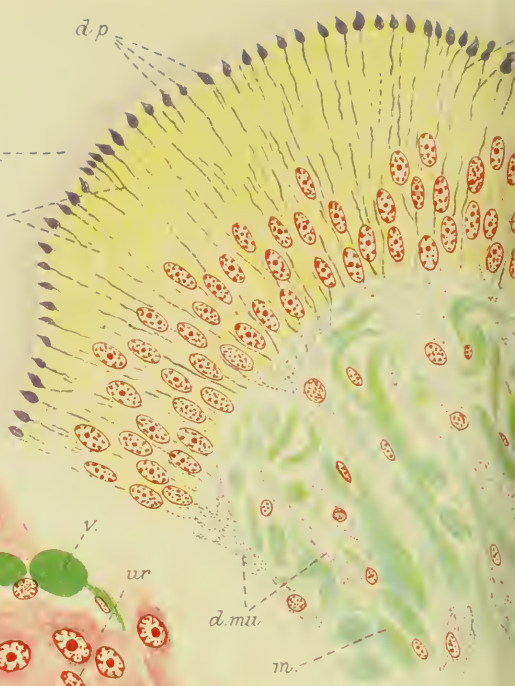


Fig.3.

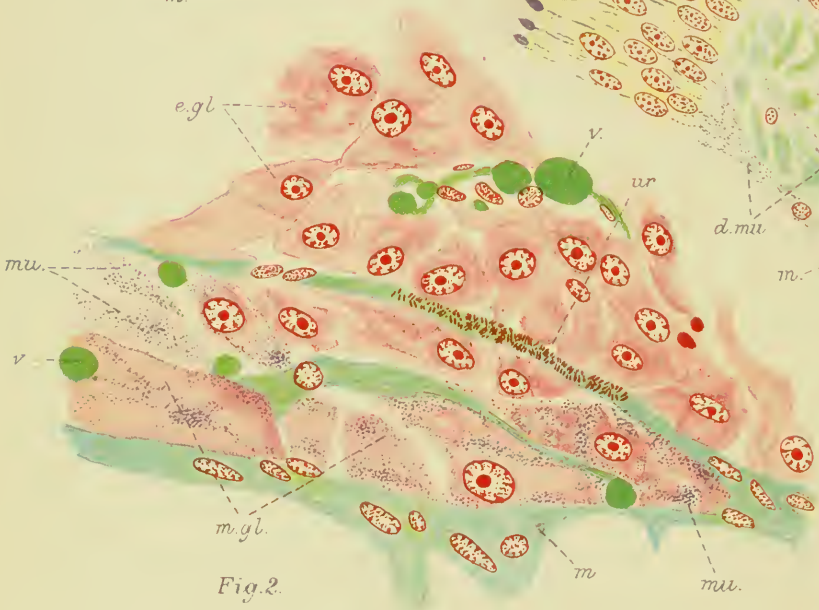
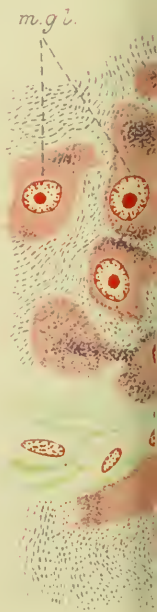


Fig.2.



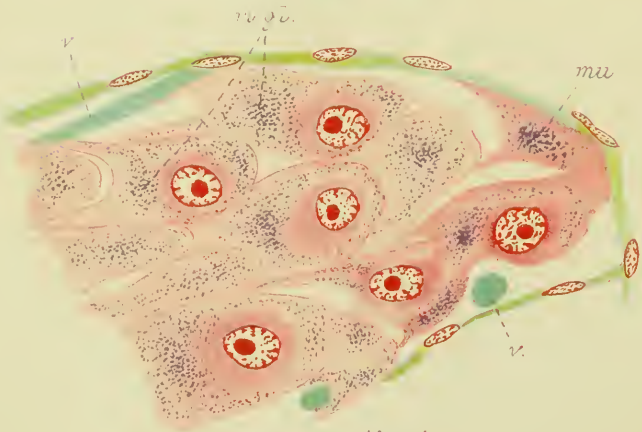


Fig.4.

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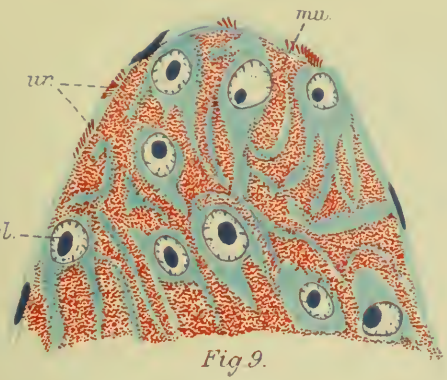

Fig 9.

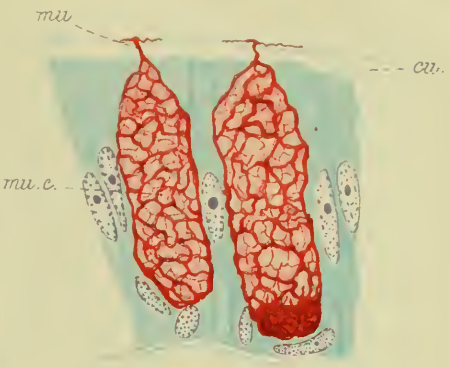

Fig. 10.

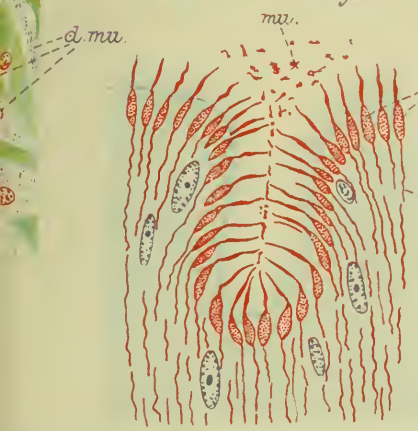

Fig. 8.

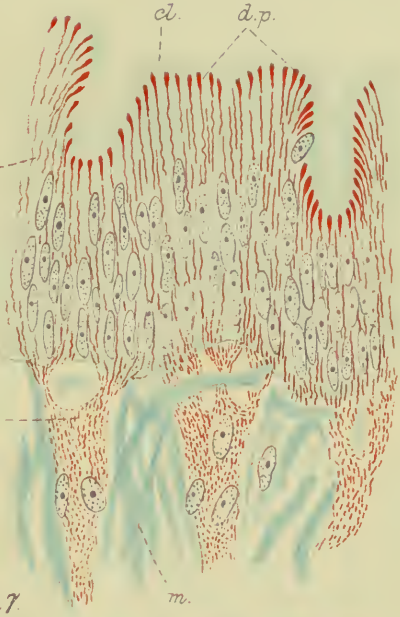

Fig. 7.

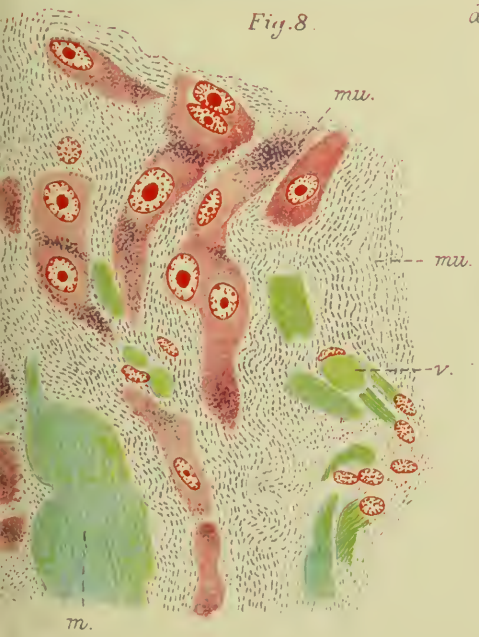

Fig. 5

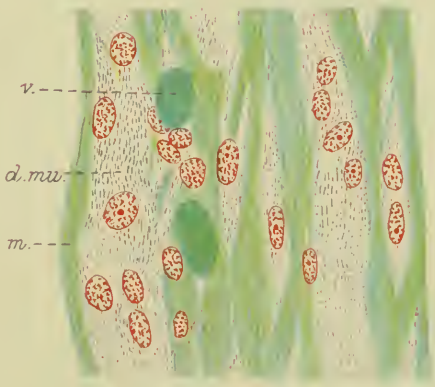

Fig. 6.

Some Observations on Caudal Autotomy and Regeneration in the Gecko (*Hemidactylus flaviviridis*, Rüppel), with Notes on the Tails of Sphenodon and Pygopus.

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With 6 Text-figures.

Contents.

Introductory.

During the rainy seasons (July to October) of 1914 and 1918 I made a large number of observations and experiments on the facts of caudal autotomy and regeneration in the common Indian Gecko, Hemidactylus flaviviridis[1] (Rüppel), a very familiar and useful 'snapper-up of unconsidered [insect] trifles' found on the walls of every bungalow in the United Provinces. In January 1915 I read a brief paper[2] on the subject at Madras on the occasion of the second meeting of the Indian Science Congress, but until the present year I have not had an opportunity of writing up a complete description of the results obtained by me.

The more conspicuous features of caudal autotomy and regeneration in Lacertilia, such as e.g. the intravertebral position of the cleavage or autotomy plane, the substitution of a continuous cartilaginous tube for vertebral centra, of an epithelial tube (an extension of the epithelium lining the canalis centralis) for the spinal cord, the change in lepidosis, absence of segmentation and subdivision of the muscles in the regenerated tail, and other features, have of course been known for many years (vide e.g. Fraisse[3] in 1885, Brindley[4] in 1895, and Tornier[5] in 1897); on the other hand, judging from recent literature on the subject known to me, there still appears to be a certain amount of uncertainty respecting even some of the main facts. E.g. in Morgan's 'Regeneration'[6] it is stated

[1] The H. coctaei of the 'Fauna of British India'. At least two other species or genera of Geckos are common in Allahabad, but the facts described in this paper apply to all.

[2] Published in brief abstract in the official account of the Proceedings of the Congress issued by the Asiatic Society of Bengal, and in several Madras daily papers.

[3] Fraisse, P., 'Die Regeneration von Geweben und Organen bei den Wirbelthieren, besonders Amphibien und Reptilien', Cassel, 1885.

[4] Brindley, H. H., "Some Cases of Caudal Abnormality in Mabuia carinata and other Lizards", 'Jour. Bombay Nat. Hist. Soc.', vol. xi, 1897–8, p. 680.

[5] Tornier, G., "Über experimentell erzeugte dreischwanzige Eidechsen und Doppelgliedmassen von Molchen", 'Zool. Anzeig.', Band xx, 1897, p. 356.

[6] 'Regeneration', T. H. Morgan, 1901.

in a foot-note (p. 198) that 'the attachment of the muscles may be the cause of the break in the middle of the vertebrae, rather than between two vertebrae', and this statement (true to a large extent), coupled with Powell White's recent assertion [1] that 'there is no special autotomy-site as in the legs of crabs, but apparently any vertebra may be involved' (also true in one sense), might very easily convey the impression that caudal autotomy is a mere mechanical fracture of any given vertebra, with the connected muscles and skin. The whole truth is, as Leydig I believe first pointed out, that instead of there being only one autotomy plane as in the crab's claw, there are as many autotomy planes, each as complex in form as that of the Crustacean, as there are caudal segments. Further, I have not yet met with satisfactory accounts of the conditions under which autotomy occurs, of the exact modus operandi of autotomy, or of regeneration under certain experimental conditions, nor with any account of the mechanism by means of which haemorrhage is stopped when autotomy occurs, and I believe, therefore, that there is justification for describing the facts as a whole in the case of Hemidactylus flaviviridis.

Naked-eye Observations on Caudal Autotomy.

(Statement 1) That caudal autotomy is very common among Geckos may be concluded from the fact that over 50 per cent. of two hundred specimens used in my experiments bore regenerated tails, and that it is an easy process may be proved by the simple expedient of catching a Gecko by any portion of the tail posterior to the unsegmented base; thus I have caught hold of the remaining end of the tail of a young Gecko five times in almost as many seconds, and on each occasion a portion came off in my fingers. In nature the animals usually shed their tails when bitten by other Geckos or other animals (e. g. out of twelve perfect Geckos placed together in a box on one occasion five had shed their tails within an hour).

(2) Geckos never shed their tails 'spontaneously' or from

[1] Vide 'Report of Brit. Assoc. Advancement Science', Manchester, September, 1915, pp. 472, 473.

mere alarm.[1] This I have proved repeatedly by catching the animals by parts of the body other than the tail. Further, mere lateral flexion (the tail is not flexed to any extent dorso-ventrally) of the tail is insufficient to cause autotomy, as may be seen when, on being chloroformed, the animal lashes its tail vigorously. In fact, an all-essential condition for caudal autotomy is that the tail should be held a little distance posteriorly to the plane at which autotomy is to occur, a fulcrum thus being provided for the action of the muscles. I have proved this by anaesthetizing (with ether) a number of Geckos and tying cotton thread round the tail in different positions, the other end of the thread being fixed. On recovering from the ether, the captive Gecko would at first try to run away (though quite unalarmed, since I observed it from a good distance away) and only find itself a prisoner by the cotton becoming taut It would then, after several further attempts at escape, suddenly stretch the cotton to its full extent and deliberately autotomize one segment in front of the segment held by the cotton. This autotomy was not a mere result of the longitudinal pull on the tail (it requires a considerable force to pull off a portion of the tail in the direction of its length,[2] though the tail can be easily broken off by sharply

[1] Gilbert White, in a foot-note on page 64 of 'The Natural History of Selbourne', states that, 'the blind-worm or slow-worm does not need a blow to induce it to cast off its tail. A sudden fright is sufficient.' This is also stated to be the case for the American Opheosaurus ventralis, the 'glass snake'. If these statements be true (and the extreme brittleness of the tail is doubtless correlated with the rigidity of the tail assumed when the animal is alarmed, all the muscles contracting strongly), it is proof that autotomy is a much easier process in these forms than in the Gecko. Such forms as Anolis principalis, the American 'Chameleon', which can usually be captured by seizing the tail, the animal only being able to autotomize by a great effort, and Uromastix spinipes, which allows its tail to be pulled off rather than release its hold on its burrow, on the other hand, lie at the other end of the scale.

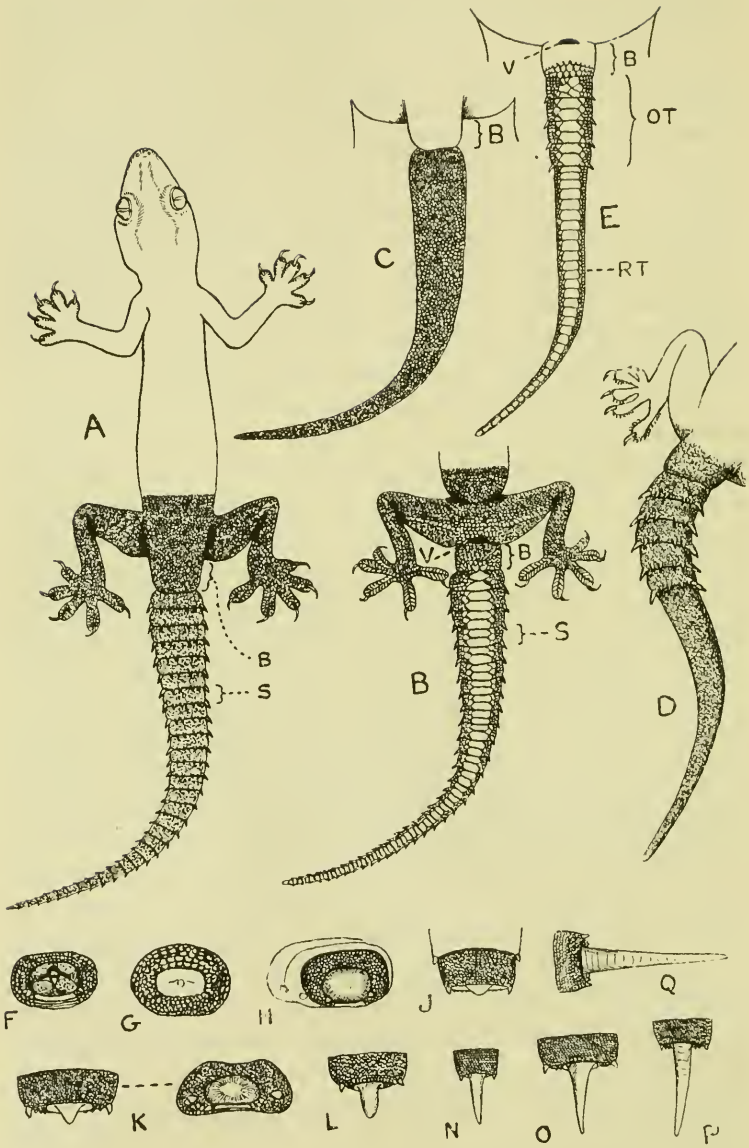
[2] In six recently killed Geckos, varying in length from 9·9 cm. to 13·4 cm., and in body-weight from 2·4 grm. to 5·5 grm., with the cotton thread suspending the weight tied midway in the length of the tail (hanging vertically), the weights necessary to break the original tail varied between 54 grm. and 129 grm., as kindly determined for me by Mr. B. K. Bas, M.Sc.

bending it at one point laterally), but was a result of powerful localized contraction of the tail muscles causing sudden flexion at one point. These observations prove that autotomy is a purely voluntary process, and this is confirmed by the further fact that Geckos, caught by the tail, sometimes refuse to autotomize when they perceive that escape is impossible (compare the refusal even to attempt to fill the gas bladder with more oxygen when a fish is over-weighted[1]). On one occasion a Gecko, tied up by thread, remained captive for three days, though it frequently tried to run away when I approached, and it was only when I held the tip of the tail that autotomy occurred—apparently the fulcrum provided by the cotton attachment was insufficient in this case.

(3) The original (non-regenerated) tail of H. flaviviridis consists of a basal unsegmented region (the 'base') covered only with small inconspicuous scales, and about thirty autotomy segments, each of which can be distinguished dorsally (Text-fig. 1, A, D) by the presence on its extreme posterior edge of six large projecting scales (three on each side of the middle line), the outermost scale on each side being the largest; ventrally each segment extends lengthwise over two of the large median transversely-elongated flat scales (Text-fig. 1, B, E). As experiments prove, autotomy can occur at the posterior edge of the base of the tail or of any subsequent segment, but cannot occur in front of the posterior border of the base. In fifty captured specimens I have found examples of autotomy having occurred naturally at every segment situated between the base of the tail and the sixteenth segment: thus in seven specimens autotomy had occurred at the posterior edge of the base, i.e. the whole of the segmented tail had been shed; in ten specimens autotomy had occurred between the first and second segments, and so on, the examples decreasing in number the more posteriorly situated the site of autotomy. In nature autotomy usually occurs in the anterior half of the segmented region (Text-fig. 1, D, E), but may of course also occur posteriorly to this.

[1] Woodland, W. N. F., 'Anat. Anzeig.', Bd. XL, 1911, p. 225.

TEXT-FIG. 1.



The Gecko usually only sheds that portion of its tail necessary for escape ; in other words, autotomy usually occurs at a segment situated not more than two segments in front of the point of seizure of the tail. This is proved by the results of the following experiments :

Thread tied between	Tail shed behind
3rd and 4th segments ;	2nd segment ;
11th and 12th ,,	10th ,,
Thread tied across middle of	
8th segment ;	7th ,,
12th ,,	10th ,.
13th ,,	12th ,,
14th ,,	12th ,,
16th ,.	15th ,,
22nd ,,	20th ,,
26th ,,	25th ,,

TEXT-FIG. 1.

The Original and Regenerated Tails of the Gecko (all figures about natural size).

A. Hemidactylus flaviviridis with original tail, dorsal view.
B. ,, ,, Original tail, ventral aspect.
C. ,, ,, Tail regenerated from the base, dorsal aspect.
D. ,, ,, Tail regenerated from the 5th segment of the original tail, dorsal aspect.
E. ,, ,, Tail regenerated from the 4th segment of the original tail, ventral aspect.

B = unsegmented base of tail ; OT = original tail ; RT = regenerated tail ; S = one autotomy segment of the original tail ; V = cloacal aperture.

F. End-on aspect of a tail segment 3 days after autotomy has occurred. The edge of the original skin shows no sign of extension over the 'wound' surface. G. The same, 6 days afterwards. The surface of the wound is now covered over with a new young skin, formed by the histogenetic cells, hiding the transverse processes of the vertebra. H. The same, 9 days afterwards. The multiplication of the histogenetic cells has now produced a slight protuberance. J. The same, 11 days afterwards. K. The same, 13 days afterwards, dorsal and end-on aspects. The protuberance is now well marked. L, N, O, P, Q represent stages of growth after 15, 17, 19, 33, and 50 days respectively.

When a Gecko is wounded on the tail, it usually subsequently sheds the tail immediately anterior to the wound as the easiest method of repairing the injury.[1]

(4) The regenerated tail, not being segmented in character (see description of structure below), cannot be shed in parts (its thin fragile extremity can, however, be easily broken or bitten off), though it may be shed as a whole either at its junction with the stump of the original tail or attached to a few segments of the original tail. This has been proved by numerous experiments which I need not record. Usually (in eleven out of thirteen experiments) when a Gecko is caught by the thick anterior portion of the regenerated tail, the whole of the regenerated tail is shed at its junction with the stump of the original tail ; in some cases, however (in two out of the thirteen experiments), the regenerated tail is shed with either one or two (rarely more) of the posterior segments of the original tail attached ; in other words, autotomy at the junction of the regenerated with the original tail is only a little more easy than autotomy at any ordinary joint of the original tail.

(5) Whenever autotomy occurs, the escape of blood from the caudal artery is practically nil. If, however, a segment be cut through in the middle, haemorrhage is a little more pronounced, and if the base of the tail be cut through (i. e. anterior to the first joint or autotomy plane) bleeding is profuse. The explanation of these facts will be found in the description of the structure of the original tail given below.

Naked-eye Observations on Normal Caudal Regeneration.

(6) Regeneration of a tail only normally occurs at the posterior surface (*a*) of the unsegmented base of the original tail, or (*b*) of a segment of the original tail, or (*c*) of the end of the regenerated tail which has had a portion broken off (not autotomized). Text-fig. 1, F–Q, shows the stages of develop-

[1] In these cases, apparently, the weakening of the joint caused by the wound renders seizure of the tail posteriorly unnecessary.

ment of the regenerated tail in H. flaviviridis up to that of seven weeks, and Text-fig. 5, J′, K′, L′, shows a second tail being regenerated on the broken-off stump of a first regenerated tail.

The exact length of time it takes in H. flaviviridis for a new tail regenerated from the base to attain the full length of the original tail I do not know, but it is certainly not less than four or five months, and is probably more.

(7) There is apparently no limit (save that imposed by the longevity of the animal) to the number of times a tail can be regenerated.

(8) The skin of the regenerated tail is not a mere extension of that of the original tail but is a new product, as shown by both lepidosis (Text-fig. 1, D) and texture. The skin of the original tail is, like that of the trunk, head, and limbs, very soft and rubs off easily (the tail in consequence not being easy to skin), whereas the skin of the full-grown regenerated tail is relatively tough and the tail is easily skinned. After autotomy the original skin shows no signs whatever of growing over the raw exposed surface, and remains quite distinct from the new skin which covers the outgrowing regenerated tail (Text-fig. 1, H–P).

The General Structure of the Original Tail of Hemidactylus flaviviridis.

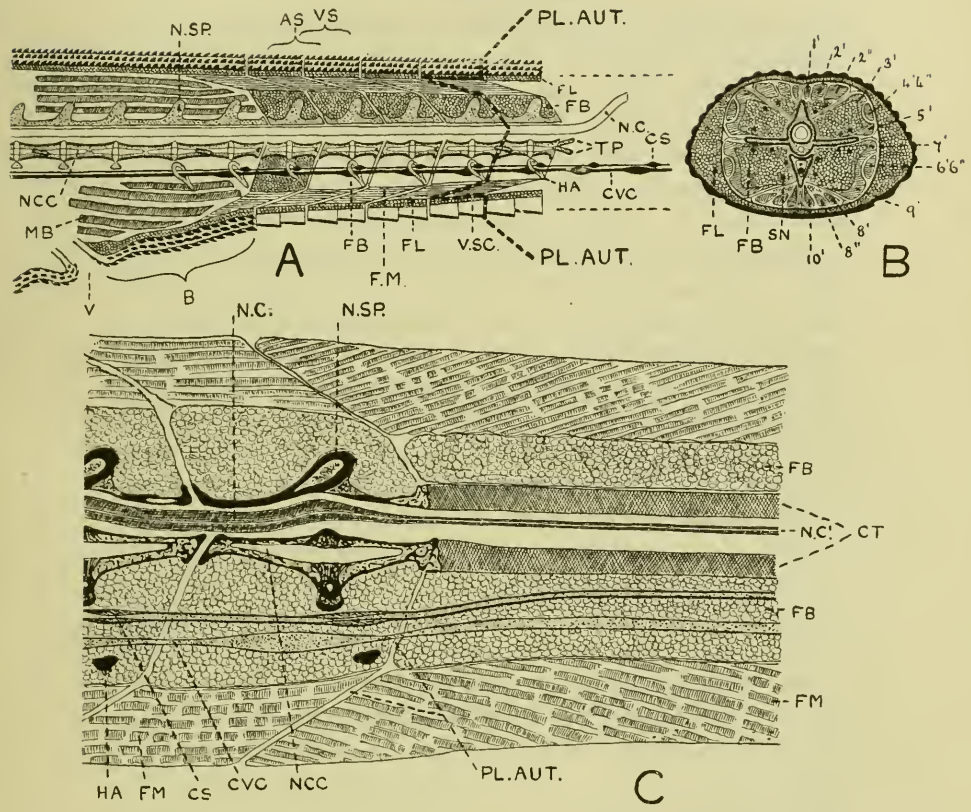
In those Lacertilia in which the tail is of distinct use to the animal for purposes other than that associated with autotomy, e. g. for prehension (as in the Gecko Ceratolophus auriculatus, Bavay, and in Chameleons), for swimming (as in aquatic Monitors, Iguanidae, Amblyrhynchus, Lophurus, and Physignathus), for steering (Basiliscus in water, Ptychozoon in air), or for balancing in air (Draco), caudal autotomy naturally does not occur, but it appears to me that the tail in the more common Lacertilia (Lacertidae, Agamidae, &c.) can be of but very little use to its owners. It is in these forms not used for swimming (as may be proved by throwing lizards into water,

when progression is seen to be effected mostly by undulations of the trunk, the tail only waving as an appendage of the trunk), nor for leverage (like the tails of hounds in turning corners), nor for balancing (lizards with amputated tails appear to be at no disadvantage in climbing or running), nor for means of offence (I have chased large Monitors in the jungles on the south coast of Ceylon and in southern India and on no occasion have they attempted to strike with their tails, though they can lash them) ; on the other hand, the tail must often be a positive disadvantage, since it is easy to catch most lizards by their tails. It is also a fact that in many lizards the tail muscles are more or less degenerate (the white muscles being valued as food in many cases), or at least incapable of exerting much force (in Central India the snake-charmers tie, without cord, the tails of small Monitors in loops round their necks, the bases of the tails then serving as convenient handles!).

These being the facts, it is not surprising that numerous Lacertilia have discovered in their tails, otherwise useless and indeed a danger, a means of self-preservation by the adoption of caudal autotomy. As we shall see in the Gecko, the whole structure of the tail is adapted for autotomy at every joint, and if, after describing these adaptations, we glance at the structure of the tails of lizards which are non-autotomous (e. g. Calotes), we shall appreciate the considerable simplification of structure which must have taken place in the ancestors of the Gecko in order to produce an autotomous tail.

If we examine longitudinal (Text-fig. 2, A, C) and transverse (Text-fig. 2, B) sections of the original Gecko tail we shall observe the following features. (*a*) The skin is divided into cylindrical regions, each covering one complete autotomy segment, by lines of cleavage (described in detail later), each of which extends round the entire circumference of the tail, and the small scales forming the uniform covering of the skin are arranged (Text-fig. 2, A) in correspondence with these regions : at the anterior or posterior edge of the region bordering the line of cleavage the small scales are arranged in a transverse circumferential line, whereas in the space between the lines

TEXT-FIG. 2.



Structure of the Original and Regenerated Tails of the Gecko.

A. Semi-diagrammatic thick sagittal section through the original tail of the Gecko (× cir. 8). AS = extent of autotomy segment through the vertebrae; B = unsegmented base of tail; CS = sphincter on caudal artery; CVC = constriction of caudal vein anterior to autotomy plane; FB = fat band; FL = subcutaneous fat layer; FM = flexor muscle; HA = haemal arch; MB = muscles of base; NC = spinal cord; NCC = notochordal canal; N.SP. = neural spine; PL.AUT. = plane of autotomy; TP = transverse processes of vertebra; V = cloacal opening; VS = extent of vertebral segment; V.SC. = transversely-elongated ventral scales. B. Semi-diagrammatic thick transverse inter-vertebral section through the original tail of the Gecko (× cir. 8); FB and FL as in A; SN = spinal nerves. The numerals indicating the flexor muscles seen in transverse section are for identification of these muscles with those shown in Text-fig. 3 (A, B, C, D, E, F). C. Sagittal section through the junction of the original and the regenerated tails (× cir. 60). Most letters as in A. CT = cartilaginous tube of the regenerated tail; N.C.′ = extension of epithelium lining the canalis centralis into the regenerated tail. The general character of the hyaline septa which mark the autotomy planes is well shown.

of cleavage, the scales of adjacent longitudinal rows alternate with each other. (*b*) Underlying the skin is a layer of fat cells, thin dorsally, thick laterally, and extremely thin ventrally (Text-fig. 2, B). This subcutaneous fat layer of the tail is also divided into cylindrical segments by lines of cleavage continuous with those of the skin ; on their internal surface the fat cells are bounded by a thin dense layer of connective tissue. Text-fig. 3, K represents the fat layer which has been cut through in the mid-dorsal line and flattened out. The extreme thickness of the two laterally-situated regions is well shown. (*c*) Lying internally to the subcutaneous fat layer is a layer of muscles, the caudal flexor muscles, the attachments of which will be described later. The laterally-situated flexor muscles are the thickest, as might be expected. On their external surfaces these muscles abut against the dense connective tissue lining of the subcutaneous fat layer, and on their internal surfaces they are for the most part attached to the outer surfaces of the submuscular fat bands. (*d*) Lying internally to the layer of caudal flexor muscles are the submuscular fat bands. These thick bands are four in number, two on each side of the vertebral column, and on each side one lying dorsally to the transverse process of the vertebra and the other ventrally. These fat bands are, like the subcutaneous fat layer, chiefly composed of fat cells, and are segmented by lines or rather planes of cleavage continuous with those already mentioned. The four fat bands are traversed by eight longitudinal radiating connective tissue septa (one dorsal, one ventral, two lateral, and four in between these), which unite the dense connective tissue layer lining the internal surface of the fat layer with the thin layer of connective tissue investing the vertebral column. These and minor septa (shown in Text-fig. 3, J, in which the fat layer has been cut through along eight lines, and the muscles and skin removed) separate the individual muscle processes from each other and serve to some extent for the attachment of the muscles. (*e*) Internally to the submuscular fat bands and forming the axis of the tail is the caudal vertebral column.

Each elongated saddle-shaped vertebra consists of an elongated centrum containing a notochordal canal (full of tissue not shown in the figures) running continuously between successive centra but closed at the planes of cleavage (autotomy planes), to be mentioned shortly. Successive centra are separated by intercentral pads of cartilage (perforated by the notochord), to which the bony haemal arches (chevron bones) are attached below. Midway in its length, and on the anterior side of the vertebral cleavage plane, each centrum gives off laterally on each side a large transverse process, which extends outwards and posteriorly to the outer surface of the submuscular fat bands. On the ventral side of each intervertebral joint and attached to the joint (not the centrum) is the haemal arch which bears a median haemal spine for the attachment of muscles. Dorsal to the centrum is the neural arch which mid-vertebrally bears a conspicuous neural spine. The well-known feature of the vertebral column in the segmented region of the tail is the presence of a vertebral cleavage plane dividing the whole vertebra (centrum and neural arch) into two pieces in the middle of its length, each autotomy segment thus containing the two halves of two successive vertebrae. This vertebral cleavage plane is marked by a hyaline septum which is continuous with the similar septa marking the cleavage planes of the skin, subcutaneous fat layer, muscular layer, and the submuscular fat bands, and it is therefore obvious that, with the exceptions of the spinal cord, spinal nerves, caudal artery, and caudal vein, and certain longitudinal blood-vessels, the whole substance of the tail is traversed at each intersegmental joint by a hyaline septum marking a continuous cleavage plane.

Nor do the adaptations to autotomy in the various systems of organs cease here. Though naturally the spinal cord and small longitudinal nerves and blood-vessels show no signs of cleavage planes, yet when we examine the two big blood-vessels of the tail we find special mechanisms for stopping haemorrhage when autotomy occurs. (*f*) The caudal

artery, when observed in longitudinal (Text-figs. 2, A, C, and 3, P) and serial transverse sections, is seen to possess in its course a number of regions in which its walls are very thick and its lumen therefore small. These thick-walled small-lumened regions constitute sphincters for the closure of the artery lumen, and each one of these sphincters is found to be situated immediately anterior to an autotomy plane (and behind the haemal arch of each vertebra) in the region of autotomy, and there is also one in front of the first autotomy plane (behind the last haemal arch of the unsegmented base of the tail), as might be anticipated. When autotomy occurs at any segment it is the closure of the sphincter on the caudal artery immediately in front of this segment that prevents haemorrhage. As far as I am aware, this is the only instance yet described of a sphincter muscle being developed on a blood-vessel. (*g*) The caudal vein does not possess sphincters and this is not surprising, since the flow of blood is towards the body and therefore away from the portion of tail which is cast off. Nevertheless, to prevent undue loss of blood when autotomy occurs, the vein becomes constricted in the region of each plane of cleavage and dilates at each in-between region (Text-fig. 2, A, C), i. e. in the region of each haemal arch, and when the tail is shed the open lumen apparently becomes temporarily plugged up by blood-clotting. (*h*) Concerning the spinal cord there is nothing worth remarking, save perhaps that it contains as usual Reissner's fibre (I have also observed this in the tail of Pygopus which is autotomous). It maintains an approximately uniform diameter throughout its course. On the ventral side of the spinal cord and in contact with its substance is a subneural vessel; also contained in the neural arch but lying external and ventral to the spinal cord are usually to be seen two lateral neural vessels, which in reality are part of a plexus of blood-vessels.

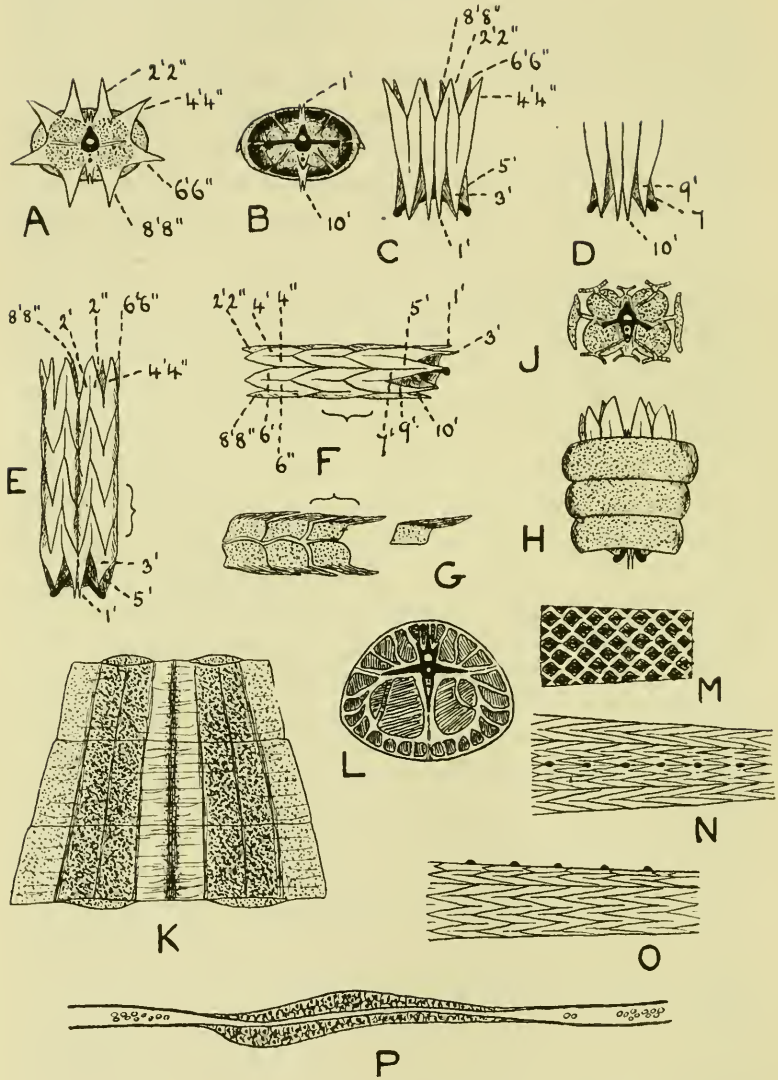
The above-named structures are to be found in the segmented portion of the original tail of the Gecko. There remains for description the unsegmented base (Text-fig. 2, A) of the tail. The

skin and subcutaneous fat layer in this region are unsegmented. The submuscular fat bands are absent, their position and that of the muscles of the tail segments being occupied by large longitudinally-disposed masses of muscle doubtless concerned with the occasional movements of the tail base. The type of muscles found in the segments of the tail is quite absent. In the base, i. e. the region between the cloacal aperture and the first autotomy plane (marking the anterior border of the first segment), two and a half vertebrae are to be found in the adult Gecko (I found three and a half vertebrae in the base of a young Gecko), and the base thus consists of the equivalents of two and a half tail segments. Only two haemal arches are present in the region of the base, these being attached to the last two intercentral cartilages, the first intercentral cartilage only possessing, like the trunk vertebrae, a small ventral nodule of bone.

The Caudal Flexor Muscles: their Attachments and Mode of Action in Autotomy.

If we catch a Gecko by its original tail and examine the front aspect of the piece shed, we see (Text-fig. 3, A) that lying external to and arising from the four submuscular fat bands are eight projecting muscle processes (numbered 2′ 2″, 4′ 4″, 6′ 6″, 8′ 8″ on each side of the segment), two arising from each fat band. If we examine the hind aspect of the portion of tail left attached to the animal (Text-fig. 3, B) we again see the four fat bands, external to which are eight cavities which, before autotomy, lodged the eight muscle processes just mentioned; there are also to be seen two pairs of small tapering muscle extremities, one in the mid-dorsal line (labelled 1′) and one in the mid-ventral line (labelled 10′). The transverse processes of the vertebra are also conspicuous. If we now remove from a single shed segment of the tail the skin and the subcutaneous fat layer, the entire musculature of the segment becomes visible (Text-fig. 3, C). Anteriorly the eight muscle processes are to be seen; posteriorly each of the four dorsal processes is seen to bifurcate, the halves of each, however, uniting with adjacent halves, except in the case of the two

TEXT-FIG. 3.


A
2'2"
4'4"
6'6"
8'8"
B
1'
10'
C
8'8"
2'2"
6'6"
4'4"
5'
3'
1'
D
9'
7
10'
E
8'8"
2'
2"
6'6"
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dorsalmost halves which are separated by the vertebral neural spine, to form altogether six posterior muscle extremities. Each of the four ventral processes (Text-fig. 3, D) end posteriorly in a similar manner. Thus on the posterior side of the segment there are altogether ten points of termination of the muscles instead of sixteen, since twelve of these fuse together in pairs and only those in the mid-dorsal and mid-ventral lines persist separately. Text-fig. 3, E, F illustrates respectively dorsal and lateral views of the musculature of several adjacent segments, from the latter of which it will be seen that the lateral posterior processes, which contract most vigorously in tail flexion or autotomy, become attached to the strong pro-

TEXT-FIG. 3.

Structure of the Original Tail of the Gecko and of the Tail of Calotes. A. Front end-on aspect of the piece of separated-off tail after autotomy (× cir. 2). Eight large muscle processes are seen which were, before autotomy, lodged in the eight interseptal recesses seen in fig. B. B. Posterior end-on aspect of the stump of the tail after autotomy (× cir. 2). Eight recesses (situated under the subcutaneous fat layer) are visible, separated from each other by radiating septa of connective tissue: these lodged the eight muscle processes seen in fig. A. The transverse processes are visible, also the extreme hind end of the haemal process. C. Dorsal aspect of the flexor muscles of a single tail (autotomy) segment (× cir. 2). D. Ventral aspect of the posterior flexor muscles of a single tail segment (× cir. 2). E. Dorsal aspect of the arrangement of the flexor muscles (× cir. 2). F. Lateral ditto. G. Attachment of the flexor muscles to the fat bands seen in longitudinal sections (× cir. 2). H. The segmented subcutaneous fat layer exposed after removal of the skin from three of the tail segments (× cir. 2). J. Transverse section through the posterior half of a tail segment showing the central septal attachments of the fat layer. The spaces between the (cut) fat layer and the fat bands are empty and form the eight recesses referred to in figs. A and B. In the anterior half of a tail segment the fat layer is attached all round to the outer surface of the flexor muscles. K. The fat layer of three segments cut through in the mid-dorsal line and spread out. Very few fat cells are present in the thin mid-ventral area (× cir. 3). Lines of cleavage are visible. L. Transverse section of the tail of Calotes (× cir. 3). The multiple subdivision of the peripheral muscles and the absence of a fat layer and fat bands are noteworthy. The large internally-situated muscles run longitudinally the whole length of the tail. M. Portion of dorsal skin of the tail of Calotes (× cir. 2). N. Dorsal aspect of muscles of tail of Calotes after removal of skin (× cir. 2). O. Lateral ditto. P. Sphincter on caudal artery of Gecko seen in longitudinal section (magnification unrecorded, but about 70 diameters).

jecting transverse processes of the vertebra. I have labelled each of the anterior muscle processes and their posterior extremities in order that the muscle masses shown in the figure of the transverse section of a segment (Text-fig. 2, B) may be compared with those of Text-fig. 3, A, C, D, E, F. In short, dissection and serial sections show that all the posterior continuations of the eight anterior muscle processes are firmly attached posteriorly to the vertebral axis, directly dorsally and ventrally to the neural and haemal spines respectively, and laterally to the transverse processes, and indirectly by connexion with the eight radiating septa of connective tissue which join the connective tissue internal lining of the fat layer with the connective tissue external investment of the vertebrae—these traversing the area of the fat bands. The muscles are also firmly attached on their inner surfaces to the fat bands (Text-fig. 3, G), which themselves are firmly connected with the connective tissue investment of the vertebrae. The eight anterior muscle processes, on the other hand, are only feebly attached to the septa separating successive muscle segments. Usually the tail of a Gecko merely depends from the body, but when the animal is excited (as when pursuing a fly) the tail can be slowly flexed from side to side. During these lateral flexions of the tail the muscles of many segments on one side of the tail contract and the strains on the slender anterior attachments of the muscles are relatively slight, however violent the flexion (as when the animal is being chloroformed), because the muscles of many segments are involved, i. e. the effect is distributed between them and the tail is freely movable. On the other hand, when the tail is seized by another Gecko, the part of the tail seized is relatively fixed, and since the body is also fixed in position, and the muscular contraction involved in autotomy is limited to one segment (see Statement 2) and is therefore proportionately violent, the contraction of the muscle, in trying to flex relatively inflexible segments, i.e. in trying to cause to approach each other the sides of two adjacent segments which, under the conditions, can only approach to a very small extent, is then

and only then able to effect the disruption of the feeble anterior attachments of the muscles. Disruption of the muscles having occurred on one side (and with it disruption of the skin, fat layer, fat bands, and vertebrae along their cleavage planes), the muscles of the other side of the segment contract violently in their turn and so complete the process of autotomy. This interpretation of the action of the muscles in autotomy explains why it is that the Gecko cannot shed its tail unless it is held, i.e. relatively fixed, a fact which I have already remarked upon.

A Brief Comparison of the Structure of the Gecko Tail with that of the Tail of a Non-autotomous Lizard—Calotes versicolor.

If we examine the tail of a typical non-autotomous lizard, such as Calotes versicolor,[1] we find conspicuous differences from the Gecko tail. In Calotes the tail is covered with equal-sized scales arranged in longitudinal rows, all the scales of adjacent longitudinal rows alternating with each other in position (Text-fig. 3, M); thus the arrangement of the scales shows no signs of segmentation, and lines of cleavage are of course absent. The annular arrangement of the scales at the ends of the autotomy segments of the Gecko tail must therefore have arisen secondarily in relation to autotomy. Internally in the Calotes tail, fat layer and fat bands are both absent, the entire space between the skin and the vertebral column being occupied by muscles.. The general arrangement of these muscles, which can be seen when the tail is skinned (Text-fig. 3, N, O) and from transverse sections (Text-fig. 3, L), is much more complicated than in the Gecko tail. In Calotes all the superficial muscles are arranged in a zigzag myotome fashion, but those internally situated are continuous (not myomeric) and run longitudinally the greater part or the whole of the length of the tail. In Varanus a similar arrangement of the muscles obtains. From these facts it will appear

[1] The cut tails of two Calotes showed no signs of regeneration after one and a half months of captivity, and I have never met with a regenerated tail in this animal in nature, nor in Varanus.

probable that in the Gecko tail the four submuscular fat bands must represent centrally-situated longitudinal unsegmented muscles which have degenerated into fat and become secondarily segmented for autotomy. It is also certain that the superficial muscles of the Gecko tail have become secondarily simplified and segmented in relation to autotomy.

Planes or Lines of Cleavage in Autotomy.

The annular lines of cleavage in the skin are indicated (1) by the arrangement of the scales in the skin, a regular straight transverse row of scales bordering each side of the line of cleavage (Text-fig. 2, A), and (2) by the presence of a line of very thin transparent substance, devoid of pigment and other cells, separating the two straight lines of scales of adjacent segments. Apparently in this line of tissue the epidermis and dermis of the integument have become extremely attenuated and practically reduced to a layer of non-cellular hyaline matrix, only occasionally traversed by capillaries and nerves passing from one segment to another. In the subcutaneous fat layer (Text-fig. 4, E) the lines of cleavage are denoted by similar lines, alone composed of this non-cellular hyaline matrix and bordered by several rows of connective tissue cells, outside which lie the cells of the fat layer. Similar sheets of matrix separate the muscle segments of the tail and the segmented parts of the longitudinal fat bands (Text-fig. 2, C). With reference to the plane of cleavage dividing the middle of each centrum and neural arch, Gadow [1] (p. 494) describes this as a 'cartilaginous septum . . . which coincides exactly with the line of transverse division of the vertebra . . . where the tail breaks off and whence it is removed'. This is a mistake; the vertebral plane of cleavage simply consists (Text-fig. 2, C), like the planes and lines of cleavage already mentioned, of a sheet of non-cellular hyaline substance which is continuous

[1] The Cambridge Natural History. Volume on Amphibia and Reptiles, H. Gadow, 1909.

with those separating the other tissue of adjacent segments; also the plane of cleavage lies immediately behind the transverse process of the centrum, which is therefore not affected by autotomy and remains projecting from the posterior surface of the portion of tail retained by the animal (Text-fig. 1, F). I have verified these statements in numerous longitudinal and transverse microtome sections, also in hand-cut sections, these latter proving, in virtue of their thickness, more useful on the whole than the former.

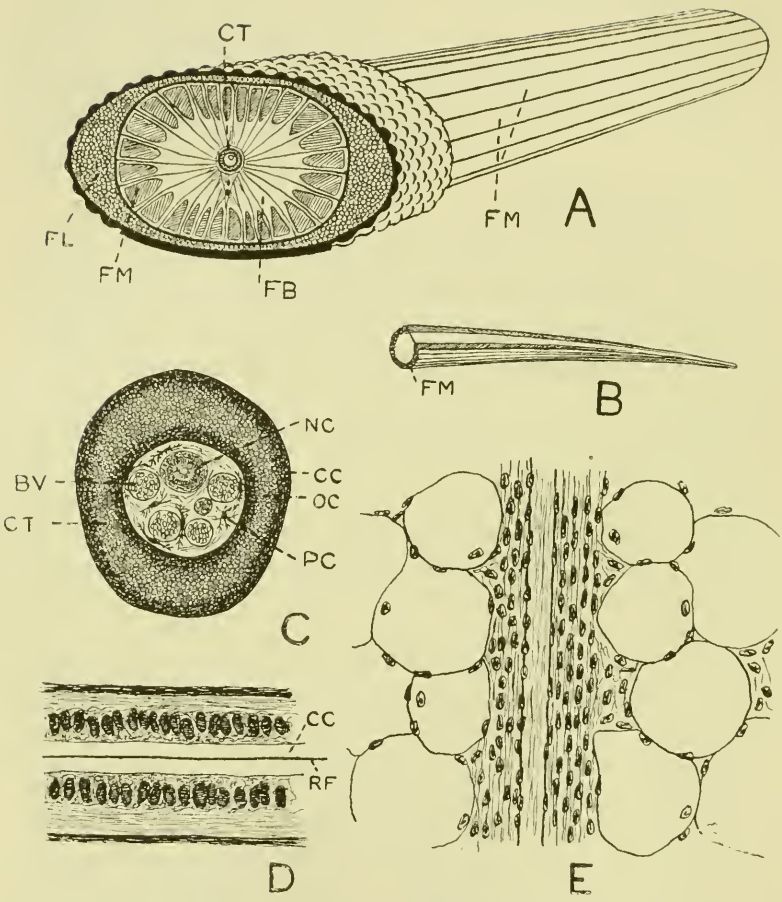
I may add here that there is apparently great general similarity between these cleavage planes in the Gecko tail and the 'breaking plane' which Paul[1] has recently described in detail in Decapod Crustacea. In fact the only conspicuous difference between the two is as regards number—in the Crustacean there is only one plane for each limb, whereas in the Gecko (as in the Ophiuroid arm) there are as many planes as there are joints. And just as there is a sphincter on the Gecko caudal artery to stop haemorrhage, so in the Crustacean there is a diaphragm developed for the same purpose. In all cases muscular action affects autotomy of the shed part along the cleavage plane.

The Structure of the Regenerated Tail of the Gecko.

The most conspicuous difference between the regenerated tail and the original tail is the total absence of any signs of segmentation in the former, either on the surface or in internal structure. On the dorsal surface of the tail the skin bears a uniform covering of the usual small scales (Text-fig. 1, C, D), i.e. the small scales are arranged in the same somewhat irregular manner throughout the length of the tail, and no larger scales are present. On the sides of the tail the scales are larger, and on the median ventral surface there is a longitudinal series of large laterally-elongated scales (Text-fig. 1, E). The subcutaneous fat layer is present (Text-fig. 4, A), very thin dorsally

[1] "Regeneration of the Legs of Decapod Crustacea from the Preformed Breaking Plane", J. H. Paul, 'Proc. Royal Soc., Edinburgh', vol. xxxv, 1914–15, p. 78.

Text-fig. 4.



Structure of the Regenerated Tail of the Gecko.

A. Semi-diagrammatic transverse section of the regenerated tail of the Gecko (× cir. 8). The multiplication of the flexor muscles (FM) seen in transverse section (and the resulting large number of radiating septa traversing the fat bands—FB) is noteworthy, also their lack of connexion with the cartilaginous tube (CT). FL = fat layer. The caudal artery and vein are seen underneath the cartilaginous tube. B. Dissection of the fat bands and flexor muscles, showing the longitudinal course of the latter (nat. size). C. Transverse section of the cartilaginous tube (× cir. 150). BV = blood-vessel;

and ventrally and thick laterally, and as usual lined internally by a thin dense layer of connective tissue ; it shows no signs of lines of cleavage, being continuous the whole length of the tail. Internally to the fat layer is the muscle layer, consisting of from twenty to thirty (in different specimens) slender muscle bands, separated from each other by a corresponding number of radiating connective tissue septa (continuations of the dense connective tissue lining of the fat layer which extend inwards through the fat band to the similar, and here thick, connective tissue investment surrounding the axial cartilaginous tube enclosing the regenerated spinal cord) and running in a straight line the entire length of the regenerated tail (Text-fig. 4, B). The fibres of these muscle bands appear to run obliquely from the central fat bands outwards to the subcutaneous fat layer and have no special connexions in their course, except that anteriorly all the bands are attached to the connective tissue septa bounding the hind ends of the muscles of the base or other portion of original tail. In autotomy the separation of the regenerated tail from the part in front of it must be solely effected by the contraction of these longitudinal muscle bands away from their connective tissue junction with the last intermuscular septum, this forcible separation causing the simultaneous separation of the slender junctions of the other organs. In other words, the tail being seized and held, these muscles contract, and since the whole body cannot be dragged back, the inevitable result is the separation of the tail.

Between the layer of muscle bands and the axial tube enclosing the regenerated spinal cord lies the substance of the submuscular fat bands already mentioned ; these are continuous from end to end of the tail (cleavage planes being

CC = calcified cartilage at periphery (the tube is also calcified on the inner edge); CT = cartilaginous tube; NC = extension of spinal cord; OC = uncalcified cartilage; PC = pigment cell. D. Longitudinal section of the spinal cord extension in the regenerated tail (× cir. 580). CC = canalis centralis ; RF = Reissner's fibre. E. Section through autotomy plane in the region of the fat bands (× cir. 250). The hyaline septum is shown, bordered by connective tissue cells, outside which lie the fat cells. Similar hyaline septa extend through the vertebrae, muscles, and skin.

absent) and are radially subdivided by the numerous connective tissue septa above described. Forming the central axis of the regenerated tail is a thick-walled cartilaginous tube (Text-fig. 4, C). The cartilage of this tube is calcified [1] on its outer surface (next the fat bands) and on its inner surface (next the spinal canal), the space between these two concentric cylinders of calcified cartilage consisting of ordinary uncalcified cartilage. Anteriorly this cartilaginous tube joins on to the ring of bony tissue formed by the centrum and neural arch of the last vertebra (Text-fig. 2, C) and so secures a continuation of the spinal canal. The cartilaginous tube is quite continuous—no planes of cleavage being present—and it bears no processes of any kind, neural spines and haemal arches both being absent. The contents of the cartilaginous tube are (*a*) a very attenuated extension of the spinal cord (about a quarter or less of the diameter of the original) which practically consists of a continuation of the cellular lining of the canalis centralis, with little or none of the external nerve-fibre substance ; (*b*) a network of capillaries which lies for the most part ventrally to the spinal cord extension ; and (*c*) an arachnoid meshwork containing pigment cells. In view of the fact that no nerves are given off from this slender extension of the spinal cord into the regenerated tail, it is evidently quite a useless structure so far as muscular innervation is concerned ; it, however, contains a well-developed Reissner's fibre (Text-fig. 4, D). It may here be mentioned that the nerves supplying the slender muscle bands are all derived from the last two or three pairs (I have not determined the exact number) of nerve roots in the stump of the original tail (according to Powell White, the nerves are, in Lacerta vivipara, derived from the last three pairs) and, as stated, have no connexion with the regenerated spinal cord. In the abstract of Powell White's paper it is stated that in Lacerta viridis the cartilaginous tube enclosing the spinal cord is 'unsegmented

[1] This calcification of the cartilage is apparent in thick unstained hand-cut sections of aceto-bichromate-fixed material ; in ordinary microtome sections it is not easily seen.

and continuous except for some perforations through which blood-vessels pass to the interior'. In the fully-regenerated tail of the Gecko no perforations at all exist in the length of the tube, not even for blood-vessels, though perforations (for vessels) are fairly numerous in the young growing cartilaginous tube, and I suspect that this is also the case in Lacerta. It certainly is so in Pygopus, sections of which Professor J. P. Hill has kindly shown to me. At the extreme posterior end, however, of the cartilaginous tube in one series of sections of a fully-developed regenerated tail I have found one median ventral terminal opening and two lateral subterminal openings through which blood-vessels pass, but these are the only openings I have discovered. In another series of sections of a young regenerated tail (6 mm. in length) I found that the spinal cord continuation actually bifurcated at its posterior extremity, one branch piercing the cartilaginous tube through a mid-ventral subterminal opening, the other branch continuing to the end of the tube, but I suspect this to be a freak.

The caudal artery extends back into the regenerated tail lying underneath the cartilaginous tube, and only differs from that of the original tail in not being enclosed in a haemal canal and in being devoid of sphincters; it gives off branches at intervals. The caudal vein extends posteriorly under the caudal artery and is uniform in diameter.

The Histogenesis of Normal Caudal Regeneration.

Under this heading I can only confirm and correct previous accounts. As Powell White says, 'The wound after autotomy is quickly covered with new skin [not derived from the old skin covering the stump of the original tail], beneath which is a mass of spindle cells [quasi-embryonic tissue] which apparently originates in the connective tissue. This cellular mass acts as a growing-point to the new tail, and from it the various structures are developed. The cartilage, fat, and blood-vessels arise by differentiation from the spindle cells. The muscle fibres arise segmentally in groups, the groups nearest the tip

being the least differentiated. The muscles in the stump play no part in the process.' It is also possible that the continuation of the lining epithelium of the canalis centralis of the spinal cord is produced by these histogenetic cells. On the other hand, it appears that the nerve trunks of the regenerated tail are produced by the growth into the regenerating tail of the torn ends of the trunks in the original tail, the posterior root ganglia of which ' are increased in size or number owing to increase in size of the nerve bundles '. The preceding account, which I can confirm in full as regards the origin of the skin, muscles, fat layer, fat bundles, and cartilaginous tube, is thus in distinct opposition to the views of Fraisse, who believed that the skin, connective tissue, cartilaginous tube, and muscles of the regenerated tail are all derived ultimately from the corresponding tissues of the original tail—that new tissues can only be reproduced from tissues like themselves. This belief is, in the main, not only disproved by actual observation, but is also contradicted by some of the results obtained from caudal regeneration under abnormal conditions now to be described.

Caudal Regeneration under Abnormal Conditions.

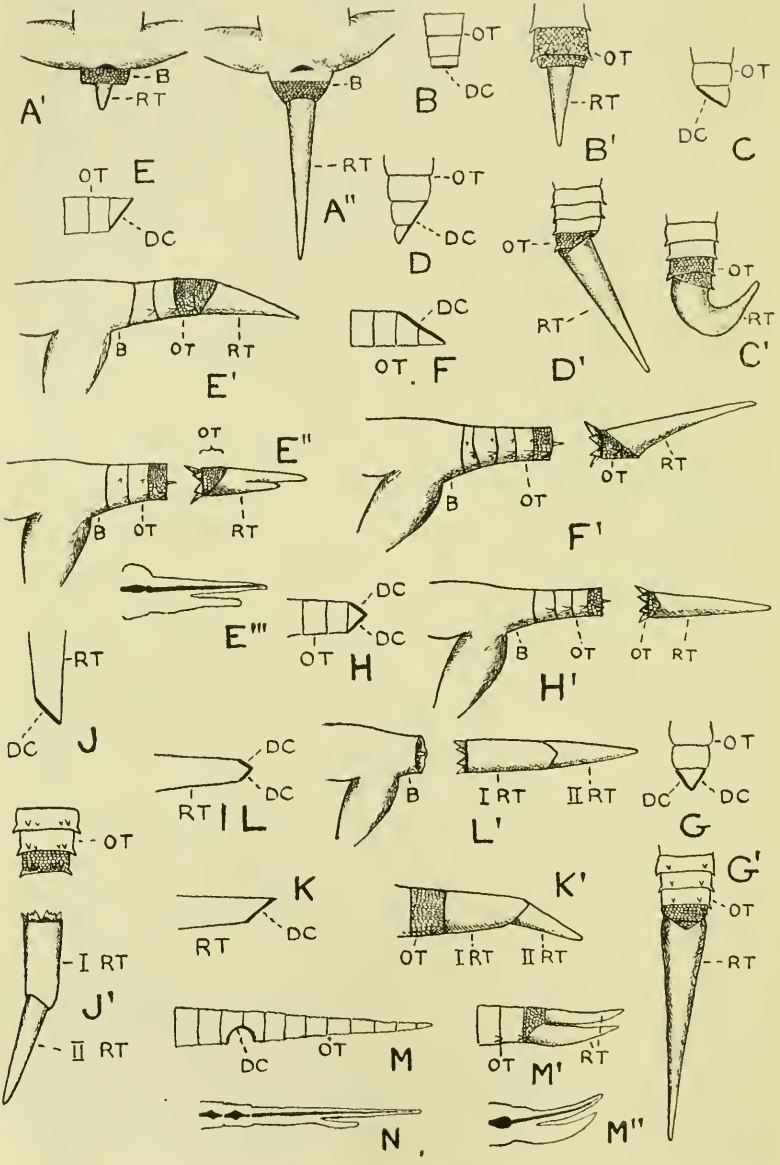
Intervertebral Regeneration. Though Fraisse rightly came to the conclusion that the remnants of the old notochord (even if these be exposed by the injury) take no part in the formation of new skeletal tissue, yet since a more recent writer like Gadow (p. 494) is of opinion that 'reproduction of centra [in the regenerated tail] is precluded by the previous normal reduction of the chorda, around which alone proper bony centra could be formed' (though Fraisse has shown that in the regenerated tail of Urodeles new vertebrae can be produced in the total absence of a notochord), it may be as well to quote, first of all, the results of my experiments on caudal regeneration from the posterior surfaces of caudal segments which were cut in half, i.e. cut transversely between any two autotomy planes, i.e. intervertebrally. These experiments were successful on four occasions (Text-fig. 5, B, B′) and in each case, though the notochord was well

exposed by the cut, the endoskeleton of the regenerated tail was of the normal cartilaginous tube type. These experiments also prove that the tissues bordering the autotomy plane are not indispensable for the regeneration of the tail—histogenetic cells are distributed throughout the tail tissues. I may add that in one of these four experiments I held the animal by the regenerated tail (of 66 days growth) but I could not induce autotomy either from the junction of the regenerated tail with the original stump or from a true autotomy plane anterior to it.

Regeneration from the Cut Base of the Tail. I have stated that the first autotomy plane in the Gecko tail is situated between the posterior surface of the base of the tail and the anterior surface of the first autotomy segment. Since we now know that caudal regeneration can occur at the surface of any autotomy segment cut intervertebrally, i. e. between two successive autotomy planes, it is of interest to inquire whether regeneration can occur from the posterior surface of the base of the tail if this be cut through anterior to the first autotomy plane. The answer to this question is also of special interest when we reflect that the structure of the base of the tail is different in several respects from that of the segmented tail proper—in the absence of segmentation, in the absence of fat bands, and in the arrangement of the muscles—and that it has been contended that (in the Gecko and the other Lacertilia which it resembles in this respect) the regenerated tail differs in type from the original tail solely because in development the former is shut off from the controlling influence of the main organism by the hyaline septa of the autotomy planes, whereas the original tail is developed before autotomy planes (which are only produced after the original tail is formed) are present.

I performed this experiment of cutting through the base of the tail four times in 1914 but in no instance did regeneration occur, though the Geckos were kept for two months. In 1918, however, when I repeated the experiment, five of the Geckos regenerated tails of the normal regenerate type (Text-fig. 5, A′, A″), as shown by the cartilaginous tube, fatty

TEXT-FIG. 5.



tissue, nerve cord, and other features seen in sections. This result is of importance because it proves (1) that regenerative cells are present in a part of the body which, under normal conditions, never reproduces a tail, and (2) that the peculiar characters of the regenerated tail are not due to mere lack of continuity with the rest of the organism. The real reason for the regenerate tail differing in character from the original tail appears to be that the organism as a whole 'knows' that the

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TEXT-FIG. 5.

Experimental Regeneration of the Gecko Tail (all figures nat. size). A'. Tail regenerated from cut base, after 20 days. A". Ditto, after 43 days. B. Diagram showing direction of cut through the middle of an autotomy segment. B'. Tail regenerated from cut-through autotomy segment (cut B), after 15 days. C. Diagram showing direction of oblique lateral cut through one autotomy segment. C'. Tail regenerated from the cut C, after 64 days. D. Diagram showing direction of oblique lateral cut through two autotomy segments. D'. Tail regenerated from the cut D, after 52 days. E. Diagram showing direction of oblique dorso-ventral cut through one autotomy segment. E'. Tail regenerated from the cut E, after 67 days. E". Tail regenerated from the cut E, after 87 days (the tail was shed when held at the point shown). E'". Diagram showing absence of endoskeleton in the lower division of the bifid tail of E". F. Diagram showing direction of oblique dorso-ventral cut through two autotomy segments. F'. Tail regenerated from the cut F, after 76 days (the tail was shed when held at the point shown). G. Diagram showing direction of two oblique lateral cuts through an autotomy segment. G'. Straight tail regenerated from the cut G, after 82 days. H. Diagram showing direction of two oblique dorso-ventral cuts through one autotomy segment. H'. Tail regenerated from the cut H, after 80 days (the tail was shed when held at the point shown). J. Diagram showing direction of oblique lateral cut through regenerated tail (cf. cut C). J'. Tail regenerated from the cut J, after 73 days (the tail was shed when held at the point shown). K. Diagram showing direction of oblique dorso-ventral cut through regenerated tail. K'. Tail regenerated from the cut K, after 73 days. L. Diagram showing two oblique dorso-ventral cuts through regenerated tail. L'. Tail regenerated from the cut L, after 73 days (the tail was shed when held at the point shown). M. Diagram showing position and extent of ventral wound made on original tail. M'. Two tails regenerated from the wound M, after 80 days. M". Diagram showing absence of endo-skeleton in the lower division of the bifid tail of M'. N. Diagram showing absence of endoskeleton in the lower division of a ventral accessory tail produced from a wound similar to M, after 80 days. B = base of tail; DC = direction of cut; OT = original tail; RT = regenerated tail.

reproduced tail is only reproduced for the purpose of being shed, and in consequence the regenerated tail is grown on cheap 'jerry-built' lines sufficient for the end in view. That this is the explanation will be clear, on the one hand, when we call to mind the regenerated tails and limbs of Urodeles, arms of Starfishes and Ophiuroids, and limbs of Crabs, Centipedes, and Plasmids (walking-stick insects), all of which, when regenerated, are required for use as integral parts of the organism and are therefore of normal type[1]; on the other hand, the fact that the organism can actively mould an autotomous appendage so as to adapt it for functions not connected with its own individuality is shown in such cases as those of the hectocotylized arm of Dibranchiate Cephalopods and the heteronereis segments of Polychaetes. According to this explanation then, the aberrant scaling of the regenerated Gecko tail is to be regarded as that form of scaling most easy to be produced under the circumstances, just as the simple longitudinal muscles (devoid of connexion with the endoskeleton) and regenerated nerve cord (devoid of white matter, ganglion cells, and nerves) are to be regarded as similar products of a 'jerry-building' policy, and not due to a mere reversion-to-type tendency, as supposed by Boulenger.[2] The type of scaling of the regenerated tail may happen to be of an ancestral type simply because this latter chances to be a 'cheaper' or 'to-hand' form of lepidosis, but it is quite evident that since the 'reversion to an ancestral type' explanation does not apply to the internal structure of the regenerated tail, it also cannot be held to be sufficient to account for the scaling.

I may mention that previous to preserving the tail (of 45 days' growth) of one of these five Geckos, I held it with my fingers

[1] The well-known examples of an antenna being generated on the eyestalk of Palinurus, of a mandible being substituted for a first antenna in Asellus, and a wing replacing the hind leg of the moth Zygaena (vide Bateson, 'Material for the Study of Variation', 1894), and other similar examples are of the same category, the 'controlling' influence of the organism as a whole, however, being at fault, the reproduced part being out of position.

[2] Boulenger, G. A., 'Proc. Zool. Soc.', Lond., 1888, p. 351.

and the animal shed it ' not very easily '. The stump bled to some extent, but not profusely.

Regeneration from obliquely-cut Ends of the Original Tail. When one or two segments of the original tail are cut through obliquely from left to right (Text-fig. 5, C, C′) or from right to left (Text-fig. 5, D, D′), the axis of the regenerated tail is usually bent out of the straight line in order to place itself at right angles to the plane of the cut (six experiments).

When one segment of the original tail is cut obliquely ventro-dorsally and postero-anteriorly (Text-fig. 5, E, E′, E″) the axis of the regenerated tail is usually bent downwards in order to place itself at right angles to the plane of the cut (three experiments).

When one or two segments of the original tail are cut obliquely dorso-ventrally and antero-posteriorly (Text-fig. 5, F, F′) the axis of the regenerated tail is usually bent upwards, the more so if the number of cut segments be two (four experiments).

In four experiments in which one segment of the original tail was cut to a point by left and right lateral cuts (Text-fig. 5, G, G′) the axis of the regenerated tail remained in the straight line.

In three experiments in which similar cuts were made dorsally and ventrally (Text-fig. 5, H, H′) the same result was obtained.

Regeneration from the Regenerated Tail. A transverse cut through a regenerated tail merely leads to a second regenerated tail being produced (two experiments).

When the regenerated tail is cut obliquely (Text-fig. 5, J, J′, K, K′, L, L′) the second regenerated tail behaves in the manner already described for regeneration from the original tail (at least six experiments).

Accessory Tails. In all the 1918 experiments chronicled above (which are only a selection of the experiments I actually performed), and in a number of similar experiments which I conducted in 1914, I only obtained four examples in which accessory tails were produced. Text-fig. 5, M, M′ shows

the result I obtained after making a wound on the ventral surface of an original tail. In this case the tail evidently autotomized at the autotomy plane separating the two segments involved in the wound, and the surface thereby exposed produced two tails. The upper tail was a normal regenerated tail in every respect, but the larger lower accessory tail differed in the essential respect that it was entirely devoid of a cartilaginous tube (Text-fig. 5, M″).

Text-fig. 5, N shows another small accessory tail produced as the result of a wound on the ventral surface of a regenerated tail. An endoskeleton was also absent in this case, as also in another similar case which I have not recorded.

In Text-fig. 5, E‴ is shown a small accessory tail produced as the result of the oblique dorso-ventral cut already described (Text-fig. 5, E). The lower lobe of the bifid tail was devoid of a cartilaginous tube.

I have described these four examples of accessory tails because, to judge from the paper by Tornier, the reader might imagine that an accessory tail without a cartilaginous tube is an impossibility. This is by no means the case, as these four examples and the examples in Anolis grahami, described by Brindley in 1898, prove. Assuming the statements of Tornier to be correct, it would appear that the injury must reach the vertebral column in order that the accessory tail produced may contain a cartilaginous tube.

Notes on Technique.

All Geckos were kept in large flower-pots, covered over with mosquito-netting, and were fed on house-flies. Tails preserved for section-cutting were fixed for 24 hours or longer in a saturated solution of potassium bichromate (100 parts), to which 5 parts of acetic acid had been added; they were afterwards washed in running water for the same length of time, and then kept in 70 per cent. alcohol until required for use. For the study of the gross structure of the tail, nothing is better than thick hand-sections (longitudinal and transverse) of the spirit-preserved material, dehydrated and mounted unstained in

balsam, the bichromate fixative acting as a stain for many of the tissues. For histology, the spirit-preserved material was first decalcified by leaving it in alcohol plus nitric acid for several weeks, and subsequently dehydrated, embedded, cut, and stained with haemotoxylin. For dissection of the muscles preliminary maceration of the tails in weak alcohol or water plus nitric acid gave good results.

Notes on the Original and Regenerated Tails of Sphenodon punctatus.

I have examined the original and regenerated tails of Sphenodon punctatus kindly given to me by Professor Arthur Dendy. The scales are arranged in the original tail in accordance with the planes of autotomy, each autotomy segment bearing dorsally one of the large mid-dorsal scales, and ventrally two transverse rows of the large hexagonal scales. The muscles, after removal of the skin, have a superficial arrangement closely resembling that of the Gecko shown in Text-fig. 3, E, F, only the muscles are more numerous. In lateral aspect, e. g., there appear to be four muscle layers (and processes) instead of two as in the Gecko (Text-fig. 3, F). In transverse section the muscles are also seen to be more numerous than in the Gecko, and they extend inwards from the skin to the vertebral column, fat bands being entirely absent. The muscles are separated from each other by thin radiating septa of dense connective tissue. I dissected out a piece of the caudal artery about 9 cm. in length and cleared it in creosote, when it was evident that sphincters were not present. The regenerated tail is of course not segmented and the scaling (irregular small scales) is quite irregular. A cartilaginous tube is present, the cartilage of which is calcified in the middle of the thickness of the ring, not on its inner and outer edges as in the Gecko. The muscles are very numerous in transverse section (about fifty bands cut across), and these are separated from the cartilaginous tube not by fat bands but by dense connective tissue, which is continuous with the subcutaneous

connective tissue by means of the radiating septa separating the muscle bands. The tails of Sphenodon, therefore, appear to be less specialized for autotomy than the tails of the Gecko, though the presence of definite autotomy planes, the evident simplification of the muscles, and the presence of the cartilaginous tube indicate that considerable progress has been made in that direction.

Résumé.

1. The Gecko original tail is made up of numerous (about thirty) autotomy segments, separated from each other by as many hyaline septa marking autotomy or cleavage planes. Autotomy can occur voluntarily at any plane provided that the tail be held a short distance posteriorly to the point of separation. Autotomy in the Gecko is never 'spontaneous' or the result of mere alarm.

2. The structure of the original Gecko tail is described. The caudal artery develops a sphincter muscle in its walls immediately anterior to each autotomy plane as a means of avoiding haemorrhage after autotomy. I am not aware that a sphincter muscle has previously been described in connexion with a blood-vessel. The caudal vein is similarly constricted in front of each autotomy plane. The base of the tail differs from the segmented portion in the absence of fat bands and in the arrangement of the muscles. The flexor muscles of each tail segment are firmly attached posteriorly to the vertebra and the outer surface of the fat bands; anteriorly, however, they are only attached to the connective tissue of the hyaline matrix in the autotomy plane and are threfore easily separated. Autotomy is effected by the strong localized contraction of these muscles separating their weak anterior attachment.

3. Comparison of the Gecko tail with the non-autotomous tail of Calotes shows that in order to effect autotomy the former has become greatly simplified. The scales have become rearranged at the extremities of each autotomy segment, the superficial muscles have also become rearranged on a more simple plan, and the internal longitudinal continuous muscle

bands have degenerated into the fat bands and become secondarily segmented.

4. The autotomy planes are marked by simple septa of a hyaline matrix bordered by connective tissue, which traverse and separate into segments the entire substance of the tail. The spinal cord, nerves, and blood-vessels are, however, continuous.

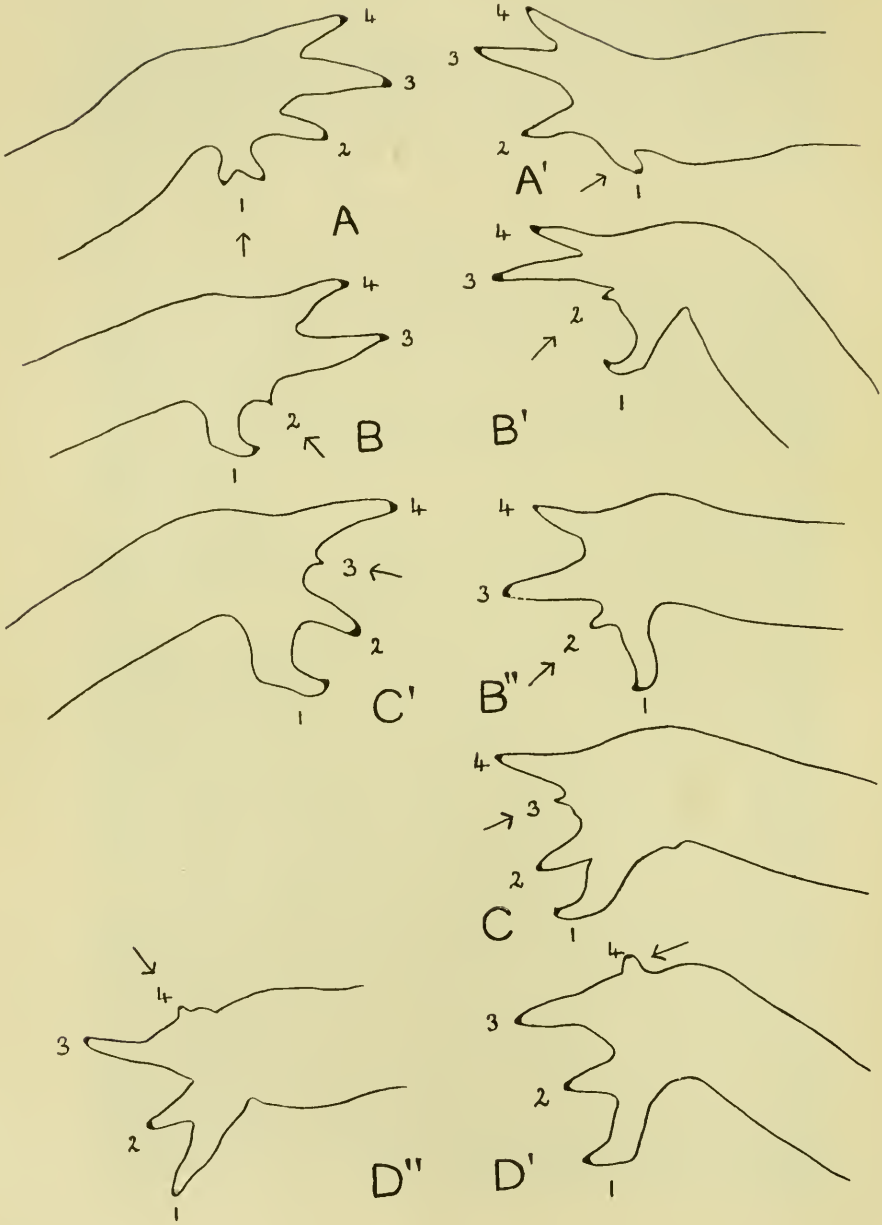
5. The structure of the regenerated tail is described. Reissner's fibre is present in the regenerated spinal cord, as in the cord of the original tail. Boulenger's explanation of the changed character of the lepidosis of the regenerated tail when compared with that of the original tail, viz. that it is a reversion to an ancestral type, does not apply to the internal anatomical features which distinguish the regenerated from the original tail. A more probable explanation of the differences between the regenerated and original tails is that the former, being merely produced for autotomy purposes, is 'jerry-built'—an appropriate description of a tail in which the muscles have no direct connexion with the endoskeleton and the spinal cord is devoid of nerves, ganglion cells, and fibres.

6. Tails of the normal regenerated type can be produced from cut surfaces situated between the autotomy planes and anterior to the first autotomy plane in the base of the tail. This is proof (*a*) that the histogenetic cells occur throughout the tail substance and quite apart from the hyaline septa, (*b*) that the peculiar features of the regenerated tail are not due to a lack of organic connexion with the rest of the body caused by the interposition of the autotomy plane septa.

7. The axis of the regenerated tail usually tends to be placed at right angles to the plane of the cut on the tail stump. In four of my experiments accessory tails were produced, none of which contained a cartilaginous tube endoskeleton.

8. The tails of Pygopus and Lacerta viridis are apparently almost identical in structure with those of the Gecko, and in Sphenodon punctatus the tails only differ essentially in the absence of the fat bands and the absence of sphincters on the caudal artery.

TEXT-FIG. 6.



In conclusion, I wish to express my thanks to Professor Arthur Dendy, F.R.S., for his kind gift of two tails (one regenerated) of Sphenodon punctatus, to Professor J. P. Hill, F.R.S., for the loan of three slides of the tail of Pygopus sp., to my pupil Mr. B. K. Das, M.Sc., University of Allahabad Research Scholar in Zoology, for much assistance in the practical work connected with caudal regeneration under abnormal conditions, and to Professor D. R. Bhattacharya, M.Sc., for some aid in 1914.

Appendix. Note on the Regeneration of Digits in an Indian Toad.

Since, so far as I am aware, only one instance[1] has yet been described of a very limited regeneration of amputated digits having occurred in adult Anura, I reproduce here drawings (Text-fig. 6) made by my former pupil, Mr. N. K. Patwardhan, M.Sc., of regenerated digits in the Indian toad, Bufo melanostictus. These digits had been removed (in all cases they were cut off with scissors to a little below the level of the bases of the adjoining digits) for purposes of identification. All the figures represent the amount of regeneration which had occurred within 73 days of amputation, excepting figs. C, B″,

[1] I refer to Gadow's statement (Cambridge Natural History, vol. on Amphibia and Reptilia, p. 67) that in two specimens of Rana temporaria in which the hand was amputated from the wrist, 'within a year this changed into a four-cornered stump and two of the protuberances developed a little further, reaching a length of about 4 mm. These specimens lived for four years without further changes.'

Text-fig. 6.

Regenerated Digits of the Indian Toad, Bufo melanostictus, from the dorsal aspect (all figures × cir. 3).
The arrows indicate the regenerated digits. B″, C, and D′ (all males) represent 94 days growth; all the others (all females) 73 days growth. It is noteworthy that in A and A′ the first digit has grown more rapidly than any of the other digits, though these animals were females, and the digit therefore was not used for the nuptial embrace.

and D′, in which the period was 94 days. The latter maximum period of thirteen weeks, three days was therefore considerably less than the year referred to by Gadow, and in this connexion I may mention that in another toad (a toad labelled J, celebrated in its way since it was the only animal in which the renal afferent veins, each cut in two, became regenerated), in which I amputated the 5th toe on both hind legs, that on the left leg became completely regenerated within fifteen months, though that on the right leg was not re-formed to any considerable extent. Unfortunately I neglected to make a drawing of this before I left India. Figures A and A′ represent the regenerated 1st digits on the left and right arms respectively, and it is noticeable that though the period of regeneration was only 73 days, and though both specimens were females (the digits therefore not being used for the nuptial embrace), yet they are better developed than any of the other digits. The other figures show the partial regeneration of the 2nd, 3rd, and 4th fingers.

On the Bionomics and Development of Lygocerus testaceimanus, Kieffer, and Lygocerus cameroni, Kieffer (Proctotrypoidea-Ceraphronidae), parasites of Aphidius (Braconidae).

By

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With 18 Text-figures.

Introduction.

The Proctotrypoidea have been less studied than most other groups of the Hymenoptera Parasitica. Ganin (1869) was the first to study the embryology of certain members of the group (**8**). In 1884, Ayers (**2**) described the development of the Scelionid, Teleas. In 1898, Kulagin (**14**) resumed the study of Platygaster; and in 1906, Marchal (**18**) published the results of his elaborate researches into the embryology and development of that family. In recent years much work has been done on this group from the systematic standpoint, notably in the monographs of Ashmead (**1**) in America, and of Kieffer (**13**) in Europe, but the life-histories of most of the families are comparatively little known.

The following is an account of the bionomics and post-embryonic development of two species of the genus Lygocerus, of the sub-family Ceraphroninae. These forms are parasites of the larvae and pupae of certain Braconidae, of the family Aphidiidae, which are themselves internal parasites of various plant-lice.[1]

I would here express my sincere thanks to Professor Stanley

[1] A preliminary note on these observations by the writer appeared in the 'Proceedings of the Cambridge Philosophical Society', 1920, vol. xix, Pt. VI.

Gardiner, who gave me facilities to carry out the work in the Zoological Laboratory at Cambridge ; and my obligations to Professor J. J. Kieffer, and to Mr. G. T. Lyle, who kindly determined the specimens of Proctotrypoidea and Braconidae submitted to them respectively.

Biological Status.

The genus Lygocerus was founded by Förster, and is included in the sub-family Ceraphroninae. Ashmead (**1**, p. 103) and Kieffer (**13**) state that the Ceraphroninae are almost exclusively parasitic upon Homoptera (Aphidae) and Diptera (Cecidomiidae, &c.). Riley is said to have reared a Lygocerus from a tortricid larva (Lepidoptera), but Ashmead considers the observation to be of doubtful accuracy. The genus contains a number of species obtained from aphides, but their bionomics have hitherto been in doubt, authorities disagreeing as to whether they are parasites or hyperparasites.

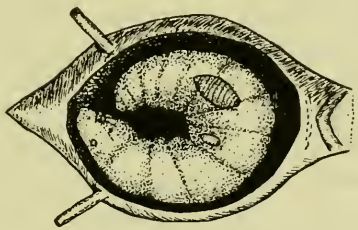
Curtis believed correctly that they were hyperparasites, and Buckton (**4**) agreed with him ; but later writers have reverted to the view that these Proctotrypids are directly parasitic upon the aphides from which they are reared. Thus Ashmead (**1**, p. 21), who says that the larvae all feed upon the host internally, continues : 'Lygocerus and allied genera living in the Aphidae, gnaw a hole through the ventral surface of the aphis, and after securely fastening the aphid by a silk-like secretion to the leaf or twig upon which it has been feeding, pupate within the body of their host, which, in lieu of a cocoon, affords ample protection to the larvae to undergo their transformations.' Gatenby (**9**) says, 'I am inclined to support the view that the Proctotrypid is a parasite and not a hyperparasite '.

The subjects of this paper, Lygocerus testaceimanus, Kieff., and L. cameroni, Kieff., are both hyperparasites. The eggs are laid and the larva stages are passed outside the body of the host. The Aphidius larva, in the course of its development, devours the internal organs of the aphis in which

it is reared ; and when it is full-fed, it lines the empty skin with silk, and pupates within it. At this time, it is itself liable to parasitisation by the Proctotrypids (fig. 1).

Lygocerus does not confine itself to Aphidiidae. Twice I have observed its larvae upon newly-transformed and dead pupae of its own species. The aphidivorous Braconidae are known to be parasitised by certain Chalcidae and Cynipidae, some of which were reared from material collected in the field in the course of this work. Lygocerus cameroni

TEXT-FIG. 1.



Skin of Macrosiphum urticae cut open to show the full-grown larva of its parasite, Aphidius ervi, which has in turn been attacked by Lygocerus cameroni. An egg, and third stage larva of the hyperparasite are represented.

occurred occasionally upon the adult larvae of a Chalcid, probably Asaphes vulgaris, and also upon a second species, not yet determined, which is possibly a Cynipid (Allotria sp.). Apart from the two cases mentioned above, where the larva had been hyperparasitised by its own species, Lygocerus was never found to be attacked by another hymenopteron.

One remarkable instance of hyperparasitisation came under notice. An aphis (Macrosiphum urticae) had been parasitised by Aphidius ervi. The latter had been hyperparasitised by an undetermined species of Chalcid. This form, after metamorphosis, had been devoured except for the frass, the head, and part of the thorax, by a second hyperparasite, whose life-history is not yet worked out. This larva was full-grown when the cocoon was opened, but it had itself

been recently hyperparasitised by Lygocerus cameroni. Hence, within certain limits, this species seems to be polyphagous.

Material.

The material used was obtained in Cambridge in the summer of 1919. At the end of June, a variety of L. testaceimanus, Kieff., was reared from the larvae of Aphidius salicis, Hal., parasitic in the sexuales of Aphis saliceti, Kalt., on the willow; and as the host material became scarce, I subsequently induced it to oviposit on larvae of Aphidius ervi, Hal., in Macrosiphum urticae, Kalt., on the nettle. In July, I reared a number of L. cameroni, Kieff., from the latter material collected round Newnham; and as the host was plentiful, and, owing to its larger size, easier of dissection than the parasites from the willow, I worked with it exclusively in July and August. The following account therefore applies especially to L. cameroni, though the life-history of L. testaceimanus is essentially the same.

Aphides parasitised by A. ervi were collected in the field, but a proportion of these were found to be already hyperparasitised by certain Chalcidae and Cynipidae. To ensure a 'pure culture' of Lygocerus, nettles infested with Macrosiphum urticae were placed in water under bell-jars in the open air insectary, and exposed to Aphidius ervi. The aphides were kept under cover during the development of the parasite, and when the latter were about to transform, the leaf was cut off, and placed in a glass tube with a fertilized female of Lygocerus. Thus the possibility of an infection by another hyperparasite was virtually eliminated.

I tried many times to cut open a flap on the dorsal side of the aphis skin, hoping by this means to follow the complete development of the hyperparasite from day to day, but the attempt always failed through the death of both the Aphidius and the Proctotrypid within a few hours.

Pairing.

No parthenogenetic ovipositions were observed, and about 40 per cent. of the imagos reared were males. Pairing took place a few hours after emergence. It was noticed that the males paired only once. Thus Lygocerus differs from its Braconid host, in which a single male will fertilise two or three females successively.

Oviposition.

The female Lygocerus, when about to oviposit, runs in an agitated manner over the leaves infested with plant-lice. Living aphides, whether parasitised or not, are ignored, and I have never seen the Lygocerus make the mistake of ovipositing on an Aphidius which had not begun to spin silk. The necessity is obvious, for until just before metamorphosis, the host is still bathed in the juices of the aphis, in which the egg of the hyperparasite could hardly develop. Sometimes a pupa is chosen instead of a full-grown larva ; but these are never attacked in the later stages when the chitin is hardening.

When a suitable host is found, the Lygocerus runs round and over it with much excitement, tapping it repeatedly with her antennae. The act of oviposition usually takes from 30–60 seconds. The Proctotrypid stands either on the thorax of the aphis skin, facing the head, or on the leaf behind it with the tip of her abdomen against its posterior part. Either way, the result is to bring the ovipositor, when exserted, into the curve formed by the body of the Aphidius as it lies, bent head to tail, in the cocoon. The ovipositor seems to penetrate the aphis skin with little effort. Sometimes it is partly withdrawn and inserted again, but only one egg is deposited on the host. Occasionally two females may be seen to oviposit simultaneously on the same Aphidius ; and, later, it is not uncommon to find two or three young larvae, but only one of the latter reaches maturity, and two imagos were never reared from the same cocoon.

The number of eggs laid by a single Lygocerus is uncertain, but from observations made on females in captivity, and from dissections of mature ovaries, it does not appear to be more than fifteen or twenty, at most twenty-five. Calculation by the latter method is difficult, as the eggs do not all mature at the same time; and if the hosts be removed from the cage of a captive female, and restored two or three days later, she will recommence and complete oviposition.

The Egg.

The egg of the hyperparasite, when newly laid, is elliptical, and measures ·25 × ·10 mm. It is white and semi-translucent, with a minute protuberance at one end. Under the high power

Text-fig. 2.

The egg immediately after oviposition. × 100.

of the microscope, the chorion shows numerous longitudinal striae. Treatment with Aman's lacto-phenol and cotton-blue reveals the presence of bodies resembling the symbiotes of the 'pseudo-vitellus' of aphides. The egg is laid upon the upper surface of the host's body, and hatches in about twenty hours. As the development of the embryo proceeds, the egg becomes more spherical, and the jaws, gut, &c., of the future larva are visible through the chorion.

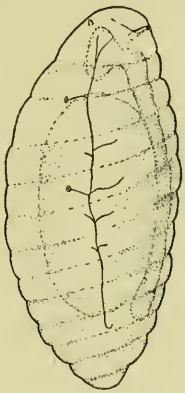
First Stage Larva.

Dimensions ·45 × ·22 mm.

The larva of the first instar is white and transparent, with a distinct head and thirteen body segments. The form is cylindrical, the greatest diameter being through the thorax, and the segments diminish regularly to the last which bears the anus. If removed to a slide, the larva can progress fairly actively by

a kind of peristaltic movement of the body, but under normal conditions it probably does not need to move from where it was hatched, provided that the host be a larva. If the latter be a pupa, the hyperparasite is generally found feeding on the posterior part of the abdomen, where the integument is still soft. As the egg, as previously described, is always deposited on the third or fourth segment of the Aphidius, the hyperparasite must needs seek the new situation for itself after hatching.

TEXT-FIG. 3.



The larva, newly hatched, showing tracheal and nervous systems. × 200.

The internal anatomy, with the exception of the tracheal system, does not change essentially during development, so that an account of it is left to the description of the fourth instar. The mouth, which is very small and transversely oval, is furnished with two slender mandibles, set behind the hood-like labrum, and the labium (fig. 5). The head is furnished with two tactile papillae. The mid-gut, which at this stage, as with the other parasitic Hymenoptera, does not communicate with the proctodaeum, is large and globose, and its contents tinge the otherwise transparent larva pale yellow.

The tracheal system consists of a pair of lateral trunks, united by an anterior commissure passing above the oesophagus in

front, and a posterior commissure passing beneath the gut, in the eleventh segment, behind. Simple dorso-lateral, and ventro-lateral, branches are given off in segments 1, 3–8. When newly hatched there are only two pairs of open spiracles, the first between the first and second segments, and the second on the anterior part of the fourth, but the spiracles of the third and fifth segments open shortly afterwards. (See 'Moults'.)

Seurat (**26**, p. 100) states that the young larva of the Chalcid, Torymus propinquis, has likewise four open spiracles, but situated on the first, fourth, fifth, and sixth segments.

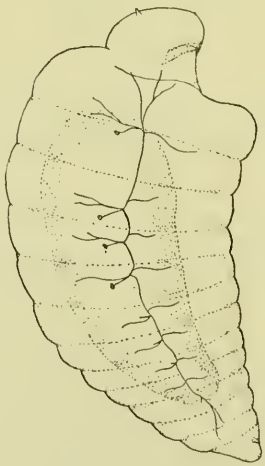
This stage lasts from twenty to twenty-four hours.

Second Stage Larva.

Dimensions ·70 × ·35 mm.

The second stage larva differs from the first chiefly in the tracheal system, and in the greater development of the anterior

Text-fig. 4.



The larva of the second instar, showing tracheal system. × 200.

part of the body in proportion to the head, so that the latter appears constricted off from the thorax, and the body resembles

a cone with the head projecting from the blunt end. The tracheal system is more complex: the ramifications of its branches are more numerous, and those of the second segment appear at this stage. The stigmatic trunks of segments six,

TEXT-FIG. 5.

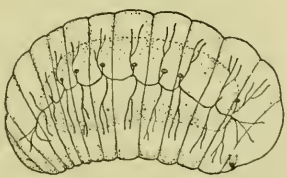
Mouthparts of second stage larva. × 400. Ventral view. *o.sal.* = aperture of salivary duct. *sk.* = endoskeleton of head. *lab.* = labium. *m. lab.* = muscles of labium. *lbr.* = labrum. *md.* = mandibles. *sal. d.* = salivary duct.

seven, and eight are visible at the junction of the dorso-lateral branches with the main stem of the tracheae, but the corresponding spiracles are still closed. This stage lasts about thirty-six hours, and during this time the host dies and becomes black and shrunken. The hyperparasite seems to feed by suction, and the skin of the Aphidius, otherwise uninjured, is gradually emptied of its contents. As the fluid from the decomposing tissues passes into the mesenteron of the Proctotrypid, the latter changes in colour from yellow to brown.

THIRD STAGE LARVA.

Dimensions 1·00 × ·75 mm.

TEXT-FIG. 6.



Larva of the third instar, showing tracheal system. × 49.

In the third stage the body becomes globose, owing to the increased proportionate development of the first seven or eight segments to accommodate the distended mesenteron. The result

of this distension is to bend the head round ventrally to form an acute angle with the long axis of the body. The papillae on the head disappear. The branching of the tracheal system is more elaborate, and the spiracles of segments six, seven, and eight open in the order named, while the stigmatic trunk of the second segment appears. This stage is longer than the two preceding, and lasts about forty hours. The parasite is bathed in the fluid that oozes from the decomposing body of the host.

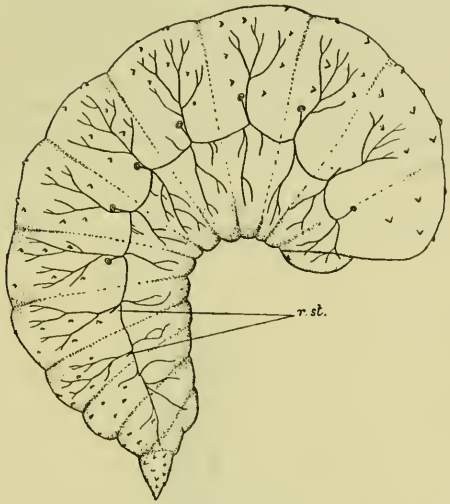
Fourth Stage Larva.

The larva in the fourth instar differs considerably from that of the preceding stages in size and form. Immediately after ecdysis, the dimensions are not much greater than those of the third instar, and the body is transparent ; but as the larva ingests the remainder of its host, it grows rapidly, and when fully fed, measures 1·67 × ·83 mm. At the same time it becomes creamy white and opaque.

The first four body segments are greatly developed. The small head is bent completely round to the ventral side, and is almost hidden by the large prothorax. The abdominal segments diminish in diameter posteriorly, and the last bears dorsally a conical caudal appendage. The function of this is unknown, unless it is used as a lever by the larva which is able to turn round freely in the cocoon. Seurat (**26**, p. 99) has described a somewhat similar appendage in a Chalcid, Encyrtus sp., and supposes that its purpose is locomotion (fig. 9, *cd.*).

Both the caudal appendage and body bear short chitinous papillae or spines. The head is without larval antennae or palpi. The mouth, which is very small and transversely oval, is bounded anteriorly by a large horseshoe-shaped labrum, and posteriorly by a smaller square labium. Between these, and deeply set within the buccal cavity, are two stout little mandibles (fig. 8). The salivary glands extend from the dorsal part of the fourth segment forwards on either side of the mid-gut as two straight tubes with a considerable lumen. They are formed of polyhedral cells with large nuclei and granular cytoplasm, which stains deeply with haematoxylin. Each gland runs obliquely

TEXT-FIG. 7.



Larva of the fourth instar, showing tracheal system. × 49. *r. st.* = rudimentary stigmatic trunks of segments 9 and 10.

TEXT-FIG. 8.

The mandibles of the full-grown larva. × 400.

TEXT-FIG. 9.

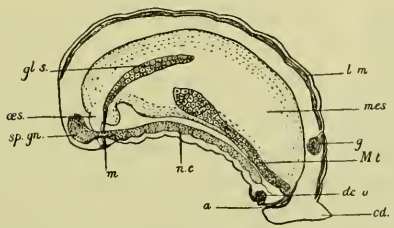


Diagram of the general structure of the fourth stage larva. *a.* = anus. *cd.* = cauda. *g.* = gonad. *dc. o.* = imaginal disk of ovipositor. *l. m.* = longitudinal muscles. *M. t.* = Malpighian tube. *mes.* = mesenteron. *m.* = mouth. *n. c.* = nerve cord. *oes.* = oesophagus. *gl. s.* = salivary gland. *sp. gn.* = supra-oesophageal ganglion.

forwards and downwards, and between the first and second segments enters a duct lined with epithelial cells, very similar to those of the œsophagus (fig. 10). The two ducts unite behind the head to form the common salivary duct, which opens just inside, on the floor of the mouth. Under high power, the ducts have the trachea-like structure found in most insects. On either side of the salivary aperture is inserted a small muscle, which runs outwards and backwards to the endoskeleton of the head. When these contract, the labium, and consequently

Text-fig. 10.

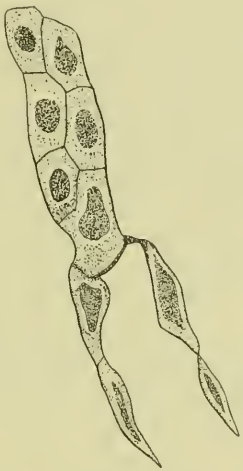
Longitudinal section through the salivary gland and duct of a larva of the fourth instar. × 300.

the opening of the salivary duct, is slightly everted from the mouth (fig. 5).

Two pairs of buccal muscles are connected with the labrum, and by their contraction enlarge the buccal cavity. The anterior, and more lateral, pair arise from the exoskeleton of the front of the head, just above the labrum, on either side of the median line, and running directly downwards (or, having regard to the position of the head, backwards) are inserted on the roof of the mouth. The posterior and median pair arise together behind the last, and, running forwards obliquely between them, are inserted on the distal half of the labrum (fig. 13). The

short oesophagus opens into the mid-gut, which fills the greater part of the body cavity, and is lined with glandular cells, rather wider than deep, with well-marked nuclei. It contains a mass of fluid food material, which is churned to and fro by incessant muscular contractions of the body, but until just before metamorphosis there is no communication with the hind-gut. Two

TEXT-FIG. 11.



Longitudinal section through the Malpighian tube of a larva of the fourth instar, showing lumen. × 300.

large Malpighian tubes extend from the fourth segment, ventral to the salivary glands, and run back on either side of the mesenteron. They are somewhat dilated at their anterior extremities, and in sections show a considerable lumen, surrounded by large flattened cells with great nuclei, resembling those of the salivary glands (fig. 11). In the posterior half of the tubes the lumen is very small and the cells are rounded. The tubes open into the ampulla of the proctodaeum, that is, the cup-like anterior end of the hind-gut, which abuts on the mid-gut in the eleventh segment (fig. 14).

The muscular system is well developed, especially the dorsal

longitudinal, and lateral muscles of the posterior segments (fig. 9).

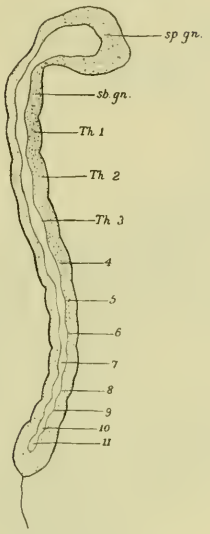
The circulatory system calls for no particular comment.

In the tracheal system of the fourth instar larva there are still seven pairs of open spiracles, for the eighth (mesothoracic) does not become functional until metamorphosis. The first spiracle is situated between the first and second segments, and the second on the posterior side of the third segment, while the remainder are on the five following segments. In addition, two rudimentary stigmatic trunks can sometimes be seen on the ninth and tenth segments, and the anterior one is occasionally visible during the third instar. It appears that these trunks are never functional, and they were not always apparent in the larvae examined. Imms (**11**) has described vestigial stigmatic trunks on the eleventh segment of the full-grown larva of Aphycus melanostomatus, which has nine pairs of functional spiracles. These do not appear in the Lygocerus larva, in which the spiracles have evidently been reduced in number from behind forwards. The aborted trunks of segments nine and ten are probably vestiges inherited from an ancestral form with ten open spiracles. The rest of the tracheal system differs from that of the preceding stage only in the greater calibre and more elaborate ramifications of the tubes. It should, however, be remarked that there is no anastomosis of the tracheal branches of the two sides of the body, such as Seurat (**26**) describes in certain Ichneumonidae and Braconidae (fig. 7).

The nervous system consists of two supra-oesophageal ganglia, united by a broad commissure, and connected with the sub-oesophageal ganglion by two short, thick circum-oesophageal commissures. The ventral nerve cord contains eleven ganglia. The four anterior are well marked ; the five following are less distinct, and appear as a wide, slightly-segmented band. The cord terminates in a bulbous swelling, composed of two ganglia, that of the eleventh segment being fused with that of the tenth (fig. 12).

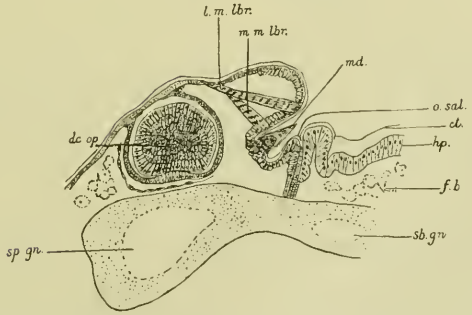
The genital organs lie above the mid-gut on either side as

TEXT-FIG. 12.



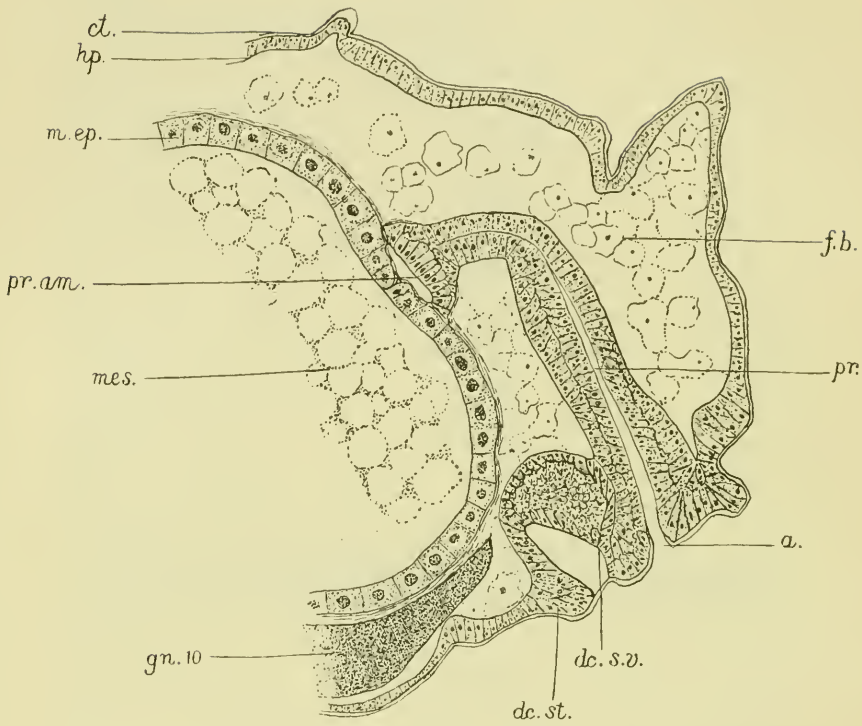
Nervous system of larva (partly diagrammatic). *sb.*=sub-oesophageal ganglion. *sp.*=supra-oesophageal ganglion. *Th.* 1–3 = Thoracic ganglia. 4–11.=abdominal ganglia.

TEXT-FIG. 13.



Vertical section through the head of a larva of the fourth instar. (The muscles of the labrum are shown somewhat diagrammatically.) ×200. *ct.*=cuticle. *dc. op.*=imaginal disk of eye. *fb.*= fat body. *hp.*=hypoderm. *l. m. lbr.*=lateral muscles of labrum. *m. m. lbr.*=median muscles of labrum *md.*=mandible. *o. sal.*= aperture of salivary duct. *sb. gn.*=sub-oesophageal ganglion. *sp. gn.*=supra-oesophageal ganglion.

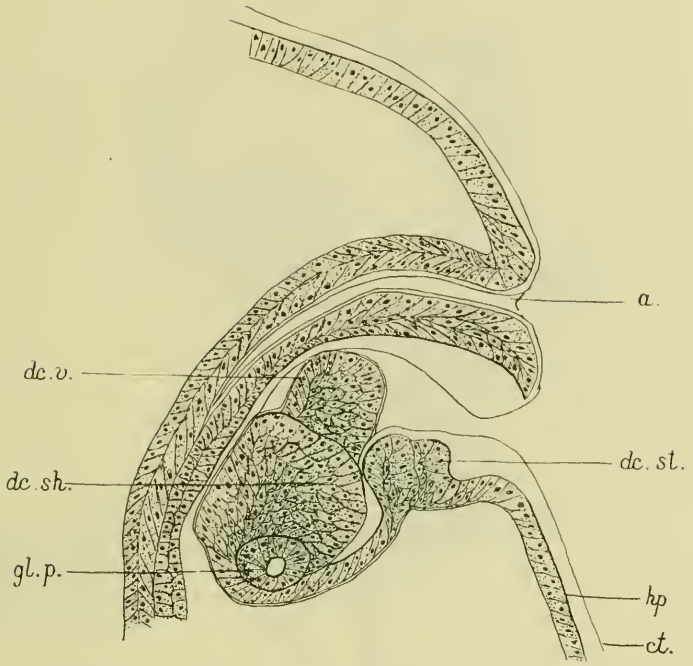
Text-fig. 14.



Vertical section through the posterior region of the body of a larva of the fourth instar. × 350. *a.*=anus. *ct.*=cuticle. *dc. st.*=imaginal disk of stylets. *dc. s. v.*=imaginal disk of sheath and valves. *fb.*=fat body. *gn.* 10.=ganglion of segment 10. *hp.*=hypoderm. *m. ep.*=wall of mesenteron. *mes.*=mesenteron. *pr. am.*=ampulla of proctodaeum. *pr.*=proctodaeum.

two oval bodies, the testis being more elongated than the ovary (fig. 9). The complete development of the accessory genital apparatus was not observed, but in the fourth instar the female armature exists as two imaginal disks on the eleventh and twelfth segments. In Lygocerus the relationship of the parts is somewhat obscured, owing to the curvature of the body and crowding together of the segments in the posterior ventral region, but my observations on the origin of the

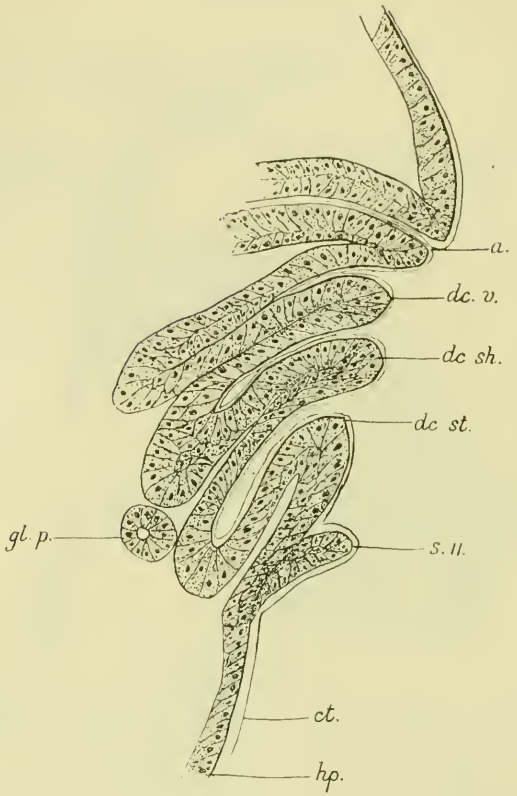
Text-fig. 15.



Vertical section through the developing genital armature of a female larva of the fourth instar. × 350. *a.*=anus. *ct.*=cuticle. *dc. sh.*=imaginal disk of sheath. *dc. st.*=imaginal disk of stylet. *dc. v.*=imaginal disk of valve. *gl. p.*='poison gland'.

ovipositor, as far as they go, are substantially in agreement with those of Seurat on Dorycteus gallicus. The stylets arise from the posterior ventral wall of the eleventh segment, and the sheath and valves are derived from the reduplication of the imaginal disks of the twelfth segment. A tubular glandular structure is formed by constriction from the hypodermal cells at the base of the latter. In its origin and position it corresponds with that described by Seurat as 'la glande à venin'. Whether this organ is actually a poison gland in the Ceraphronidae I am unable to say. Saunders, quoted by Woodward (Ashmead, 1), records that he was stung by a female

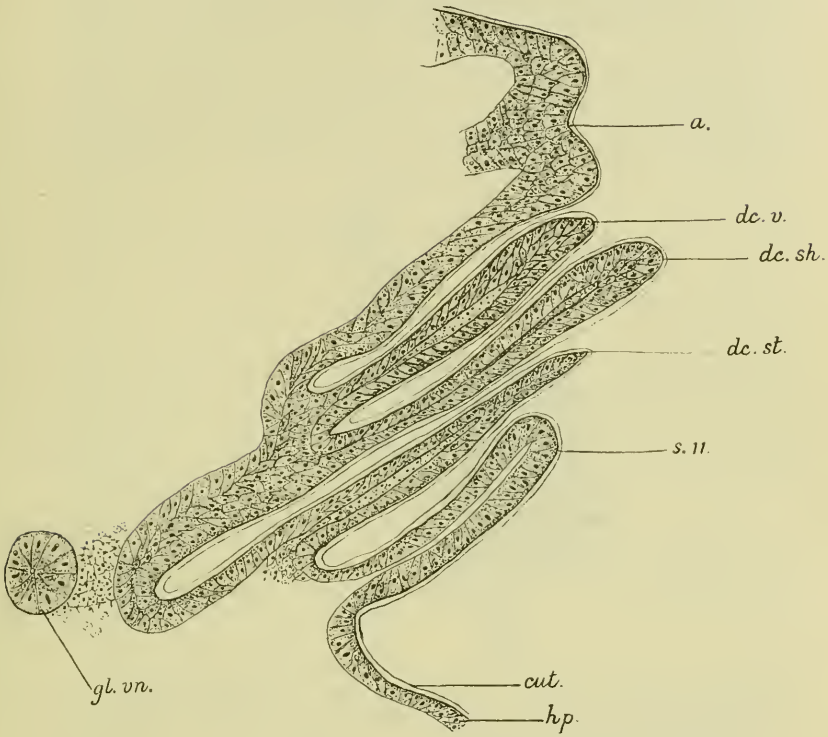
Text-fig. 16.



The same as fig. 15, more advanced. × 350. *a.*=anus. *ct.*= cuticle. *dc. sh.*=imaginal disk of sheath. *dc. st.*=imaginal disk of stylet. *dc v.*=imaginal disk of valve. *gl. p.*='poison gland'. *hp.*=hypoderm. *s.* 11.=sternite of segment 11.

of Scleroderma linearis; and of other parasitic Hymenoptera, the female Ichneumonid, Ophion, will sometimes pierce with the ovipositor when handled. The pain is more severe and persistent than a mere mechanical stab would produce, so that presumably some secretion enters the wound. Bordas and others have described structures in various Terebrantia which appear to be homologous morphologically with the poison glands of the Aculeata, but their function is

TEXT-FIG. 17.



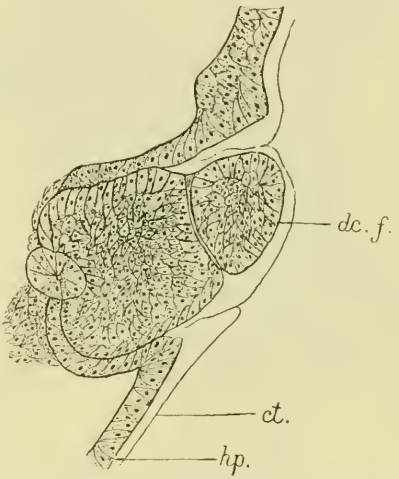
The same as in figs. 15 and 16, shortly before metamorphosis. × 350. *a.* = anus. *ct.* = cuticle. *dc. sh.* = imaginal disk of sheath. *dc. st.* = imaginal disk of stylet. *dc. v.* = imaginal disk of valve. *gl. p.* = 'poison gland'. *hp.* = hypoderm. *s.* 11. = sternite of segment 11.

still uncertain. The tubular gland, 'glande tubuleuse', that Seurat describes in Dorycte s, I have not traced in Lygocerus (figs. 14, 15, 16, 17).

Owing to lack of suitable material, the whole ontogeny of the male genital armature was not followed, but it appears to arise, as described by Seurat, from the imaginal disks of the twelfth segment only. In the fourth instar two terminal lateral processes appear at the end of the disks, and probably represent the future stipites (forcipes) (fig. 18).

The fourth instar lasts between two and three days. Lygocerus does not spin silk, but pupates within the cocoon previously woven by the Aphidius. Just before metamorphosis, the mid-gut opens into the hind-gut, and the contents are voided. The larva is active, and by its movements the frass, together with the now empty skin of the host, are welded into

Text-fig. 18.



Vertical section through the developing genital armature of a male larva of the fourth instar. × 350. *dc.f.* = imaginal disk of forcipes. *ct.* = cuticle *hp.* = hypoderm.

a compact, moist pellet at the ventral side of the body. The frass of the Proctotrypid, Chalcid, and Cynipid parasites of Aphidius can readily be distinguished from one another, for that of Lygocerus is invariably a single black mass, whereas that of the Chalcidae and Cynipidae consists of several pieces of a different form and colour.

Moults.

The determination given of the number of moults, and the duration of the instars, is based on the examination of many individuals of different ages, and may be somewhat arbitrary; but it was the only practicable method to employ, since it

proved impossible to keep one larva alive for observation from day to day. The reasons for determining the different instars thus are as follows :

The newly hatched (first instar) larva of Lygocerus possesses only two pairs of open spiracles, but examples twelve hours old have four. At one time I believed that these forms were separated by a moult, though it was never observed. On the other hand, I noticed a larva twenty-four hours old which had the cast skin attached to the hind part of the body. The exuviae were too much torn to show the spiracles, but the larva itself had four (fig. 4). For purposes of convenience, therefore, I have referred all stages up to that represented in that figure to first instar, and assumed that the spiracles of the third and fifth segments opened as the stadium proceeded ; but it may well be that there is a moult between the forms with two and those with four spiracles. We should then have five larval stages, separated by four moults.

Similarly, the actual ecdysis between instars two and three, as here described, has never been observed, but the differences in the external form and respiratory system seem sufficient to place them in separate instars.

The difference in size and form between instars three and four is so great that, if a large number of larvae had not been examined, there would have been doubt in referring them to the same species. The fourth instar, immediately after the moult, is transparent, and half the size of that represented in fig. 7. But the caudal appendage and tracheal system are unmistakable, so that although the actual ecdysis has not been seen, this form has been described as the fourth instar.

Pupation and Emergence.

The period of pupation is from fourteen to sixteen days. If disturbed, the pupa jerks its abdomen vigorously from side to side. It is possible that this habit, which is marked in both the larva and the pupa, and in which they differ from the Aphidius itself, and from its Chalcid and Cynipid parasites.

may in some degree protect them from oviposition by the females of their own and other families.

When ready to emerge, the imago gnaws a hole somewhere on the dorsal side of the cocoon and creeps out. As Gatenby (**9**) has remarked, this hole differs from that made by Aphidius in having irregular edges, and is not necessarily placed in the dorso-posterior region of the aphid's skin.

The number of broods occurring in one year is not known, and probably depends on the number of species of Aphidius upon which the hyperparasites can live. Two broods were reared from Aphidius ervi in 1919; but the host did not appear in any numbers before July, and it is possible that earlier broods may have occurred with a different host. All the imagos of Lygocerus had emerged by the end of August, and there is no evidence to show whether the species over-winters as larva or pupa.

In captivity the imagos generally live five or six days, but sometimes as long as ten. They were observed to feed on sugar and water, on honey-dew from the aphides, and on sap oozing from cut leaves, but they seemed to live as long, and to remain as vigorous, when no food was supplied.

Comparison of Larval Characters with those of other Sub-Families.

The most complete comparative account of the larvae of entomophagous Hymenoptera is that of Seurat (**26**), who studied certain Ichneumonidae, Braconidae, and Chalcidae. Unfortunately he did not include the Proctotrypidae, and our knowledge of the larval morphology of this family, as already remarked, is very scanty. Seurat emphasized the importance of the tracheal system in determining the larvae of the different groups, but, as Lichtenstein and Picard have recently pointed out (**15**), increased knowledge has somewhat modified this view.

Some authorities have considered that the Proctotrypoidea are allied to the Chalcidoidea, but Ashmead (**1**) disputes this, and thinks them in every respect more nearly related to the Hymen-

optera Aculeata, and among Terebrantia, to the parasitic Cynipidae. The discussion of the affinities of the group is outside the scope of this paper, but it should be pointed out that the larval form of this particular genus of Ceraphroninae differs from the Chalcid larvae described by Seurat (**26**), Imms (**11**), Embleton (**6**), &c., in several respects. As regards the tracheal system, the late opening of the spiracle of the second segment is common to many larvae of the entomophagous Hymenoptera. On the other hand, the larva of Lygocerus is remarkable for the reduced number of abdominal spiracles, and the rudimentary nature of the stigmatic trunks of segments nine and ten, and differs from the Ichneumonidae and Braconidae studied by Seurat in the absence of anastomosis of the tracheal vessels of either side; though as Lichtenstein and Picard (**15**) have shown for the Braconid, Sycosoter lavagnei, this is not an invariable character of the external feeding Braconidae.

The reduction in the number of spiracles is carried still further in Platygaster. Marchal (**18**) figures four spiracles in Platygaster ornatus, the first between the first and second segments, and those succeeding on the third, fourth, and fifth. The spiracle of the fourth segment (the propodaeum of the imago) differs from the others in its larger size, and the greater proliferation of the hypoderm cells surrounding it. 'Il est pareil à une sorte d'histoblaste aux dépens duquel devra se former plus tard le grand stigmate du segment médiaire de l'adulte.' Further, in Platygaster, the main tracheal trunks are not joined posteriorly by a commissure. In Lygocerus a posterior commissure exists, and the spiracle of the fourth segment is indistinguishable from the rest.

Likewise M'Colloch (**20**) describes 'four or five pairs of well-developed spiracles' in the larva of the Scelionid, Eumicrosoma benefica; but Ganin (**8**) states that there are nine spiracles in the third stage larva of the form of Platygaster that he studied, and that spiracles are lacking only on the first, second, and three last segments.

Kulagin (**14**) for Platygaster, and Ayers (**2**) for

Teleas, do not describe the later stages of the larvae, and say nothing about the tracheal system. Keilin and Thompson (12) describe nine pairs of spiracles in a Dryinid larva, parasitic in Typhlocyba (Homoptera). The relative positions are not determined, but from the figure it seems as if the meso-, or possibly the metathorax, bears no spiracles.

I can find no other account of the tracheal system of the Proctotrypoidea, and until we have more knowledge of the hymenopterous larvae which live upon their hosts as external parasites, we cannot tell how far the characters observed indicate true phylogenetic relationships, or are merely secondary adaptations. Moreover, it is unwise to compare a highly modified internal parasite, such as Platygaster, with the more generalized external forms; though in this connexion it may be significant that the third stage larvae of Platygaster and Eumicrosoma have a certain resemblance to the early larva of Lygocerus.

The differences are not confined to the tracheal system. Marchal describes ten ganglia in the nerve cord, and three Malpighian tubes, in Synopeas rhanis. Keilin and Thompson observed thirteen ganglia, and no Malpighian tubes, in the Dryinid that they studied. This diversity of structure indicates either that little reliance can be placed on larval characters, which are often adaptive, or that the Proctotrypoidea as at present understood are, in some respects, an arbitrary group.

Economic Status.

From an economic standpoint Lygocerus must be regarded as an injurious insect. Parasitisation by Braconidae is an important natural check upon the increase of plant-lice; and this Proctotrypid, like the hyperparasitic Chalcidae and Cynipidae, is an enemy of the beneficial Aphidius. Unless, as seems improbable, it confines its attacks to a single species, it must destroy considerable numbers of Aphidiidae.[1] Aphi-

[1] Kieffer records that L. testaceimanus has been reared from a rose aphis (? Macrosiphum rosae) (13, p. 51).

lius ervi, and the nearly related species A. *avenae*, are parasites of such pests as *Macrosiphum granarium*, the grain aphis, and according to Marshall (**19**) are polyphagous, preying indiscriminately on various species of aphides. If their parasites follow them to other hosts, their efficiency as controls of plant-lice must be seriously impaired. For instance, two collections of A. *ervi* from M. *urticae*, made from different places round Cambridge in August, gave the following results :

	Number examined.	Parasitised by other families.	Parasitised by *Lygocerus*.	Total % parasitised.	% Parasitised by *Lygocerus*.
I	50	12	16	56	32
II	38	6	17	60	44

Other collections, of which exact records were not kept, likewise showed a high percentage of hyperparasitisation by these Proctotrypids.

Aphidius is at least twice as prolific as its parasite, and each female destroyed by the latter means the loss of thirty or forty ovipositions, which would kill, or at least impair the fertility of, the same number of aphides. If this high rate of hyperparasitisation should occur in a grain crop infested by *Macrosiphum granarium*, attacked by *Aphidius*, the efficiency of this natural control might be lowered by 50 per cent.

Summary.

1. *Lygocerus testaceimanus*, Kieff. is a hyperparasite of *Aphis saliceti*, Kalt., through the primary parasite, *Aphidius salicis*, Hal. ; and L. *cameroni*, Kieff. is similarly a hyperparasite of *Macrosiphum urticae*, Kalt., through the primary parasite, *Aphidius ervi*, Hal.

2. The *Aphidius* is attacked immediately before or after metamorphosis, when lying within the empty skin of the aphis within which it is reared.

3. The egg is laid, and post-embryonic development takes place, outside the body of the host.

4. The evidence points to the conclusion that there are four larval instars and three moults.

5. The larvae differ in several particulars from those of the families of Proctotrypoidea previously described, and there is considerable difference in form between the early and later instars.

6. During development, which lasts about six days, the larva devours its host, and then pupates within the skin of the aphis for a further period of two weeks.

7. Two, and possibly more, broods are reared in the season ; and it is probable that the hyperparasite is a considerable check on the Aphidius in its control of plant-lice infestation.

8. Lygocerus, though occasionally attacked by its own species, was never found to be parasitised by another hymenopteron. This immunity is probably due to the active movements with which the larva and pupa in the cocoon respond to external stimuli.

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On the Terrestrial Planarians from the Islands of Mauritius and Rodrigues; with a Note upon the Canal connecting the Female Genital Organ with the Intestine.

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With Plate 4 and 6 Text-figures.

Few terrestrial planarians are as yet known to occur in the islands remote from continental land in the Indian Ocean. Such are Pelmatoplana mahéensis von Graff and P. braueri von Graff (**12**, **20**) from the Seychelles, Placocephalus isabellinus Geba (**11**) from Mauritius, Geoplana whartoni Gulliver (**1**) from Rodrigues, and Rhynchodemus ceylonicus von Graff (**17**) from Male Atoll.

The material serving as a basis of the present report was collected by Mr. H. P. Thomasset in October, 1918, in the island of Mauritius, and by Mr. H. J. Snell in November and December of the same year in the island of Rodrigues. The specimens were sent to Professor J. S. Gardiner, who kindly turned them over to me for examination.

In this communication it may not be out of place to add a brief account of G. whartoni Gulliver, known as occurring in the Rodrigues Island. I am much indebted to the Director of the British Museum of Natural History for the privilege of studying this species.

The following is a list of the species dealt with in the present paper :

Geoplana whartoni Gulliver ;
Placocephalus isabellinus Geba :
Rhynchodemus ceylonicus von Graff ·
Amblyplana trifuscolineata, n. sp.

The planarian fauna of the islands mentioned above is regarded by von Graff to be derived from that of the Aethiopian region, whilst the species referrable to Rh. ceylonicus has been clearly brought from Ceylon through the agency of man.

Before proceeding further, it gives me great pleasure to express my deep indebtedness to Professor J. S. Gardiner for his suggestions and kind assistance throughout this work in his laboratory. I deem it my duty to mention my indebtedness to Dr. Sir A. E. Shipley for his kind help in many respects. My best thanks are also due to Dr. H. A. Baylis for providing me with opportunities and accommodation for the examination of the Museum material.

Geoplana whartoni Gulliver.

(Text-fig. 1.)

Geoplana whartoni Gulliver (1), pp. 561, 562, Pl. lv, fig. 1.—von Graff (12), p. 347, Pl. iv, figs. 12–14, Pl. xxvi, fig. 4.

This species, according to Gulliver's statement, occurs in situations similar to those in which the nemertean, Tetrastemma rodericanus, lives, and, indeed, is often found together with it. He collected some specimens on rotten wood.

External Characters.—The body is elongate, slender, and for the most part nearly uniformly broad, though it tapers off considerably in front. The sole on the mid-ventral surface is slender, and corresponds to about one-ninth the width of the body. Well-grown specimens in the preserved state measure 15–20 mm. long by about 2 mm. broad.

The ground colour of the dorsal surface is cream, with three dark-brown stripes which run almost throughout the whole length of the body, and anteriorly merge into the general colour of the head-end, without revealing a dark tip. The ventral surface is a somewhat paler shade of the same colour as the dorsal, without any markings.

The numerous eye-spots are arranged in a single row round the anterior tip, and continue sparsely for some distance down the sides.

The mouth-opening, which leads into the peripharyngeal chamber, is placed somewhat behind the centre of the body, in the mid-ventral line.

The common genital opening lies nearer to the posterior end of the body than to the mouth-opening.

Epidermis and Body-glands.—The following account is based on a single specimen received from the British Museum. The epidermis consists of a layer of columnar cells, which are about equally high on the dorsal and ventral surface, and possess cilia, which, however, are confined to the latter surface. It contains spindle-shaped rhabdites on the dorsal surface only, where they are found in enormous quantities, evidently situated between the epidermal cells. Immediately beneath the superficial muscular system there occur such rhabdites as are still contained in their mother-cells. These are scattered in sparse numbers in the parenchyma. There are enormous quantities of slime glands, deeply situated in the parenchyma, opening not only to the exterior all over the surface of the sole, but in a narrow zone of the ventral surface along and just within the margin of the body.

Muscular System.—The musculature of the body presents no noteworthy features, consisting, as it does, of two systems, superficial and deep, which are rather more strongly developed on the ventral than on the dorsal side, doubtless in relation to the movements. Dorso-ventral fibres occur also in the usual manner.

Digestive System.—The mouth-opening is situated somewhat behind the middle of the body and at nearly the centre of the peripharyngeal cavity, with the pharynx horizontally disposed. The pharynx is a cylindrical tube, terminating conically at the free end. Embedded in the parenchyma in front of the pharynx-insertion are numerous salivary glands, which continue their way to the free end of the pharynx.

All the three main trunks of the intestine give off numerous lateral branches, which are sometimes bifurcated and sometimes ' multifurcated '. The epithelium consists, as usual, of a single

layer of high cylindrical cells. So far as I have observed, special glands are altogether absent in the lining epithelium.

Nervous System.—The exact arrangement of the nervous system could not be ascertained, but it seemed to be quite similar to that previously observed in several forms of this genus. Each half of the bilobed brain-mass is continuous posteriorly with one of the longitudinal nerve cords, which proceed, running nearly parallel to each other, to the hind end of the body, and are connected together by transverse commissures. Lateral nerves are given off from the cords towards the nerve plexus, which lies directly beneath the superficial muscular system.

The eye consists simply of a small pigment cup, partly filled with a peculiar cellular substance, whose true nature could not be ascertained from any of the sections available.

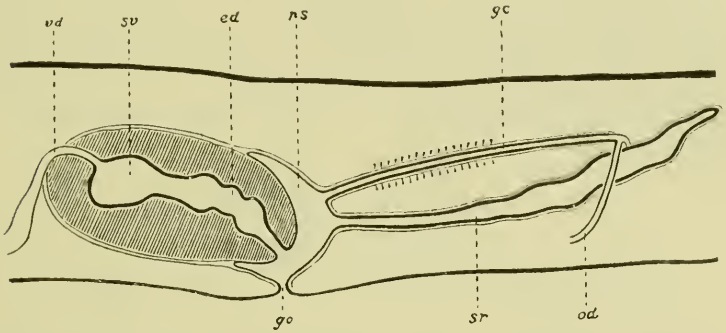
Reproductive Organs.—The genital organs are in accordance with those described by von Graff. The common genital opening leads directly into the penis-sheath, which receives from behind the openings of the seminal receptacle (uterus) and the glandular canal. The cavity is lined with a single epithelium resting upon a fine basement membrane, beneath which are found circular and longitudinal muscular layers.

Male Organs.—The numerous testes occur close together in the ventral parts of the body, arranged in two longitudinal lateral zones which extend from behind the ovaries to nearly the region of the copulatory organs. Each testis is, as usual, made up of sperm-mother-cells and spermatozoa in all stages of development, surrounded by the tunica propria. Probably they are all connected by testicular ductules, but these could not be definitely made out. Not far in front of the penis the vasa deferentia rise obliquely upwards to enter the penis-bulb separately at the upper lateral sides and finally open into the lumen of the penis or the seminal vesicle. The vas deferens, which is filled with spermatozoa, has a wall consisting of an epithelium and an outer layer of circular muscular fibres.

In the penis there can be distinguished the conical intromittent part, lying nearly horizontally in the penis-sheath, and the

bulbous part of muscular nature, which contains a cavity of somewhat irregular contour, the seminal vesicle. The vesicle gives rise to the moderately wide ejaculatory duct which opens at the tip of the penis. The muscular fibres of which the penis is composed are arranged in two principal sets, circular and longitudinal, the fibres of the two sets occurring intermingled with one another. Embedded in the parenchyma of the penis are numerous glands, the ducts of which open into its lumen lined by a layer of small columnar cells.

Text-fig. 1.



Diagrammatic representation figure of the sexual organs of Geoplana whartoni Gulliver. *ed.* = ejaculatory duct. *gc.* = glandular canal. *go.* = genital opening. *od.* = oviduct. *ps.* = penis-sheath. *sr.* = seminal receptacle. *sv.* = seminal vesicle. *vd.* = vas deferens.

Female Organs.—The paired ovary occupies a ventral position somewhat behind the brain. It is a nearly oval body made up of egg-cells in several stages of development. From the lateral aspect of the ovary the oviduct starts as an ampullaceous passage, which soon takes the character of a narrow duct and proceeds backwards just outside the longitudinal nerve cords, receiving the vitelline glands at numerous points. The vitelline glands are represented by branching cellular masses, which are extensively distributed in the interstices between the gut diverticulae. The mode of the connexion of the glands with the oviduct is effected by means of the short branches of the

latter. Far behind the genital opening the oviduct rises obliquely upwards, to unite with its fellow of the opposite side into a single common duct, the glandular canal, which opens into the penis-sheath from behind, after receiving numerous glands. The duct exhibits a distinct lumen throughout the entire length. Its direct wall is lined by a ciliated epithelium, outside which is a layer of circular muscular fibres.

At a short distance below the opening of the glandular canal the penis-sheath gives rise to a narrow passage, which pursues a somewhat tortuous course obliquely backwards and upwards, becoming gradually wider at the same time. Beyond the junction point of the oviducts it extends further backwards. This organ, which doubtless represents the seminal receptacle, has a wall consisting of a non-ciliated epithelium and a fine muscular coating; in the cavity are found enormous quantities of spermatozoa.

Placocephalus isabellinus Geba.

(Pl. 4, figs. 1, 2.—Text-fig. 2.)

Placocephalus isabellinus Geba (11), pp. 385, 386.

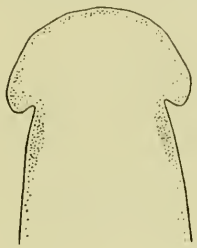
Three specimens of the species, which I identify with Placocephalus isabellinus described by Geba from the Mauritius Island, were collected by Mr. Thomasset under half-rotten logs and rocks in damp places in the same island.

The head in the preserved state is of a semilunar shape and not wider than the trunk, from which it is distinctly marked off by a constriction. The trunk is dorso-ventrally depressed, elongate, and nearly uniformly broad for the most part of its length, though it tapers in the hind parts down to the bluntly pointed end. The sole, scarcely raised above the general level, extends from the neck to the posterior extremity, its width being about a quarter that of the body. The large specimen was 120 mm. long by 4 mm. broad, while the small was 50 mm. long by 3 mm. broad.

As mentioned by Geba, the ground colour of the dorsal surface is an umber brown with five longitudinal black stripes, a median

and two pairs of laterals. The median stripe is very fine, extending from the neck to the posterior extremity, and widening slightly above the pharyngeal region. The inner pair are much the strongest of all, and the outer pair at the edge of the body become indistinct as they approach the hind end; on either side both coalesce at the neck into a black patch. The head is marked with a crescentic black pattern. Ventrally, the worm is similar in coloration to the dorsal surface, with a darker shade at the outer edge and also next to the surface of the sole; this latter is very pale.

Text-fig. 2.



Eyes of Placocephalus isabellinus Geba.

The numerous eye-spots are distributed all round the head, and are continued sparsely for a considerable distance along the sides of the body. At the sides of the neck they extend somewhat to the ventral surface and form a patch, as seen in Text-fig. 2.

The mouth-opening, which leads into the peripharyngeal chamber, is placed at some distance in front of the centre of the body. In the specimens examined the pharynx was protruded through the mouth-opening as a creamy frill.

The genital organs were unfortunately yet undeveloped in the individuals examined. Like some other forms, this species may to some extent reproduce asexually by transverse fission, as stated by von Graff. On two occasions the severed hind end presented a concave edge, apparently forming the new tail-end.

Rhynchodemus ceylonicus von Graff.

(Pl. 4, figs. 3, 6–8.—Text-figs. 3, 4.)

Rhynchodemus ceylonicus von Graff (**12**), pp. 499, Pl. xv, figs. 35–38.—Laidlaw (**17**), p. 579.

The material was collected by Mr. Snell in the island of Rodrigues. At a glance it appeared to be identical with Geoplana whartoni described above, as dealt with by the collector, but a closer examination has revealed the fact that this is not so. After some hesitation I have referred it to von Graff's Rh. ceylonicus, which has not been adequately described, as Laidlaw referred a worm from Male Atoll to this species, but with some doubt.

This species appears to be fairly common in this island, as it has been procured in enormous quantities at Grande Montagne and also at Mount Malartic. According to Mr. Snell's statement, it is found under decaying logs, on the bark, under the bark, or in the wood ; the nemertean appeared to exist in far greater quantities than the terrestrial planarians, but these often live together in the same place.

External Characters (Pl. 4, fig. 3).—The body in the preserved state is nearly oval in transverse section, elongate, slender, and for the greater part of a uniform width, though it gradually tapers off towards the anterior and posterior ends, which are bluntly pointed. The ventral surface is made up of the median somewhat raised sole, on which the animal creeps. It extends over almost the whole length of the body and is rather less than one-fourth the width of the body. This species is wholly devoid of any trace of a sensory pit at the anterior tip. In length the animals range from 22 mm. to 45 mm. ; the difference in length depending upon the state of contraction. The 45 mm. specimen was not less than 3 mm. across.

Von Graff is speaking of the coloration of the body as a whole when he states in his description : ' Die Grundfarbe ist lebhaft gelb (sulphureo-citrinus) und der Rücken mit drei sehr kräftigen schwarzbraunen Streifen versehen, von welchen aber die beiden

lateralen mehr als doppelt so breit sind als der mediane. Hinten convergiren die feiner werdenden Längsstreifen, ohne aber zusammenzufliessen, vorne verschwimmen sie in der graubraunen Pigmentirung des nur an der äussersten Spitze farblosen Vorderendes. Eine gleiche Trübung findet sich auch auf der Bauchseite des Vorderkörpers. Sie verschwindet erst gegen die Mitte der Körperlänge und erstreckt sich vom Aussenrande der Seitenstreifen des Rückens bis an die Kriechleiste, in deren Umgebung sie am dunkelsten wird.'

In the specimen I have examined, the dorsal surface is of a uniform orange colour with a slight touch of grey and marked with three fine black longitudinal stripes, comprised of one

Text-fig. 3.

Eyes of Rhynchodemus ceylonicus von Graff.

median and two lateral, these latter converging towards the extremities of the body and meeting the median one. At the anterior end the lines thicken and then coalesce, revealing a dark tip unlike von Graff's form, in which the anterior tip is light. In most instances the lateral lines are much thicker than the median. Sometimes the former get slightly lighter and are less strongly marked than the latter. The ventral surface is much paler than the dorsal, except on the sole, where the colour is nearly white.

The eyes, which are only two in number, occur on either side near the anterior tip of the body.

The mouth-opening which leads into the peripharyngeal chamber lies nearly in the middle of the body, differing from von Graff's form, in which it is situated at the commencement of the posterior fifth of the body. The pharynx in the normal condition is usually completely retracted and hidden within the

peripharyngeal chamber. In some preserved specimens, it was protruded through the mouth-opening as a cylindrical organ of a creamy or white colour.

The common genital aperture is situated about half-way between the mouth-opening and the posterior extremity of the body.

Epidermis.—The specimens had not been preserved in a condition satisfactory for the purpose of minute examination. The epidermis is not of the same thickness all over the body, being thickest on the dorsal surface, gradually becoming thinner as it passes round to the mid-ventral surface. The cilia, though stated by some investigators to exist over the entire surface of the body, in this species are present on the surface of the sole only. Dorsally and laterally the epidermis, as is well known, is made up of closely packed, elongated, columnar cells resting upon a basement membrane, each with an oval nucleus at its base. Apparently wedged in between these cells, except those that are on the head-surface, are found spindle-shaped bodies, the rhabdites, which originate from their mother-cells, scattered in fair abundance in the parenchyma beneath the dermal musculature. In some cases the rhabdites are seen to be in connexion with their mother-cells. Also there are some unicellular glands which open to the exterior here and there. Between the epidermal cells are found some ' gland cells ' with granular contents. These, though having been regarded by Dendy (**9**) as masses of hardened mucus originating from the rhabdite-forming cells, appear to me to be masses of mucus derived from the glandular cells. Except on the surface of the sole the epidermis on the ventral surface is constructed in the same manner as that on the dorsal. Embedded in the parenchyma are unicellular glands, which are much more abundant on the ventral than on the dorsal surface, and these make their way to the surface generally, instead of opening on the ventral surface, more especially submarginally, as they do in some other terrestrial forms as well as in all the freshwater and marine Triclads. The epidermis on the surface of the sole, as has been already indicated, is composed of closely packed,

short, columnar cells, each bearing a large number of short cilia on its outer surface. In no cases have I been able to demonstrate rod-like bodies, wedged in between the cells. Deeply situated in the parenchyma there are enormous quantities of slime glands, which open to the exterior all over the surface of the sole.

Basement Membrane.—The basement membrane, which is in connexion with the epidermis, is distinctly visible as a very thin, structureless, homogeneous layer. It is perforated at various points by the passages of the rhabdite-forming cells and the glands which lie deep down in the parenchyma.

Muscular System.—The musculature of the body, as is well known, is differentiated into two systems, superficial and deep.

The superficial muscular system consists, as usual, of circular, transverse, and longitudinal fibres. Immediately beneath the basement membrane is a thin muscular layer made up of closely apposed circular fibres. The transverse fibres, crossing those of the other set obliquely, are just inside the circular layer. The longitudinal fibres form a thick layer, the external longitudinal layer, which is more strongly developed on the ventral surface than on the dorsal. The muscles appear separated into a series of bands, each made up of a few fibres. Through the intervals between the bands the rhabdites and the glands make their way to the surface.

The deep muscular system, separated from the superficial by a zone of tissue, forms a layer thicker than the latter, and consists principally of two distinct sets of fibres, longitudinal and circular, which occur intermingled in the same mass, without being arranged in definite layers. The longitudinal fibres are more strongly developed than the circular. In addition to these dorso-ventral muscles are found, which run between the branches of the intestine.

Parenchyma.—The tissue filling all the interspaces between the various organs and structures assumes, as usual, the appearance of an irregular network, in the ground substance of which is found a number of nucleated cells of a more or less stellate shape. Embedded in the superficial parts of the dorsal

parenchyma are the fine pigment granules in enormous quantities, which are of an irregular outline and of a dirty olive-like colour. The pigments, though rather few, occur on the ventral side also.

B o d y - g l a n d s.—Situated in the intervening zone between the superficial and deep muscular systems are two distinct kinds of glands, the mother-cells of the rhabdites and the unicellular glands, as already mentioned. On some occasions the mother-cells of the rhabdites have a very stout, horny-looking cell-wall with a greatly elongated narrow tube tapering off into a long process, each of which makes its way between the epidermal cells at various points. Due to the action of reagents, the cells vary in appearance. In some cases there occur such rhabdites as are still contained in the mother-cells.

The rhabdites vary in form and appearance. Some present a slender spindle-like shape, while others are nearly oval in shape. In no cases have I been able to demonstrate the vermiform bodies which were described by Dendy and others. Sometimes the rhabdites appear almost homogeneous, and sometimes finely granular, but I have no doubt that they are all one and the same thing. In some sections the dorsal surface of the worm, outside the epidermic cells, is seen to be partly covered with a layer of hardened mucus which reveals a character quite similar to the rhabdites. They may possibly, by making the animal extremely unpalatable, serve as a protection for its own body, and also help to hold its prey more securely.

Scattered in sparse numbers in the parenchyma are unicellular glands, which have the finely granular contents and open to the exterior at various points of the body-surface, as mentioned above.

Besides those glands there are slime glands which occur deeply embedded in the parenchyma along the median plane of the body and open out on the surface of the sole. They occur in enormous quantities, and are distinguished from the glands opening out over the whole surface of the body by a closer affinity for borax carmine. In the terrestrial planarians the movements are effected by the action of cilia in mucus which is

constantly being secreted in greater or less quantities, and gives rise to a thin layer between the ventral surface of the body and the substratum. In this case rhythmical wavy motions of the muscles stand, of course, in intimate relation to the movements.

Digestive System.—The mouth-opening, which lies nearly in the centre of the body, leads, as usual, into the wide peripharyngeal cavity with the pharynx horizontally disposed. The cavity is lined with a single layer of epithelial cells made up of pear-shaped cells of a glandular nature, as has been stated by Dendy in G. spenceri. The epithelium rests upon a fine basement membrane, beneath which are two layers of circular and longitudinal muscular fibres. Situated in the parenchyma around the cavity are unicellular glands which open into the cavity

The pharynx is a short, tubular body of a cylindrical shape, which arises from the dorso-anterior wall of the peripharyngeal cavity, with its free end posteriorly directed. The outermost layer of the wall is represented by a very thin, richly ciliated epithelium, immediately beneath which come, as usual, two thin layers of external longitudinal and internal circular muscles. The circular layer is followed, after an interval in which glandular and nervous tissues exist, by a very thick layer of longitudinal muscular fibres. Just external to this layer comes a layer of circular fibres, immediately surrounding the lumen of the pharynx, which is lined by a single layer of non-ciliated cells. Besides the muscles mentioned above, there are found a number of radial fibres, running from the inner circular layer towards the outside.

The lumen of the pharynx leads anteriorly into the intestinal canal, which is of the triclad type. The anterior trunk extends to a point above the brain and usually gives off on each side numerous lateral branches, which are sometimes bifurcated and sometimes trifurcated. The posterior trunks proceed backwards nearly to the hind end of the body, one on each side of the middle line, and are provided with numerous outwardly directed, subdivided branches. The wall of the intestine is a single epithelium made up of high cylindrical cells, which are

placed very closely together and rest on the surrounding tissue. The cells, each with an oval nucleus in its basal portion, contain a great number of coarse, highly refractive granules in the finely granular protoplasm. In some cases the cells were observed to be vacuolated in the distal portion of the cell. So far as I have observed, any special glandular cells are altogether absent in the epithelium.

Nervous System.—The brain is a bilobed organ, situated at the anterior end of the body between the ventral wall and the anterior termination of the intestinal canal. From the brain-mass arise numerous nerves which are distributed over the various parts of the anterior end of the body. But their arrangements were not clearly made out. Each half of the organ is formed of a very finely granular ground-substance, in which small nerve cells occur much more abundantly towards the periphery than in the central part. At various points the mass is perforated by fine muscular fibres in the dorso-ventral direction.

Each half of the brain-mass is continuous posteriorly with one of the longitudinal nerve cords, which proceed straight backwards, until finally they join together at the posterior end of the body. The cords themselves are very thick and usually present, in cross-section, the characteristic spongy or finely reticulate appearance. Small nerve cells are scattered in sparse numbers in the substance of the cords. Throughout their entire course the longitudinal nerve cords are connected by very numerous transverse commissures. Laterally they give off numerous branches towards the nerve plexus, which lies beneath the outer longitudinal muscles of the body and extends completely round the body. The plexus consists of a close network of fine fibres.

Eyes (Pl. 4, fig. 6).—The only special sense-organs which I have seen in the present species are the eyes. Each consists, as usual, of a pigment cup and of numerous visual rods. The pigment cup is of a bell-like shape with its opening directed outwards and upwards, and is as usual formed of very minute, closely packed, spherical granules, of a dark-brown colour.

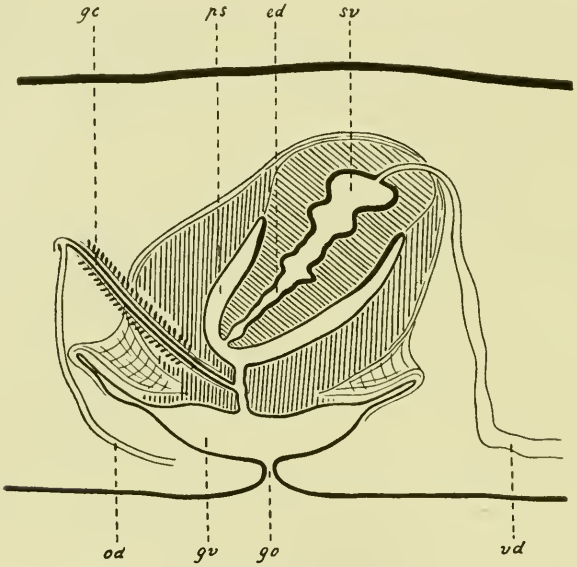
Enclosed in the cup is a mass of visual rods, the outer extremity of which projects for a short distance beyond the margin of the pigment cup. Between the pigment cup and the mass of the visual rods, and also just in front of the outer surface of the rods, small spaces are visible, doubtless caused by shrinkage of the tissues. Each rod is an elongated, faintly staining, very finely granular body, which at the periphery shows a closer affinity for borax carmine than in the central part. In front of the opening is a collection of nervous matter, viz. granular substance and fibres surrounded by numerous cells, apparently belonging to nerve cells. The fibres pass over into the cavity of the pigment cup, but how the nerves stand in connexion with the visual rods I was unable to determine.

Reproductive Organs (Pl. 4, figs. 7, 8).—The common genital aperture, lying nearly mid-way between the mouth-opening and the posterior extremity of the body, leads into the wide, annularly outbulged vestibulum, which receives the opening of the penis-sheath from above. Both the vestibulum and the penis-sheath are lined with a single epithelium resting upon a fine basement membrane, beneath which are found circular and longitudinal muscular layers. Especially around the penis-sheath the muscular layer presents a thick, compact mass, which chiefly consists of circular fibres and is continuous with that of the penis. In the diaphragmatic part between both the cavities just mentioned the radial muscular fibres are present in a strongly developed condition.

Male Organs.—Numerous follicular testes are placed close together in the ventral parts of the body, arranged in a single row on either side of the anterior main gut trunk, just on the dorso-lateral side of the longitudinal nerve cord. The row begins on each side slightly behind the ovary, and extends backwards nearly to the insertion of the pharynx. Each testis, of an oval shape, is made up of sperm-mother-cells and spermatozoa in all stages of development, surrounded by the tunica propria. In contact with the epithelium accumulations of the mother-cells occur, which contain very large, deeply staining, highly granular nuclei. In the cavity of the testis, and separated

from the accumulations of the mother-cells lie some compact masses of metamorphosing spermatozoa. The spermatoblast in a further stage of development presents an elongated, pear-shaped protoplasmic body, in the broad end of which the nucleus is visible as a distinct, deeply staining spot. It is then changed into a spermatozoon, the nucleus forming the head and

Text-fig. 4.



Diagrammatic representation of the genital organs of Rh. ceylonicus von Graff. *gv.* = genital vestibulum. Other letters as in Text-fig. 1.

the protoplasm having greatly stretched out and elongated itself into a thin thread to form the tail of the spermatozoon.

Each testis gives rise, on its lower side, to a short canal which communicates soon with the vas deferens. The vasa deferentia, proceeding backwards close along the dorsal sides of the longitudinal nerve cords, rise obliquely upwards to enter, each separately, the bulbous part of the penis at the upper lateral sides, and finally open into the lumen of the penis or the seminal vesicle. The vas deferens, which is filled with spermatozoa, is

lined by a thin, flattened epithelium of nucleated cells resting upon a basement membrane.

The penis consists of two parts, viz. the free, conical intromittent part lying subvertically in the penis-sheath, and the bulbous basal part of muscular nature. Enclosed in the latter part is a wide cavity of somewhat irregular contour, the seminal vesicle, into the anterior extremity of which open the vasa deferentia ; posteriorly this is continuous with the ejaculatory duct which opens into the penis-sheath at the tip of the penis. The cavity is lined by a layer of columnar glandular cells, beneath which is a circular muscular layer. Embedded in the parenchyma of the penis are numerous glands which open into the seminal vesicle and the ejaculatory duct. Externally the penis is covered with a thin epithelium which becomes thicker towards the proximal portion, and at the same time is provided with cilia. The epithelium surrounds a muscular layer consisting of external, thick, circular, and internal, thin, longitudinal fibres. On some occasions the penis at the proximal parts gives rise to special processes which are covered with an epithelium made up of ciliated, columnar cells.

Female Organs.—The paired ovary is situated far behind the brain, one on either side close to the dorso-lateral side of the longitudinal nerve cord. Each ovary is nearly oval in shape, and its cavity is lined with a thin epithelium, composed apparently of a single layer of flattened cells. In the interior of the ovary, ova in various stages of development are met with. Occupying the periphery of the ovary occur numerous young ova, each with an oval, large, and highly granular nucleus. In the successive stages of development the ovum assumes a nearly spindle-like shape, as has been mentioned by Dendy. The large nucleus sometimes shows a very distinct chromatin network. Situated in the central and lower regions of the ovary are the ripe ova, which present a round shape and enclose a very large nucleus, revealing a transparent, vesicular aspect.

The vitelline glands are represented by irregularly ramified masses of cells, which are extensively distributed in the interstices between the diverticulae of the intestinal trunk and stand

at many points in connexion with the oviduct. The vitelline glands consist of large round cells closely packed, each of which contains a highly granular nucleus and highly refractive protoplasmic bodies. Probably, at the time when the ova are passing down, the cells break down and make their way into the oviduct. They are considered to take part in connexion with the nutrition of the ova and also with the formation of the cocoon capsule.

The oviduct arises from the mid-ventral aspect of the ovary as a wide passage ; this soon assumes the character of a narrow canal, which proceeds straight backwards, just along the outside of the nerve cord. In the region of the genital opening the oviduct nears the median line, rising upwards at the same time, and finally unites with its fellow of the opposite side, at a point behind the penis, to form the rather wide glandular canal. The oviduct shows a distinct lumen along its entire length. Its actual wall is made up of a layer of distinctly nucleated columnar cells, with well-developed cilia projecting into the lumen of the oviduct. Immediately external to the layer mentioned comes a layer of circular muscular fibres.

As already indicated, the oviduct receives the vitelline glands at several points of its course. The mode of connexion seems nearly similar to that described by Moseley (22), Dendy, von Graff, and others, in several forms. The glands stand in communication with the oviducts by means of the short branches of the latter, which are situated at tolerably regular intervals.

The glandular canal, mentioned above, runs anteriorly and obliquely downwards to open from behind into the atrial passage, between the penis-sheath and the vestibulum. The canal is constructed in the same manner as the oviduct, and is lined with an epithelium made up of ciliated columnar cells resting upon a fine basement membrane, beneath which exists a muscular layer composed of circular and longitudinal fibres. Numerous glands are found all round the canal, into which they open.

The present species is wholly devoid of any trace of the organ representing the seminal receptacle. As already indicated, the

vestibulum is supplied with an annular outbulging, which extends more deeply backwards than forwards. To me, this outbulging appears to serve as a seminal receptacle during copulation.

Amblyplana trifuscolineata, n. sp.

(Pl. 4, figs. 4, 5.—Text-figs. 5, 6.)

This new species is represented by a single specimen which was taken by Mr. Thomasset under a half-rotten log in the island of Mauritius.

External Characters (Pl. 4, figs. 4, 5).—The body, which is nearly circular in cross-section, is rounded at the posterior end, and has the lateral margins even and nearly parallel for a large part of its length, but tapering in front to the bluntly pointed extremity. The sole corresponds nearly to one-third the width of the body, extending to both extremities. It measures 25 mm. long by about 3 mm. across in the broadest part.

In coloration this species nearly resembles Geba's Amblyplana tristriata, described by that author from the Comoro Island. The dorsal surface is of a dark colour with a touch of olive-like brown, and marked with three longitudinal black stripes, a median and a pair of laterals, the latter converging towards the extremities of the body, without coalescing. Ventrally, the colour is similar to that of the dorsal side, except for the creeping surface which is pale, while each side of it has a diffused brownish black tinge.

Near the anterior tip of the body lie the eyes, one on each side, as shown in Text-fig. 5.

The mouth-opening, which leads into the peripharyngeal chamber, is situated at a short distance behind the centre of the body. I could make out its position by a slight protrusion of the pharynx.

The common genital opening lies at the hind end of the first third of the distance from the mouth-opening to the posterior extremity of the body.

Epidermis and Body-glands.—The epidermis consists, as usual, of a layer of columnar cells, which are of a greater height on the dorsal than on the ventral side. Wedged in between these cells, except on the ventral surface, are spindle-like rhabdites which appear almost homogeneous. In some sections they are seen to be discharged on to the exterior, revealing a layer of hardened mucus over the epidermis. The rhabdites enclosed in the subcutaneous cells occur widely

Text-fig. 5.

Eyes of Amblyplana trifuseolineata, n. sp.

distributed on the dorsal side of the body. In addition to the glands deeply situated in the middle of the body and opening to the exterior on the surface of the sole, there are some glands which open in scattered distribution all over the ventral surface.

Muscular System.—Immediately beneath the fairly well-developed basement membrane is the superficial muscular system composed of the outer circular and the inner longitudinal layers. The deep muscular system, which chiefly consists of longitudinal fibres, is well developed all round in the parenchyma as a thick and continuous sheet surrounding the intestine and the nerve cords.

Digestive System.—The mouth-opening is placed at about the centre of the peripharyngeal chamber, in which is disposed the pharynx of a cylindrical shape. It is conically pointed at the free end. The gut trunks are provided with numerous subdivided branches, the epithelium of which presents no noteworthy features, consisting, as it does, of high columnar cells.

Reproductive Organs.—The genital apparatus is nearly similar in appearance to that of Am. tristriata Geba. The genital opening leads into the vestibulum, which forms an

oblique upwardly directed, annular outbulging, and which receives the penis-sheath from above. The vestibulum has a wall consisting of a single epithelium and a muscular layer, while the penis-sheath is lined with a ciliated epithelium, outside which is a thick muscular coating, chiefly composed of circular fibres.

Male Organs.—The numerous testes, containing spermatozoa in several stages of development, are arranged in a row on each side of the body close to the upper side of the longitudinal nerve cords, extending from behind the ovary to the insertion of the pharynx. The vasa deferentia run backwards, just along the inside of the nerve cords. Shortly in front of the penis they gradually bend inwards and upwards, finally to open as a rule separately into a moderately wide seminal vesicle. The vas deferens shows a definite wall consisting of a thin epithelium and a feeble muscular layer of circular fibres.

The penis is a conical body, hanging from above subvertically in the pear-shaped penis-sheath, and encloses a cavity, the seminal vesicle, which gives rise to the ejaculatory duct, opening into the sheath at the tip of the penis. The vesicle is coated internally with a thick glandular epithelium, which projects into the lumen of the organ in folds. Embedded in the body-parenchyma around the penis-bulb are numerous glands, the ducts of which enter the penis at the base and open into the penis-sheath over the surface of it.

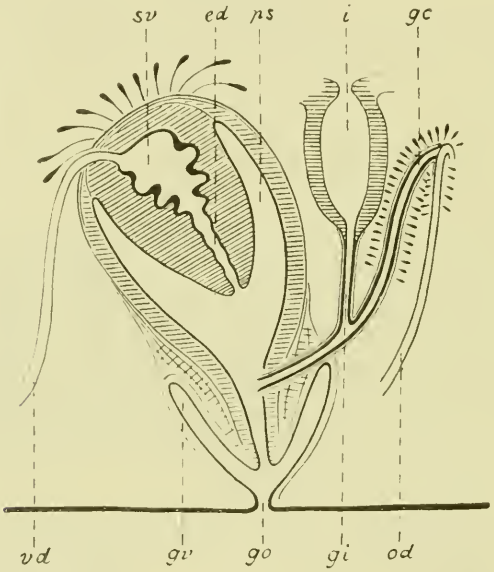
Female Organs.—I am unable to give an account of the ovary, as I have been reluctant to sacrifice the anterior half of the body to the microtome. Probably the paired ovary occurs in the usual manner. The vitelline glands, which are composed of large cells closely packed, extensively fill up the interstices between the gut diverticulae. They are in connexion with the oviduct at numerous points by means of a short cylindrical duct.

The oviducts lie close to the dorso-lateral side of the nerve cords, one on each side, in which position they proceed straight backwards, receiving the contents of many vitelline glands. Behind the genital opening they near the median line, slightly

rising at the same time, and finally join into a single median duct, the glandular canal. The oviduct is characterized by the possession of ciliated epithelial cells, beneath which comes a thin muscular coating, and between which open numerous glands for some little distance before forming a common duct.

The glandular canal pursues a course obliquely forwards and downwards, and finally opens into the vestibulum at a point on the right side, after receiving in its course a short duct from

Text-fig. 6.



Genital organs of Am. trifuscolineata in sagittal section, diagrammatically shown. *gi.*=genito-intestinal canal. *i.*= intestine. Other letters as in Text-figs. 1 and 4.

above, which stands in communication with one of the intestinal coeca, so that there is, as in the Heterocotylean Trematodes, a genito-intestinal canal. This is similar to that described by Geba (**11**) in Am. tristriata and Am. mediostriata. The canals are constructed in the same manner as the oviduct, and are lined with an epithelium composed of ciliated columnar cells; outside this is a thin muscular layer.

The intestinal coecum is coated internally with an epithelium made up, as usual, of high columnar cells, which near the junction point of the canal exhibit a close affinity for borax carmine ; in the cavity are contained spermatozoa enveloped in a coagulum of the secretion. This organ seems to me to serve as a seminal receptacle.

As stated above, the present species closely resembles Am. tristriata described by Geba. But it differs from this in the arrangement of the parts of the genital organ.

Note upon the Canal connecting the Female Genital Organ with the Intestine.

The peculiar canal connecting the female genital organ with the intestine is of somewhat frequent occurrence in other terrestrial planarians, as is the case with Rhynchodemus terrestris Müll., Rh. attemsi Bendl, Pelmatoplana mahéensis von Graff, and P. braueri von Graff. In Rh. terrestris, according to von Graff (**12**), the two ducts, one on each side, spring from the anterior parts of the seminal receptacle and take a course obliquely upwards and backwards, finally opening into the posterior trunk of either side. But these connexions appear to be inconstant in occurrence and arrangement, for on some occasions there exists, according to Bendl (**2**, **3**, **5**), a right connexion only, which is well developed. He has also placed on record a case of Rh. attemsi, in which the receptacle is in direct connexion with the left posterior trunk of the intestine, without passing by any distinct duct. According to Mell (**20**), the vagina in both P. mahéensis and P. braueri is continuous with a canal which communicates with the right posterior trunk of the intestine.

An arrangement of this kind is also known to occur in other Turbellarian groups. Such are Oersted's Phaenocora unipunctata, an Acoela (**4**, **5**), and Haswell's Enterogonia pigrans, a Polyclad (**13**, **14**). In the former the receptacle communicates with the intestine by a short median duct, while in the latter the dorsal passage of the vagina, after receiving on

its ventral side the common duct formed by union of the lateral uterine ducts, proceeds backwards as a narrow tube, which opens into the median posterior branch of the intestine. To me, such frequent occurrence of the genito-intestinal connexion appears in favour of the view that this is certainly not abnormal. The discovery of the canal in question helps to connect more definitely the seminal receptacle of some Polyclads and Triclads with parts that occur in other Platodes. It cannot well be doubted, it seems to me, that this canal corresponds to the similarly named canal in the Heterocotylean Trematodes. In this group the duct passes from the oviduct, opposite the opening of the yolk-duct, to the right limb of the intestine.

Now let us proceed to review the arrangement of the terminal part of the female genital organ, which is of interest from the morphological point of view. The vaginal canal, after almost invariably receiving the unpaired common uterine duct, either ends blindly, as in Stylochus and some others, or proceeds backwards to join the seminal receptacle, as in some Triclads, which is unpaired in most, but paired in some, genera (Discocelis, Woodworthia, Shelfordia, and Diplosolenia). This agrees closely with the condition of the duct found in the Aspidocotylean Trematodes, which are provided with a duct, arising from the oviduct, near or opposite the opening of the yolk-duct and leading to the vitelline receptacle.

On some occasions the dorsal passage of the vagina, instead of swelling into a receptacle and opening into one of the intestinal coeca, pursues a course backwards, finally to open to the exterior at a certain point of the surface of the body. In Cryptophallus and Bergendalia it proceeds backwards and downwards, describing an arched course, and finally opens into the female atrium closely behind the vaginal aperture and just inside the external female aperture. In the case of Trigonoporus, Copidoplana, and Tripylocelis the duct terminates behind on the ventral surface of the body by the second female aperture. In Polyporus the second female opening lies near the hind end of the body, while in Laidlawia it occurs, occupying a position on the dorsal, but not on the

ventral, surface. Such an opening dorsally situated is also known to occur in Acoelean forms, such as Cylindrostoma quadrioculatum Jens, and C. klostermanni Jens.

The discovery of Laidlawia (**15**) mentioned above may be regarded as of some importance, as it may constitute an additional link in the chain of evidence against the homology of the part of the duct, as has been suggested by Lang (**18**). He, in his monograph, has the following passage : ' In morphologischer Beziehung erinnert der Canal, insofern er eine Verbindung zwischen der Einmündungsstelle des Uterus in den Eiergang einerseits und der Aussenwelt anderseits darstellt, einigermassen an den Laurerschen Canal der Trematoden und Cestoden.' A comparison with the Laurer's canal of the Malacocotylean Trematodes, which passes up from the oviduct, in the neighbourhood of the ootype, and opens by a minute pore on the dorsal surface, obviously suggests itself.

Great interest is attached to the existence of some Polyclads having the dorsal passage of the vagina, which opens either to the exterior on the surface of the body, or into one of the intestinal coeca, as stated above. The homology between the genito-intestinal canal of the Heterocotylea, the Laurer's canal of the Malacocotylea, and the duct leading to the receptacle in the Aspidocotylea, though it may be open to question, seems to have the balance of evidence in its favour. Haswell (**14**) has put forward the view that there can be regarded as representing Laurer's canal in the Polyclads not only the genito-intestinal canal of Enterogonia, but the seminal receptacle of the Acotylea in general and the posterior female passage, which opens to the exterior, as has been observed in some forms. I am inclined not only to concur with him, but further to develop to a certain extent this view even to the Triclads. In this communication, however, I have intentionally abstained from making any such attempt, leaving the problem to future consideration.

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EXPLANATION OF PLATE 4.

Fig. 1.—Placocephalus isabellinus Ceba in the preserved state, seen from the dorsal side. About natural size.

Fig. 2.—Ditto. Ventral view.

Fig. 3.—Rhynchodemus ceylonicus von Graff in the preserved state, seen from the dorsal side. About 1·5 ×.

Fig. 4.—Amblyplana trifuscolineata, n. sp. in the preserved state, seen from the dorsal side. About 2 ×.

Fig. 5.—Ditto. Ventral aspect.

Fig. 6.—Rh. ceylonicus. Longitudinal section of an eye.

Fig. 7.—Ditto. Transverse section through the ovarian region.

Fig. 8.—Ditto. Median sagittal section through the region of the copulatory organs.

Abbreviations used in the Explanation of Plate.

ed. = ejaculatory duct. *gc.* = glandular canal. *gv.* = genital vestibulum. *i.* = intestine. *ln.* = longitudinal nerve cord. *n.* = nerve. *od.* = oviduct. *ov.* = ovary. *p.* = pigment. *ps.* = penis-sheath. *s.* = sole. *sv.* = seminal vesicle. *vd.* = vas deferens. *vr.* = visual rod.

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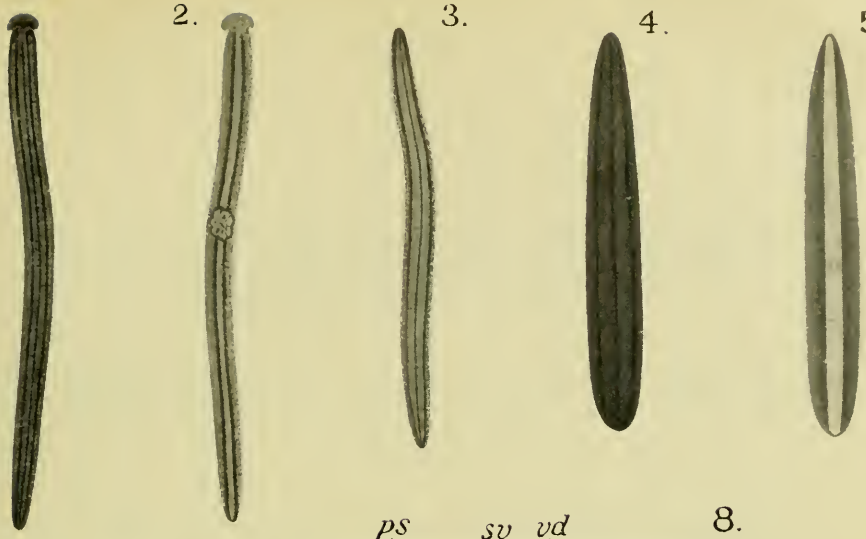

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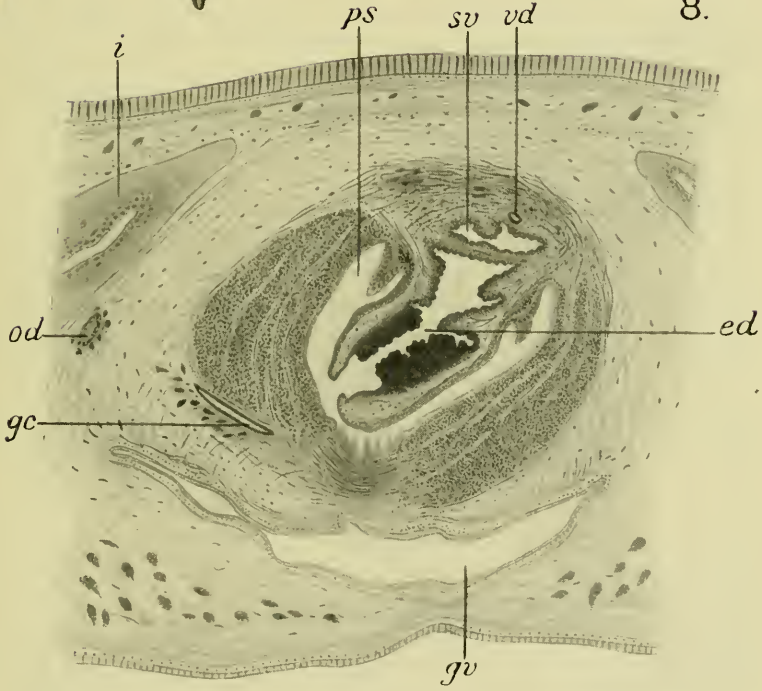

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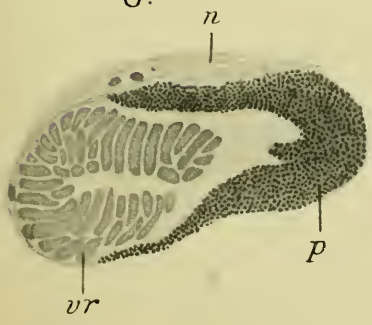

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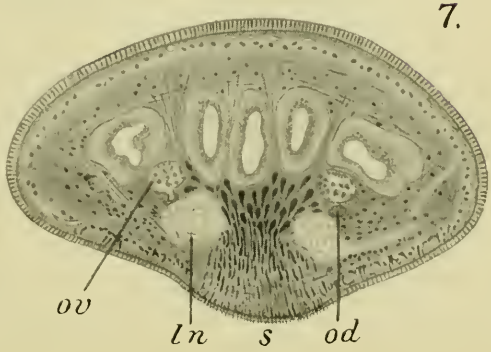

Kaburaki del.

Gonospora minchinii, n. sp., a Gregarine inhabiting the egg of Arenicola.

By

Edwin S. Goodrich, F.R.S., and H. L. M. Pixell Goodrich, D.Sc.

With Plates 5 and 6.

When examining the contents of the coelom of an Arenicola ecaudata Johnston, at the Marine Biological Laboratory in Plymouth last winter, we discovered a new Gregarine of considerable interest, since it appears to be the first instance on record of such a parasite inhabiting the ovum of its host.[1]

This gregarine belongs to the genus Gonospora. It does not seem to occur at all in the male worm, and of the females examined only about 30 per cent. were infected. However, since the parasite was not found in any but female worms whose ovaries were fairly ripe and had begun to shed their products into the coelom, it is probable that it often inhabits less mature hosts, but in some situation not yet determined. We have looked for it without success in the immature ovary. Frequently it occurs simultaneously with the larger and well-known coelomic gregarine Gonospora (Kalpidorhynchus) arenicolae Cunningham.

The immature ovary of Arenicola ecaudata is a lobulated organ with finger-shaped processes (see Gamble and Ashworth, 1). Inside it the germ-cells multiply, accumulating in its lumen, and later bursting through its wall. The ova thus escape into the coelom at various stages in development; some quite small and oval, others larger, more rounded, loaded with yolk, and surrounded by a thick covering. This shell is formed of two

[1] Since this was written we have learnt from Sir Ray Lankester that many years ago he discovered a somewhat similar parasite in the eggs of Thalassema. From an inspection of some unpublished drawings of the trophozoite, which he kindly sent to us, we conclude that it is not the Gregarine described in this paper.

distinct layers : an outer thin refringent membrane, the original vitelline membrane ; and an inner much thicker and probably less dense perivitelline layer (fig. 10). A full-grown ovum with its covering is about 120 to 130 microns in longest diameter.

Young Trophozoites.—The youngest stages of the parasite observed were small rounded trophozoites embedded in the egg close to its nucleus. Fig. 1 shows such a stage where the gregarine is 12 μ in diameter ; far smaller than the nucleus of the immature egg it has invaded, and indeed only about twice the diameter of its nucleolus. It will be noticed that even at this early stage the nucleus of the parasite is distinguished from that of the ovum by the possession of two karyosomes, while the latter is almost invariably provided with only one nucleolus. The trophozoite continues to grow at the expense of the egg, enlarging and becoming stored with granules of paraglycogen (figs. 2, 3). As it acquires the shape and size of the adult (fig. 7) the egg and its nucleus become more and more compressed against the surrounding membranes.

Penetration into the egg.—It has been stated above that the ovum of Arenicola is protected not only by a vitelline membrane, but also when full-grown by a thick perivitelline layer. How does the parasite penetrate into the egg ? is a question which at once suggests itself. Now it is probable that fully-developed eggs are safe from invasion, since infected eggs are rarely, if ever, found with the perivitelline layer fully formed. By far the greater number of eggs infected are provided with a vitelline membrane only (figs. 4, 7), or with but a thin perivitelline layer as well (figs. 6, 11). The parasite enters the egg by boring a round hole through these membranes, and usually the margin of the hole is found turned inwards (figs. 2, 4). The aperture so formed may remain open ; but sometimes it seems to close up almost entirely (fig. 6), presumably when the egg is invaded at a very young stage.

Position and growth of trophozoite in eggs.—It is often very difficult to decide whether the parasite, having pierced the egg-membranes, really enters the egg-cell or merely bulges into it. Except perhaps in the very earliest stages it

certainly lies as a rule outside the egg-cell, between it and the membranes (figs. 2, 4). It compresses the egg more and more as it grows and is separated from it by a space, except at that one region opposite the point of entrance where the epimerite of the parasite adheres closely to the egg-cytoplasm near the germinal vesicle (fig. 9). Here are developed, in that part of the gregarine which is fixed to its host, small club-shaped bodies staining deeply in haematoxylin or fuchsin. They appear to be hollow, with long narrow necks reaching to the surface (fig. 9). These strange structures somewhat resemble the 'lamelles mucoïdes' described by Léger and Duboscq in Nina (2); but their function would appear to be connected with the absorption of nutriment from the egg, or possibly merely with fixation. Meanwhile, as the parasite grows it enlarges the deep depression it causes in the egg; the margin of this hollow is at first smooth (fig. 2), it soon becomes notched, and finally drawn out into delicate protoplasmic processes converging towards the point of entrance (figs. 3, 4, 5).

Effect of parasite on host egg.—The very young ovum has little or no yolk; but with advancing age the yolk granules increase in number until the fully-developed egg becomes so heavily loaded that it looks quite opaque. In parasitized eggs, however, the yolk is absorbed by the gregarine almost as fast as it is laid down, so that in late stages the compressed ovum is relatively clear, while the parasite on the contrary is densely granular (fig. 4). The nucleus of the egg is also influenced, for its nucleolus, instead of undergoing the orderly series of changes seen to occur in normal eggs, lags behind in differentiation, remaining in fact apparently at that stage of development it had reached when the egg was invaded. Thus the nucleolus in most parasitized ova resembles that of the quite young ovum when it is still small and has but little yolk (figs. 2, 7).

Another peculiar and somewhat similar effect is seen on the egg-envelopes. There is no reason to think that the perivitelline layer when once formed can be reabsorbed, and since it is, as a rule, almost or quite absent from parasitized eggs,

even when these have reached full size, there can be little doubt that the presence of the gregarine checks its deposition. Never have we observed full-sized eggs without parasites in which this layer was not present.

Emergence of parasite from egg.—When the trophozoite has completed its growth it emerges from the egg-shell by a round hole, which is probably the enlarged original opening through which it entered, or at least formed afresh in the same place (figs. 5, 8). The gregarine first pushes out its pointed ' tail ' end, the rest of the body following after.

Fate of parasitized egg.—As soon as the parasite has thus abandoned the egg, leaving a large space partially surrounded by the emaciated host-cell and communicating with the exterior by an aperture of considerable size, leucocytes from the coelomic fluid make their way in (figs. 8, 11, 12). They gather in large numbers in the cavity, and proceed to attack the already depleted ovum, the cytoplasm of which becomes vacuolated. Strange thread-like structures, which stain in acid-fuchsin, are now visible round the edge of the egg (*th.*, fig. 12) before its final breaking up.

The free trophozoite.—The full-grown trophozoite free in the coelomic fluid is usually pear-shaped, the epimerite being at the blunt end. As a rule the nucleus is provided with two conspicuous karyosomes, but additional small granules may be present. Often the gregarines hang together in groups, sometimes in masses of ten or twelve individuals.

Association and spore-formation.—The association of two trophozoites is terminal (fig. 13), the ' head ' end of one penetrating deeply into that of the other in the manner so characteristic of the genus Gonospora (3, 4). At the extremity of the embedded epimerite may be seen in sections a cap of dense substance tipped with a deeply-staining granule, possibly of nuclear origin (fig. 14). At this stage, before the formation of a cyst, the two associates can still be separated by pressure. As soon as the cyst wall is secreted round the pair their opposed faces flatten out. Gamete formation and syngamy then take place as usual in these gregarines.

A spore with its eight sporozoites is shown in fig. 15 ; it is from 8 to 10 μ in length. The sporocyst is thin, one pole being rounded and the other provided with a slight thickening, but there is no well-developed funnel such as occurs in Gonospora glycerae (**3**).

For this new gregarine we propose the name Gonospora minchinii.

Summary.—The new species of gregarine described above, and to which we have given the name Gonospora minchinii, occurs in the coelomic fluid of the female Arenicola ecaudata. The adult trophozoite is pear-shaped, and the ripe spore has a thin cyst without distinct funnel. The young trophozoite lives in the egg floating in the coelomic fluid of the Arenicola, where it grows at the expense of the food-material stored in the ovum. To reach the ovum it pierces the vitelline membrane and perivitelline layer. The growing trophozoite occupies a deep depression it causes in the egg, to which it adheres by its epimerite. The margin of this depression becomes drawn out into delicate protoplasmic processes. The cytoplasm and nucleus of the host-cell, and also the development of the perivitelline layer, are affected by the presence of the parasite. When full-grown the trophozoite escapes from the egg by a hole pierced in its envelopes, and leucocytes then enter the space so left to complete the destruction of the ovum.

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EXPLANATION OF PLATES 5 AND 6.

Fig. 1.—Young egg of Arenicola with small trophozoite inside it. Whole preparation ; Formol-iodine, Paracarmine. × 500.

Fig. 2.—Later stage showing opening in vitelline membrane, and depression in egg in which lies the parasite. Whole preparation ; Formol-corrosive, Paracarmine. × 500.

Fig. 3.—Nearly full-grown parasite in egg ; from the living. × 500.

Fig. 4.—Semi-diagrammatic optical section of egg with contained parasite. × 500.

Fig. 5.—Trophozoite emerging from egg ; from the living × 500.

Fig. 6.—Portion of a section of an infected egg showing the young trophozoite. Bouin, Iron-Haematoxylin. × 1,100.

Fig. 7.—Optical section of whole preparation of egg with full-grown trophozoite. Formol-corrosive-acetic, Paracarmine. × 500.

Fig. 8.—Infected egg from which the parasite has escaped. Leucocytes are making their way into the cavity. From the living. × 500.

Fig. 9.—Portion of a section of full-grown trophozoite which is fixed to host-cell near flattened nucleus, and showing deeply-staining bodies, *a.* Chrom-osmic : Iron-haemat., Light-green. × 1,100.

Fig. 10.—Part of section of uninfected egg, showing normal development of vitelline and subvitelline membranes. Bouin, Iron-haemat. × 1,100.

Fig. 11.—Section of an infected egg from which parasite has escaped. Chrom-osmic ; Iron-haemat. × 500.

Fig. 12.—Similar egg at later stage showing its destruction by invading leucocytes. Chrom-osmic, Iron-haemat., Light-green.

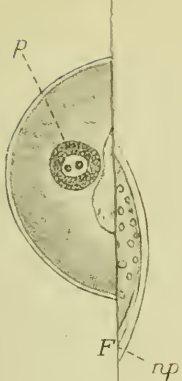
Fig. 13.—Two Gonospora minchinii in association. Whole preparation. × 120.

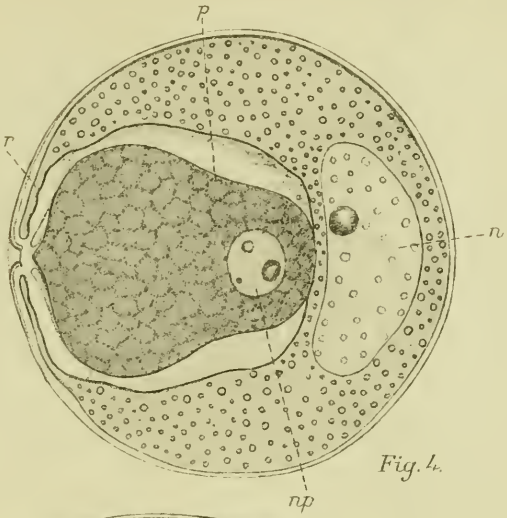
Fig. 14.—Section through dovetailing epimerites of associates. Chrom-osmic ; Iron-haemat., Light-green. × 1,100.

Fig. 15.—Spore with eight sporozoites. × 3,000.

Reference Letters.

a. = deeply-staining bodies at edge of trophozoite fixed to ovum. *c.* = cytoplasm of ovum. *cp.* = cytoplasm of parasite. *l.* = leucocyte in cavity vacated by parasite. *lt.* = limit between associated trophozoites. *mp.* = minute pore, probably contracted pore of entrance. *n.* = nucleus of egg. *nc.* = nucleolus. *np.* = nucleus of parasite. *o.* = ovum. *op.* = opening. *p.* = parasite. p^1 and p^2. = associates. *pr.* = protoplasmic process. *sp.* = space left by parasite. *sv.* = perivitelline layer. *t.* = 'tail' end of trophozoite *th.* = threadlike structures in outer zone of egg. *v.* = vitelline membrane





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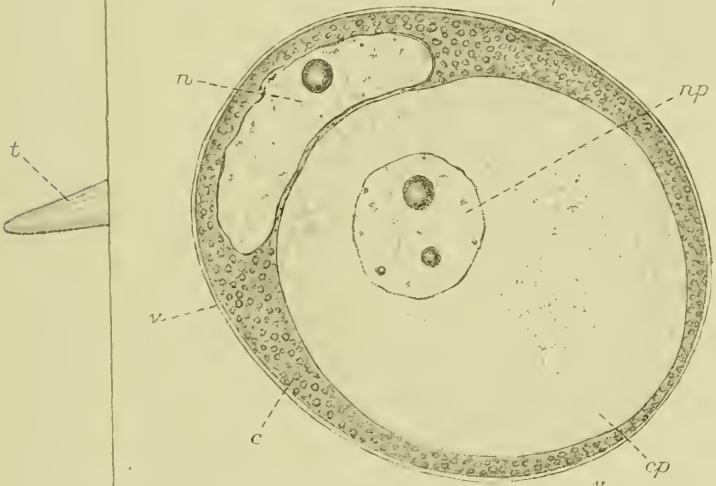

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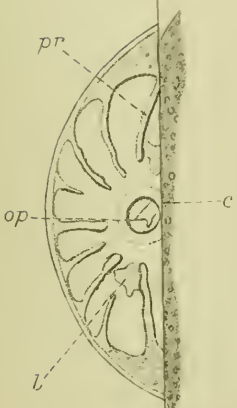

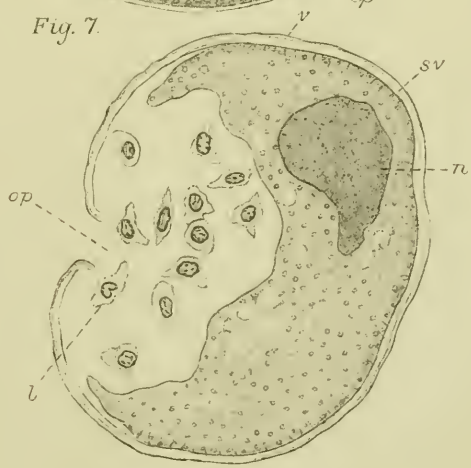

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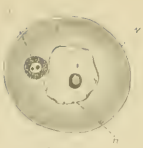
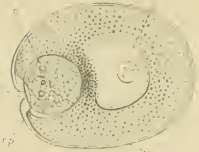


Fig 1



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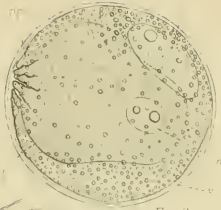


Fig 3

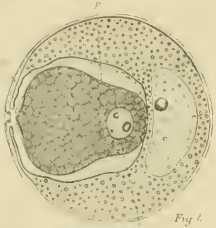


Fig 4

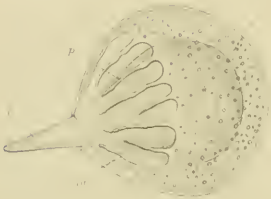


Fig 5

Fig 6

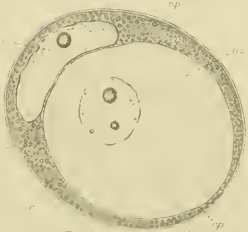


Fig 7

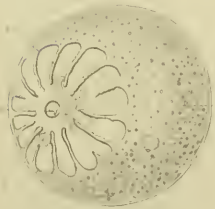


Fig 8

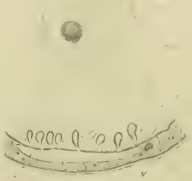


Fig 9

Fig 10

Fig 11

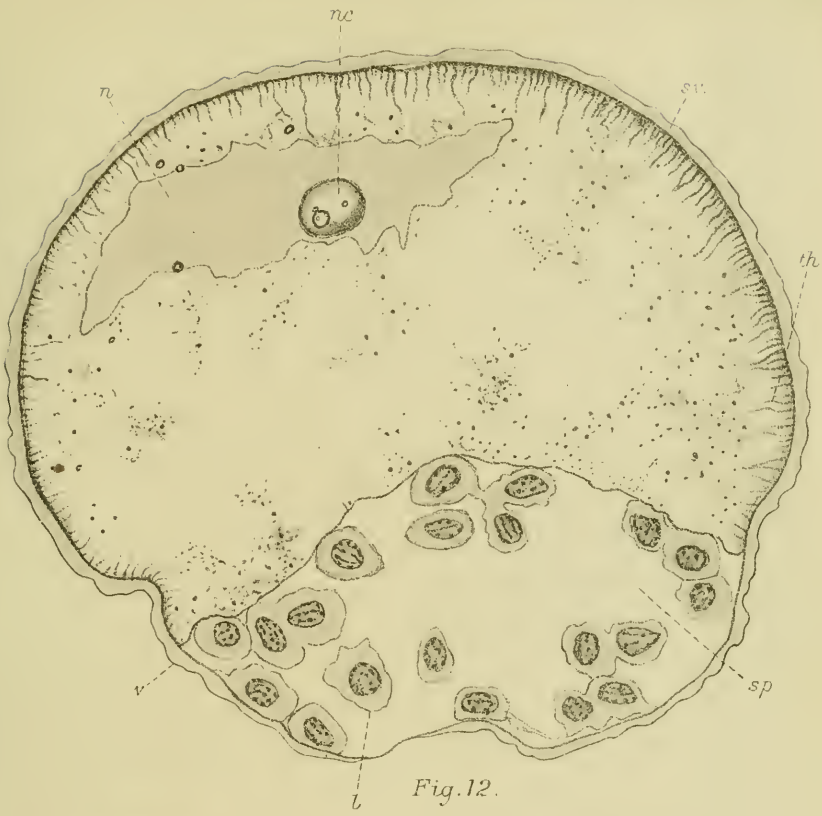


Fig. 12.

Fig. 13.

Fig. 15.

Fig. 14.

The Eye of Peripatus.

By

William J. Dakin, D.Sc., F.L.S., F.Z.S.,

Derby Professor of Zoology, University of Liverpool;
late Professor of Biology in the University of Western Australia.

With Plate 7 and 3 Text-figures.

The first description of the minute structure of the Eye of Peripatus was given by Balfour (1) in his memorable paper on the anatomy of Peripatus capensis. So far as I am aware nothing has been added to our knowledge of the structure since that date, despite the advances in microscopical technique, and the rather thorough investigation of invertebrate visual organs. Other arthropod eyes have received considerable attention, and this seems strange at first because a comparison of the Peripatus eye with that of other arthropods should be highly interesting by reason of the phyletic position occupied by the Onychophora.

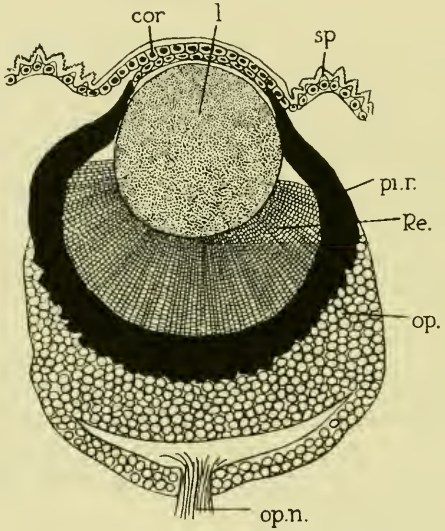
The development of the eye was followed by Sedgwick (4), but nothing was added to the previous knowledge of the structure of the adult eye, although the origin of the different parts was very clearly shown.

In Balfour's illustration, the structure of the eye of Peripatus capensis is shown in longitudinal section through the head. This figure has been often recopied, and it will be well to take note of the details brought out (see Text-fig. 1, which is a copy of that after Balfour in this Journal, vol. 23). The general cuticle of the body wall is continued as a thin layer over the eye. Below this is the cornea—a layer of epithelial cells, which are continuous with the epidermis. Between the cornea and the lens there is another cell layer which appears to terminate peripherally against the region marked pigment. There is no evidence to show that the structures masked by the pigment were ever brought to light.

From the illustration it would appear as if the pigment formed a separate layer which acted as a kind of capsule enclosing the retina and bounding the eye internally. This impression is strengthened by the fact that the cells below the pigment are marked ' optic ganglion '.

The space within the structures enumerated above is occupied by the lens, and by a layer termed the rods.

TEXT-FIG. 1.



Longitudinal section of the Eye of Peripatus capensis after Balfour, 'Quart. Jour. Micr. Sc.', vol. 23, plate 18, fig. 24. *cor.* = cornea; *l.* = lens; *op.* = optic ganglion; *op.n.* = optic nerve; *pi.r.* = pigment; *Re.* = rods; *s.p.* = secondary papilla.

Now let us turn to the results of the present investigation. The species utilized was Peripatoides occidentalis from Western Australia. A large number of preparations had to be made, including sections and maceration preparations. No single method can be singled out, the usual series of fixatives and stains must be adopted, one method giving a little information, another a little more (see Dakin, "Eye of Pecten", 'Quart. Journ. Micros. Sci.', 1909).

The Eye of Peripatus is not stalked although the distal

surface forms a dome-shaped protuberance on the skin. The whole of this bulge appears to be occupied by the lens. In sections which have not been depigmented (see left side of fig. 1) the eye appears to be made up of three regions—the lens, the region previously known as the retina (or rod region), and the so-called optic ganglion. Now it will clear matters up at once if we state that the rod layer does not consist of cells but only of parts of cells—i.e. the distal halves of cells whose nuclei lie internally to the pigment. In other words, the so-called optic ganglion plus the rod layer together make up the retina. The units of these layers are not separated by a layer of pigment; the pigment is actually enclosed within the cells (see fig. 2).

The Cuticle overlying the eye (fig. 1, *Cut.*) differs from that of the surrounding regions in being free from the small projections so characteristic elsewhere. Not only are the minute spines absent, but the dermal papillae which are present over the entire body wall are missing here.

The Epidermis is continued over the eye to from the Cornea (fig. 1, *Cor.*). Most of the cells of the general epidermis are somewhat cubical or pyramidal in form, with large nuclei. The corneal cells are very different, being quite flat. The nuclei are decidedly compressed and the protoplasm is reduced in amount.

The Subcorneal layer of cells may be said to form a capsule which encloses the lens. It is seen as a well-marked layer where it covers the lens and extends down over the rod layer (fig. 1, *Sub. Cor.*). There is nothing of importance to add further regarding it except that in the development of the eye it formed the outer portion of a complete vesicle, the proximal cells of which have given rise to the retina (see fig. 5).

The Lens is non-cellular and forms a homogeneous mass which stains readily with eosin. The face towards the retina appears almost flat in well-preserved sections, whilst the distal surface is highly convex, so that the entire structure is practically a dome. In all the well-preserved sections the proximal

surface of the lens was in contact with the face of the retina. A delicate non-nucleated sheath appears to bound the lens, but it is in all probability only the outermost layer of the lens substance.

The Structure of the Retina.

Very little trouble will suffice to show quite clearly the structure of the dioptrical part of the eye described above. The elucidation of the structure of the retina is a much more difficult task, and it is quite natural that this essential part of the eye has remained misunderstood.

As we have already seen, the pigment band does not enclose the retina, but is made up of pigment granules lying within the retinal elements. We shall keep the term Rods for the real constituents of the rod layer, the part marked *Re.* in Balfour's figure. This rod layer in poor, or even in moderately good sections, appears to be made up of rather long 'rods' separated by clear spaces. The 'rods' also have a peculiar broken-up appearance even when not cut obliquely, as appears most frequently to have been the case. Now as a matter of fact these dark-staining bodies a r e n o t t h e r o d s. Maceration preparations, but still more certain, transverse sections in the plane of the retina, show quite clearly that the rod layer is not exactly what it seems. It comes as a surprise, in fact, to discover that the dark-staining part of the rod layer appears in transverse sections as a grating or net (see fig. 3). It now requires the study of depigmented longitudinal sections and maceration preparations to explain the above. Really the explanation is simple. The retina is built up of one kind of unit only, and there are no supporting cells or other non-visual elements. Each visual unit consists of a rod-cell bearing a rod.

The R o d - c e l l s a n d R o d s. A rod-cell (see fig. 2, and fig. 1, *Rod-cell*) consists of a columnar portion containing finely-granular protoplasm and crowded with pigment granules, and a proximal constricted and unpigmented part swollen out by the nucleus. As the rod-cells are numerous and the nuclei

rather large, the latter are arranged at different levels in the cells. It is the nuclei of the rod-cells which collectively have been mistaken for an optic ganglion.

Proximally the rod-cells are continued as nerve fibres, which form the very short optic nerve. The distal portions of the rod-cells are hexagonal in section, so that all fit together closely to form a mosaic (fig. 4).

The rods are projections from the rod-cells, but the main part, the axis, of the rod is composed of a rather non-staining material. Thus in longitudinal sections the axes of the rods lie between the stained column-like bodies, whilst in transverse sections the rods would be the meshes of the grating (see fig. 3). The next question is, naturally, what is the 'grating' itself, the part so easily mistaken for the rods in longitudinal section. It would appear as if this staining substance was simply the peripheral portions of the rods.

Each rod can be seen in maceration preparations to bear peripheral 'Stiftchen'—short processes very characteristic of invertebrate visual cells. These 'Stiftchen' clothe each rod completely, and it is the 'Stiftchen', or the 'Stiftchen'-borders, of the rods which stain up so readily and actually appear to be the rods in longitudinal sections. This explains why they show up as a kind of grating when cut transversely, for the 'Stiftchen'-borders of adjacent rods touch each other (see figs. 1 and 3).

Underlying the layer of rod-cells is a collecting region of nerve fibres—the prolongations of the sensory cells. These collect to form a short optic nerve (fig. 1, *Op. N.*) which enters the brain. The optic tract is traceable for some distance within the 'Punktsubstanz'. A delicate layer of connective tissue forms a capsule bounding both retina and optic nerve.

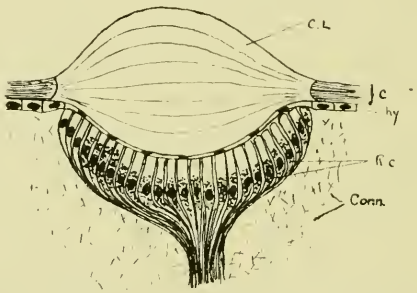
Comparison of the Eye of Peripatus with that of other Arthropods, and with the Polychaete Eye.

The Eye of Peripatus is in reality a very simple structure compared with some insect ocelli. It is developed, as was discovered by Sedgwick (**4**), as a simple vesicular

invagination of the ectoderm. The vesicle cut off gives rise to the subcorneal layer and the retina (see fig. 5). The lens is secreted within this vesicle and is non-cellular. It has no connexion directly with the cuticle of the body-wall, nor is the latter thickened as it passes over the cornea.

The description already given shows clearly that we can exclude the complicated compound eyes of the Insects and Crustacea so far as our comparison is concerned. No information regarding the origin of the compound eye of the arthropoda is likely to be obtained by the study of the Eye of Peripatus. Comparison must be made, then, with the lower and more

Text-fig. 2.



Insect ocellus (Helophilus) after Hesse, somewhat modified. *C.* = cuticle; *C.L.* = cutic. lens; *Conn.* = connective tissue; *hy.* = hypodermis; *R.c.* = rod-cells of retina. Note difference in character of lens from that of Peripatus. The formation of lens by thickening of cuticle over eye is very characteristic in Insecta.

simple arthropod visual organs, the simple eyes. We shall also exclude the Arachnoid eyes, the structure of which (see Lankester (**6**), and Watase) is again different in type. We are left with the Myriapod eyes and the larval eyes and ocelli of insects.

A marked difference is easily recognized between the Eye of Peripatus and the above. In the ocelli of insects (Helophilus, Ceratopsyllus, &c., see Text-fig. 2) and in the larval eyes, we usually find that the ectoderm is invaginated to form the retina (see literature **2** and **3**). We do not find a complete vesicle. The ectoderm does not give

rise to a completely separated vesicle, part of which becomes a subcorneal layer. On the other hand the retinal layer can be traced into the ectoderm.

With this marked difference we must also note that the lens in the Insecta and the Myriapoda is directly continuous with the cuticle and is indeed a local thickening of the same, whilst in Peripatus it is secreted within the vesicle.

The modern work confirms, therefore, the statements of Lankester (**5**), when in his article on the structure and classification of the Arthropoda he adds, ' . . . the Chaetopod eye, which is found only in the Onychophora where the true Arthropod eye is absent. The essential difference between these two kinds of eye appears to be that the Chaetopod eye (in its higher developments) is a vesicle enclosing the lens, whereas the Arthropod eye is a pit or series of pits into which the heavy chitinous cuticle dips and enlarges knobwise as a lens '.

Thus whilst we can homologize the cuticle, cornea, subcorneal layer, &c., of Peripatus with parts of the simple eyes of the Myriapoda and Insecta, the Peripatus eye is not primitive so far as the dioptrical parts are concerned, but has developed along its own lines and resembles that of the highly-developed Chaetopoda. The Eye of Peripatus has, however, not evolved very far, and its retina is quite simple and indeed not at all unlike that of the median ocelli of Helophilus (one of the Diptera) or of the eye of Scolopendra. In both these examples we have retinas consisting solely of visual cells. These cells bear rods which are remarkably like those of Peripatus and have the same marginal (lateral) ' Stiftchensaum '. Indeed, the rods of the Scolopendra retina stain very like those of Peripatus.

Hesse speaks of the retinal elements of these eyes as being of a very original type. It is particularly interesting, therefore, to find the agreement with Peripatus.

The histology of the Polychaete eye has been investigated in some detail by R. Hesse (**3**). We can find material for comparison in his papers.

Eyes are to be found of very varying form and complexity of development. In a great many cases an open cup-shaped retina is to be seen (resulting from ectodermal invagination), but there is no lens, cuticular or otherwise. The retina in nearly all cases consists of rod-cells bearing rods which are directed distally. In a large number of the eyes, the histology of which has been investigated, the details are not very similar to the Eye of Peripatus. Hesse's figure of the eye of Siphonostomum diplochaetos is, however, curiously like that of the early illustrations of the Peripatus eye so far as the retina

TEXT-FIG. 3.

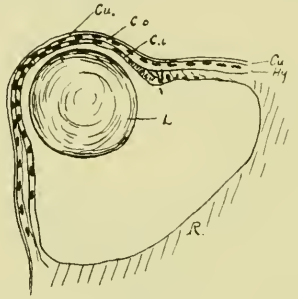


Diagram of lens and corneal layers of eye of Polychaete (Vanadis formosa), modified after Hesse. Note similarity of arrangement of layers to that found in Peripatus. *C.o.* = outer cornea; *C.i.* = inner cornea; *Cu.* = cuticle; *Hy.* = hypodermis; *L.* = lens; *R.* = retina (structure not shown).

is concerned. Both the vertical sections and those taken in the plane of the retina indicate this, and no doubt the structure is almost exactly the same as that of the Eye of Peripatus. A detailed re-examination with up-to-date methods would be necessary to make it certain.

The remaining features (dioptrical) of this Polychaete eye are quite unlike those of Peripatus. The eye is not nearly so well developed as that of the latter.

One of the best-developed Polychaete eyes is found in the group Alciopidae. We have here a vesicular eye (see Text-fig. 3) with enclosed and well-developed lens. There are many resemblances to the Eye of Peripatus. The cuticle, for

example, is continued over the eye without thickening. Below this, and between it and the lens, there are two cellular layers—an outer cornea and an inner cornea. These correspond exactly to the corneal and subcorneal layers in Peripatus. The lens is non-cellular.

We need not carry our comparisons further ; they may be summed up as follows : (1) The retina of the Eye of Peripatus is of a simple and primitive type, and is found again in the ocelli of certain Diptera and in the eyes of some Myriapoda. It is also not unlike that of some Polychaeta. (2) The dioptrical parts of the Eye of Peripatus (lens and corneal layers) are well developed and, as pointed out by Lankester, are arranged in a manner quite unlike that met with in the Diptera, Myriapoda, or Crustacea. These parts, on the other hand, resemble very closely the similar structures of the Polychaete Vanadis. (3) The Eye of Peripatus possesses some features of a simple type met with in other Arthropod groups and in the Polychaeta, but so far as the Arthropoda are concerned it has followed its own line of evolution and remains quite distinct.

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3. Hesse, R.—" Untersuch. ü. die Organe der Lichtempfindung: Polychaeta ", ' Zeit. f. wiss. Zoologie ', Bd. 65. 1898–9. " Arthropoda ", loc. cit., Bd. 70. 1901.
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5. Lankester, E. Ray.—" On the Structure and Classification of the Arthropoda ", ' Quart. Journ. Micro. Sci.', Vol. 47. 1904.
6. —— " On the Structure and Classification of the Arachnida ", ' Quart. Journ. Micro. Sci.', Vol. 48. 1904.

EXPLANATION OF PLATE 7.

Illustrating Prof. W. J. Dakin's paper on 'The Eye of Peripatus'.

Fig. 1.—The Eye of Peripatoides occidentalis in vertical section (longitudinal through the eye). The right half of the retina is represented in the depigmented condition, the left side in the natural state. × 740. *Cor.* = cornea; *Cut.* = cuticle; *Sub. Cor.* = subcorneal layer; *Op. N.* = optic nerve; *Epid.* = epidermis; *Mus.* = muscle-cells; *L.* = lens.

Fig. 2.—Complete rod-cell with rod isolated from the retina. Maceration preparation. × 1,500. *Pig.* = pigment; *Nuc.* = nucleus of rod-cell.

Fig. 3.—Transverse section through retina in plane of the rods (stained haematoxylin, Ehrlich). × 1,500.

Fig. 4.—Transverse section through retina, in plane of rod-cells in the region where pigment is present. (Depigmented section.) × 1,500.

Fig. 5.—Diagrams illustrating the development of the Eye of Peripatus.

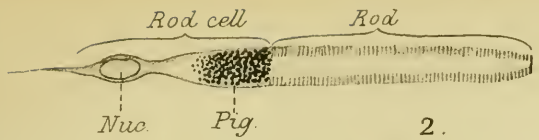
(*a*) Invagination of ectoderm.

(*b*) Invagination of ectoderm complete.

(*c*) Ectodermal vesicle cut off.

(*d*) Proximal cells give rise to retina, the distal becomes the subcorneal layer.

(*e*) Retina developed, lens secreted by cells of vesicle.



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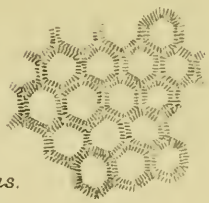

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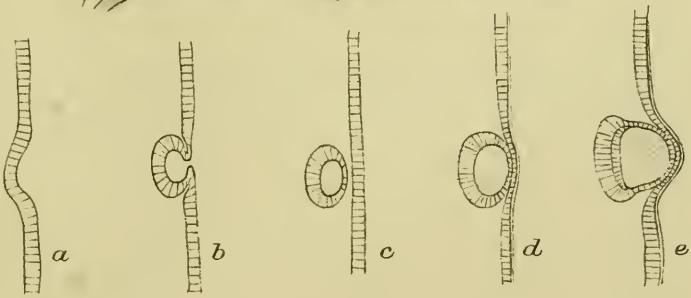

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Huth, London.

PERIPATUS.

On the Development of Cucumaria echinata v. Marenzeller.

By

Hiroshi Ohshima.

With Plates 8 and 9 and 11 Text-figures.

Contents.

1. Introductory Remarks.

The following passages, which are to be found in the diary of the Marine Biological Station of Misaki, concern the spawning of Cucumaria echinata, and are written chiefly by the late Professor Dr. Kakichi Mitsukuri.

'June 18, 1899. Mitsukuri arrived at the Station to-day, being informed of the fact that in these days Messrs. Aoki and Tsuchida had observed the spawning of Cucumaria echinata.'

'June 20. If some freshly caught specimens of C. echinata are kept in a glass vessel, it is almost certain that they will spawn in the evening.'

'July 21. C. echinata spawned!'

'July 29. C. echinata caught in the morning began to spawn at 5 p.m.'

'August 11, 1902. Kuma Aoki dredged several scores of C. echinata at the mouth of the inlet of Koajiro. They began to spawn at 7.30 p.m. and went on till about 10.30 p.m.; a very large number of eggs were spawned. Mitsukuri engages in the study of them.'

In his memoir on pedate holothurians (**31**, 1912, p. 242) the late professor has recorded with reference to the above facts as follows: 'In the breeding season (the summer) the ripe individuals throw out reproductive elements. The males shoot forth the spermatic fluid, after which the females begin to shed eggs, which easily undergo development under observation.'

So far as the results of his study are concerned, unfortunately no report was made, except his two short addresses delivered at the monthly meetings of the Tōkyō Zoological Society. The contents of those addresses can now be recovered only from unpublished notes made by a member who attended the meetings.

The first address was given on December 16, 1899, under the title 'General Account of the Embryology of Holothurians'. The notes may be translated as follows:

'To obtain eggs of C. echinata it is necessary to tease the animal and not to change the water. Animals captured in the morning will lay eggs in the evening. They are in an extended posture while laying eggs; the genital products are emitted from the interradially situated genital pore in the form of a streak. The amount of the products of each emission is very remarkable. No peculiar points are noticeable in the segmentation of the eggs. In the five-tentacled stage a pre-oral

hood is formed containing a mass of food-yolk of a green colour. The first pair of pedicels appear on the ventral side, and at this stage large calcareous plates, which differ in shape from those found in the adult, appear in the interradii. Next to the five primary tentacles (Text-fig. 1, I) three more (II_1, II_2, II_3) arise, of which in most cases the right-hand one (II_1) appears first. The larva attains about 2 mm. in length without having developed the two remaining tentacles (III). The manner of the branching of the tentacles seems to follow a certain rule, just as in the

TEXT-FIG. 1.

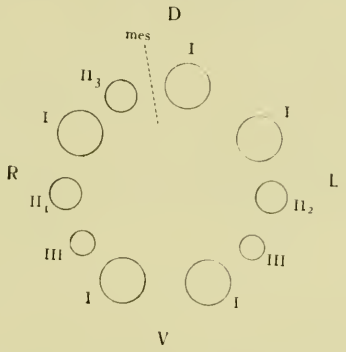


Diagram showing the order of appearance of the tentacles. Anterior view. Larger circles (I) represent the primary tentacles; medium-sized (II), secondary ones appearing next; and smallest ones (III), those last to appear. *mes.* = position of the dorsal mesentery. (After Mitsukuri.)

phyllotaxis among plants, and the second pinnule is the largest of all (Text-fig. 2).

'The first pair of pedicels are followed by the third (Text-fig. 3, 3) appearing on the left side of the midventral radius, then the fourth (4) appears on the ventral side of the right ventral radius. A further increase of pedicels may be seen in the diagram. To some extent the pedicels increase forwards from the height of the first pair, while later some appear behind it.'

The second report was contained in his address on the change

of calcareous deposits in Holothuria vagabunda, read on February 21, 1903. Here he spoke again on the sequence of the appearance of the secondary tentacles. Judging from the figure he indicated a difference from his former statement, since the dorsal tentacle (Text-fig. 1, II_3) was shown as the first to appear among the secondaries.

Having been engaged in the study of holothurians since the lamentable death of Professor Mitsukuri on September 17, 1909, I have made a new and careful examination of the subject. On August 12, 1910, my first attempt ended in failure because the spawning season was already over in that year. During

TEXT-FIGS. 2 & 3.

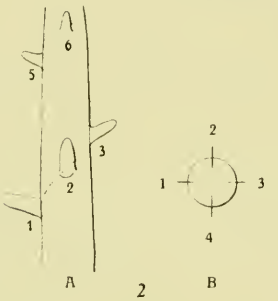


Diagram showing the manner of branching in tentacles of the young. A = Side view of a part of the stem to show the spiral arrangement of 'pinnules' 1, 2, 3. B = Profile of the stem to show positions of pinnules. (After Mitsukuri.)

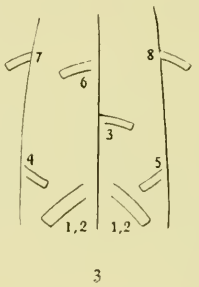


Diagram showing the order of appearance of pedicels in the young. Ventral view. Numbers 1–8 indicate the order of appearance. (After Mitsukuri.)

the next few years I was unable to visit Misaki at the right season, but at last, on July 25 and August 1, 1916, I was fortunate enough to observe the animal spawn and to rear the larvae. By the kindness of Professor Dr. I. Ijima I was permitted to examine the valuable material left by the late professor, on which, and on my own collection, my studies have been based: and incomplete though it may be, owing to several gaps in the developmental stages in the materials available for examination,

I believe I have been able to throw some light upon the embryology of the group.

The present work was started while in the Zoological Institute of the Tōkyō Imperial University, but the greater part of it was carried on in the biological laboratory of the Fifth High School in Kumamoto, and it was completed in November of 1918. Owing, however, to the pressure of various affairs it could not have been published before I had left Kumamoto the next summer. The manuscript was then brought with me to London, and has been subjected to Professor E. W. MacBride's kind and careful revision. It is my pleasant duty here to express my hearty thanks to Professor I. Ijima and Professor S. Goto for their kindly supervision, and to various others for their help. Further, I extend my gratitude to Mr. K. Yoshioka, Director of the Fifth High School, by whose favour I have been able to enjoy many facilities to assist me in my studies. Lastly, to Professor MacBride, to whose courtesy the appearance of this paper is entirely due, I beg to tender my deepest indebtedness.

2. Previous Studies on Allied Forms.

So far as the embryology of apodous holothurians and our knowledge of typical auricularіae are concerned, the famous works of J. Müller, Metschnikoff, Semon, Bury, Mortensen, and Clark are unrivalled; but our knowledge of the pedate forms is meagre and, for the most part, fragmentary. This meagreness is due, I think, partly to the fact that artificial fertilization is very difficult,[1] and partly to the shortness of the larval stage, the auricularia stage being usually omitted,[2] so that the larva easily escapes the eyes of investigators of the

[1] Clark (**7,** 1898, p. 58), Mitsukuri (**30,** 1903, p. 11), and Edwards (**12,** 1909, p. 212) never succeeded, while Selenka's experiment (**45,** 1876, p. 157) resulted in the malformation. Only one case of successful artificial fertilization is recorded by Mortensen in Holothuria nigra, though in this case only a small percentage of the eggs developed (**35,** 1913, pp. 17–18).

[2] Holothuria tubulosa and H. nigra pass through a typical auricularia stage (Selenka, **45**; Mortensen, **35**).

plankton. Among pedate holothurians, the forms which offer the materials for the study of embryology belong to two families only, i.e. Holothuriidae and Cucumariidae, both of which are chiefly dwellers in shallow water. Östergren (**42,** 1912, p. 338) attributed the shortness of larval life in Cucumariidae to the fact that they live near the coast. Any longer period of larval life, he thought, would expose the larvae to greater danger of being swept away by violent currents to destruction.

Again, our knowledge of their development is fragmentary, because, first, many of the observations have been made on those forms which have brooding habits in which it is not easy to secure a complete series of developmental stages, and, secondly, there are some difficulties in the technique of investigation, the egg being yolky and the larvae mostly opaque.

The embryology of pedate holothurians was first studied by Danielssen and Koren (**11,** 1856). Their material was identified as Holothuria tremula, but Ludwig (**21,** 1889–92, pp. 249, 251) and Mortensen (**33,** 1898, p. 24) alike denied this and suggested that it was dendrochirote. Later, Louis des Arts (**2,** 1910, pp. 9–10) and Östergren (**42,** 1912, p. 338) have both identified it as Cucumaria frondosa. Kowalewsky (**17,** 1867) was the second who had the good fortune to observe the spawning of C. planci (=Pentacta doliolum) and C. kirchsbergii (=Psolinus brevis); he managed to rear larvae for over ten days, which attained the pentactula with a pair of primary pedicels.

Selenka's work (**45,** 1876) on Holothuria tubulosa and Cucumaria planci (=C. doliolum) entered into a more detailed account than those of his forerunners, since he employed the paraffin-section method. Ludwig (**22,** 1891) most excellently explained the processes of organ formation, and elucidated many points upon the origin of various organs in C. planci. He escaped the failure which befell Selenka, since he was successful in obtaining well-orientated serial sections, whereas Selenka adopted the method of 'Masseneinbettung', i.e. embedding large numbers of embryos

together and trusting to chance to obtain some orientated in the right direction. But unfortunately his materials were fixed only once every day, so that there were gaps between the stages he obtained. He was able to publish only preliminary notes without figures, and no final report. In his 'Holothurien' of Bronn's 'Klassen und Ordnungen des Thierreichs' (**21,** 1889–92) he summarized the facts known up to that time chiefly in Labidoplax digitata, Holothuria tubulosa, and Cucumaria planci.

Mortensen (**32,** 1894) described in detail the young of a brooding form, Cucumaria glacialis. Another brooding form, Phyllophorus urna, was then studied by Ludwig (**24,** 1898). Lo Bianco's skill managed to keep the young of that species alive for two months outside the mother's body in a small aquarium.

In Edwards's study (**12,** 1909) on Holothuria floridana much attention was paid to the development of the ambulacral appendages, some important details in the early changes of other organs being left unnoticed. Des Arts (**2,** 1910) succeeded in rearing larvae of Cucumaria frondosa and observed some early changes and pathological accounts caused by change of temperature. The most promising work has been carried on by Newth on C. saxicola and C. normani, yet we know of his results only through a preliminary note (**36,** 1916).

Thus far the important works so often referred to in the present paper. Other works left unnoticed in the above enumeration will be cited later on as occasion may require.

3. Methods of Investigation.

The newly-shed eggs were transferred to a larger glass vessel by a pipette. The vessel was cylindrical in shape, 28 cm. in diameter and 18 cm. in height, and was filled with clean filtered sea-water. The water was changed the next morning, and was afterwards left unchanged. The vessel was tightly covered with a glass plate, and was soaked in cool well-water which was changed once or twice a day.

To kill and fix the larvae I used a chromo-acetic mixture containing a slight amount of osmic acid. Later examination showed that this fixative proved very satisfactory for the study of tissues, but for embryological purposes it is bad, since the free cells contained in the hydrocoele and enterocoele are fixed in a very expanded state, almost filling up the lumen of these vesicles. Newth used Bouin's picro-formol-acetic and Flemming's strong solution, and seems to have experienced a similar difficulty, judging from his following remarks: 'Even after the tentacles are well established, and can be protruded and retracted, their lumen is obliterated in some places by the vacuolated inner ends of their cells', and 'there is a complete suppression of the typical curved hydrocoele crescent owing to the large size and close crowding together of its lobes and to the thickness of their walls' (**36**, p. 636).

The late Professor Mitsukuri seems to have used acetic sublimate, and his materials proved very good for the study of those internal cavities, which remained very wide and distinct. Some larvae of C. planci which he obtained at Naples on March 25–7, 1898, labelled as fixed with acetic sublimate, are in a precisely similar condition to his material of C. echinata. Ludwig (**22**, p. 604) doubted the wisdom of Selenka's employment of chrom-osmic mixture, and recommended a careful alcohol method. For observations on calcareous deposits in somewhat advanced larvae the latter were simply killed in alcohol.

For the orientation of the material to be sectioned, the double embedding in celloidin-paraffin gave good results. First, the material was put in celloidin-clove-oil mixture, and then hardened with chloroform. The hardened block was then clarified with carbol-xylol and transferred into melted paraffin. Even by employing the celloidin-paraffin method of sectioning, shrinkage of the material by about at least 15 per cent. diameter is unavoidable. That is why the Text-fig. 5, which is drawn from a whole mount, is larger than other figures obtained from sections. Sections were cut of a thickness of 5 μ, except in the case of the egg and quite advanced young, which were cut into sections 8 μ thick.

The sections were stained with Heidenhain's haematoxylin and orange-G, and very satisfactory results were obtained even from the material which had been preserved for nearly twenty years, enabling pictures to be taken by photomicrography.

For the reconstruction of sections, the graphical method with a glass-plate with parallel lines proved very simple and quite satisfactory.

4. Breeding Season.

As mentioned above the late Professor Mitsukuri observed the spawning of Cucumaria echinata on July 21 and 29, 1899, and on August 11, 1902, and Messrs. Aoki and Tsuchida seem to have observed it previous to June 18. I myself observed the same phenomenon on July 25 and August 1, 1916, but as stated above, on August 12, 1910, I was too late to secure the spawning individuals.

Though the animal occurs in April and May 'in such abundance that many boats dredge for them day after day, and by evening each one is loaded down with them' (Mitsukuri, **31,** p. 241), no spawning takes place in the evening, and an examination of the gonads reveals the fact that they are still immature. It is strange that in July and August the animal does not occur in such abundance as in April and May. Further, from the latter part of July to the early part of August we meet with minute young provided with five to ten tentacles and pedicels of varying numbers, mingled with sand and broken shells, in which adult animals are found embedded. These young measured only 1·5–4·5 mm. in length on August 12–17, 1910, while on July 20–5, 1916, they were remarkably large and in a far advanced state of development (see Table V).

From these facts we may conclude that the breeding season begins about the middle of June and comes to an end in the early part of August, though it varies to some extent according to different years. The larger young found in the latter part of July may have developed from eggs spawned quite early in the season.

Sex-Ratio.—Selenka (**45,** p. 157) noticed that in

Holothuria tubulosa both sexes occur in approximately equal numbers. Lo Bianco (**19**, 1899, p. 476) found that in a certain locality males of Phyllophorus urna occur much more abundantly than females. I could pay no special attention to the sex-ratio of C. echinata during the breeding season. But of the specimens collected on April 9, 1914, an examination of over 3,000 individuals showed that there were 1,627 males to 1,596 females, or that the ratio of males per 1,000 females is 1,019. Both sexes thus occur in almost equal numbers.

5. Genital Organs.

In addition to the statements of systematists, such as that given by v. Marenzeller (**28**, 1881, p. 128), I may describe some points about the genital organs.

Genital Tubes.—Both in males and females the genital tube consists of an external epithelium, a connective tissue layer, and an internal germinal epithelium. The external epithelium, which is continuous with the peritoneum, is very thick in an inactive stage of the gonads (Pl. 8, fig. 2, *ep*), and the connective tissue layer is very thin. I have no positive evidence to prove the presence in C. echinata of the muscle layer which was found in C. glacialis (Mortensen, **32**, p. 715) and C. laevigata (Ackermann, **1**, 1901, p. 731). In Pseudocucumis africanus, however, I found in the specimens fixed on July 28, 1915, when the breeding season was over, rather scattered circular and longitudinal muscle fibres, a connective tissue layer, and a very high external epithelium. Though having the same habit of carrying the brood inside the body-cavity, the structure is totally different from the peculiar feature found by Clark (**7**, 1898, pp. 58–9) in Synaptula hydriformis. Here he observed a very thin external epithelium and scanty connective tissue, which, according to that observer, probably break so as to allow the ripe eggs to escape into the body-cavity.

Female Gonad.—In a still unripe condition, as was met with in the specimens collected on March 27, 1914, the female gonad is light purplish grey in colour, with a very thick wall

owing to the high external epithelium as stated above. It contains eggs of various sizes. The egg is slightly flattened and is attached to the wall of the genital tube by its broad surface (Pl. 8, fig. 2), not by a slender stalk formed of the follicular epithelium, as described by Semper (**47**, 1867–8, p. 144), Jourdan (**16**, 1883, p. 52), and others in Holothuria.[1] The germinal vesicle (*n*) is large and spherical, lying eccentrically near the free end of the egg. The centre of the free surface is indicated by a minute conical process of the cytoplasm (*ma*).

The gonad in the breeding season (Pl. 8, fig. 3) has become thin-walled and yellowish in colour, containing still very small eggs as well as large ones, which latter are ready for spawning. The germinal vesicle (*n*) has now approached much nearer the free end than in the foregoing stage, leaving a thin layer of yolky cytoplasm between it and the egg-membrane. Germinal spots (*gs*) are more minute and fewer compared with those in the early stage.

The centre of the free surface is now very peculiar in structure. Here we see a compact cytoplasmic mass, of a somewhat fibrous nature, forming a short rod-like body protruding through the egg-membrane, with its free enlarged knob-like end attached to the follicular epithelium (Pl. 8, fig. 3, *ma*). Usually the proximal end reaches the germinal vesicle and becomes continuous with its membrane, but in rare cases it ends apart from the germinal vesicle, where the latter is not very near to this pole of the egg. The space between the egg-membrane and the follicular epithelium (*j*) was perhaps occupied by a gelatinous layer. This remarkable structure, which I may call a micropyle appendage,[2] is only conspicuous in full-grown eggs,

[1] According to Hamann (**15**, 1884, p. 89) H. tubulosa is exceptional; here a fibrous bundle of connective tissue serves to fasten the egg.

[2] As early as in 1851, J. Müller remarked that the ovarian egg of Pentacta doliolum is flat, and that at the centre of a flattened surface there is formed a yolky process which passes through the thick jelly layer investing the egg. He further observed a similar structure in some other Holothurians—Thyone fusus, Holothuria tubulosa, &c. ('Monatsber. Akad. Berlin', April, 1851; 'Phys. Abhandl. kön. Akad. Wiss. Berlin', 1852 (1850), p. 77, Taf. IX, fig. 8, 9; Müller's 'Arch. f. Anat. u. Physiol.', 1854, p. 60).—Jan. 28, 1921.

while in the very early stages no such structure appears (fig. 1). In a rather small one I only once made out a slight indication of this structure.

A similar feature was observed by Semper (**47**, p. 144). He found that in Holothuria impatiens and others the jelly-canal ('Mikropylkanal') of all the egg is directed without exception to the internal lumen of the ovarial tube, and in his figs. 6 and 7 of Pl. xxxvi it is shown that the 'Stiel des Kernes' is penetrating the canal. On the formation of the egg and the significance of the micropyle he writes as follows:

'Eine der Zellen des einfachen glatten Epithels vergrössert sich und hebt dabei die anliegenden Zellen etwas mit in die Höhe. In diesem Stadium scheint das Ei lediglich aus einem Keimbläschen mit sehr geringer Dottermasse zu bestehen. Wie schon vorher die einzelnen Zellen des Epithels miteinander zusammenhingen, so bleibt auch jetzt noch die Eizelle in inniger Verbindung mit den nächstliegenden Epithelzellen, welche sie bei stetem Wachsthum mehr und mehr mit sich in die Höhe zieht. . . . die Mikropyle ist allerdings ein Stigma, nämlich die bis zur völligen Reife bestehende Verbindungsstelle mit den zur Eihaut umgewandelten Epithelzellen, und der Stiel, an welchem die Eier hängen, erklärt sich auf die einfachste Weise durch allmäliges Auswachsen und Abtrennen von der inneren Follikelhaut' (pp. 144–5).

I could not find the youngest stage of eggs showing the relationship with the follicular epithelium, as figured by Semper (fig. 10, *a*, *b*, *c*). But, as stated above, this peculiar structure develops quite late, apparently simultaneously with the appearance of the gelatinous coating. I feel justified in thinking that this has something to do with future changes of the egg, above all with the maturation, and is not a mere vestige of the attached part of the egg. Hamann (**15**, pp. 88–9, fig. 3) observed a similar structure in H. tubulosa. He noticed a cytoplasmic cord, which breaks through the transparent albuminous layer, ending in a round nucleus-like body outside the follicular epithelium. In my case it was different in that the rounded end always lay inside the epithelium. I could find no trace in any of my specimens of the peculiar feature observed by Mortensen (**32**, p. 715, Pl. xxxi, fig. 22) in C. glacialis, viz. that the

chromatic substance gathered on one side of the egg in the form of a dish.

Male Gonad.—I am unable to give any detailed account of the male gonad. In the specimens collected during March I found spermatogonia and spermatocytes near the germinal epithelium, while the central part of the tube was filled with unripe spermatozoa or probably spermatids. In the breeding season the internal space of the tube is filled with ripe spermatozoa, only a thin layer of larger cells—probably spermatocytes—being found near the germinal epithelium. Radiating bunches of spermatozoa, termed by Mortensen Spermatogemmae, were found in C. glacialis (**32,** p. 715, Pl. xxxi, fig. 23) and were also observed in C. ijimai.

Genital Papilla.—The genital papilla is situated immediately behind the tentacular crown along the mid-dorsal line. A very singular case is noticed by Ludwig (**20,** 1887, p. 1233), who observed that in C. crocea the papilla is located far backwards, in extreme cases 8 mm. back and far from the tentacular crown in an individual, which measured 40 mm. in length, and 8·5 mm. in another individual, 42 mm. long ; that is one-fifth or more of the body length.

But a more noteworthy fact about the genital papilla was discovered by Edwards (**13,** 1910, pp. 338–9, Pl. xiii, figs. 2–5). He found in C. frondosa that the male genital papilla is subdivided into from four to thirty or more parts with a general average of ten, each branch ending in a terminal pore. The papilla in females is usually simple, ending in a single pore, but sometimes, though rarely, two or five pores may be met with. Further, the same author (**14,** 1910, pp. 599–608, Pl. xix, fig. 1) proved the presence of a similar feature in such allied species as C. californica, C. miniata, and C. fallax.

C. echinata offers another example of the same thing. In a male specimen which I examined the genital pores were at least fifty in number, while in females there were from five to about twenty-five pores. Previous to branching, the end of the genital duct is dilated into a wide cavity just below the cluster of minute papillae. This cavity, as well as the branched canals, is lined with a ciliated epithelium followed by a thin

layer of connective tissue and a layer of loose irregularly arranged muscular fibres.

6. Spawning.

The late Professor Mitsukuri observed on one occasion that the spawning had begun at 5 p.m., and in another that it had continued from 7.30 to 10.30 p.m. My experience is as follows :

At 5 p.m. on July 25, 1916, several specimens were brought to the Station, and soon after that I found two individuals emitting spermatic fluid. Half an hour afterwards, at 5.30, another individual began to lay eggs. In the morning of August 1 of the same year some few specimens were got and brought into the Station at 1.15 p.m. Soon afterwards two individuals were found emitting sperm, but in this case no laying of eggs was seen to follow. At about 2.30 p.m. of the same day plenty of large specimens were brought in. Here the emission of sperm by a male was found to begin at 5.25 p.m., and a female which was lying about 5 cm. distant from the former began to shed eggs at 5.40, which continued till about 6.30 p.m. In the specimens of the same lot kept in another jar the emission of sperm by one began at 4.50 p.m. followed by several others, but no shedding of eggs took place here.

These specimens were all quite big, and later examination showed that all of them were sexually ripe and contained spermatozoa or eggs in abundance. In the individuals which missed the chance of shedding genital elements, I noticed that it never took place the next evening or at any later time. Even exposing the animals in a warm sunny place with very little sea-water, or putting them in the dark, could not cause them to spawn. According to Mitsukuri (*ante*, p. 174) unclean water makes the animal lay eggs.

The male, while emitting spermatic fluid, stretched out its tentacles half-way and kept them very quiet. The spermatic duct could be seen through the body-wall as a white streak which appeared to perform a peristaltic movement. In consequence of the subdivision of the spermatic duct at the genital papilla, that white streak could be seen divided into five or six

branches, and a white thread-like spermatic fluid flowed out from each. Being heavier than sea-water, the spermatic fluid sank down as a milky white cloud on to the bottom of the vessel.

In females the shedding of eggs seemed to be accompanied by no waving of tentacles, but eggs were thrown out intermittently on the hinder aspect. The egg is much heavier than sea-water and very soon sinks to the bottom.

According to Kowalewsky (**17**, p. 1), who put about fifty freshly-caught individuals of C. kirchsbergii into a large vessel through which fresh sea-water flowed, the emission of sperm by males occurred within two hours. The spermatic fluid formed a white thread streaming out of a pore situated between tentacles, and was then stirred up by the waving movement of the latter. The emission of sperm lasted for about an hour, and in the next hour a female lying near began to shed eggs. He seems to have believed that the egg was fertilized inside the mother's body and was expelled through the pore in the body-wall, and that in a viviparous form, Phyllophorus urna, this pore serves as a birth-pore. Selenka (**45**, p. 166) observed no waving of tentacles in the male C. planci while emitting sperm, and according to him the females which began to shed eggs as soon as the males emitted sperm moved their tentacles very actively. Des Arts (**2**, 1910, p. 3) put a great number of C. frondosa into an aquarium, and the first night saw the laying of eggs which were soon fertilized. According to Newth (**36**, p. 633) the spawning of C. normani takes place in the night, and generally near midnight. On one occasion males and females lying together in the same tank began to spawn within a few minutes of one another. Isolated individuals of both sexes are said to have spawned too, but he was never successful in fertilizing the egg so shed by adding sperm suspension, and I cannot help doubting whether he was careful to ascertain that the females really spawned without being stimulated by spermatic fluid.

Of other pedate holothurians records are given by Selenka (**45**, p. 157) on Holothuria tubulosa, and by Edwards (**12**, p. 212) on H. floridana. Among some dozens of big

specimens of H. tubulosa kept in a large box, the males emitted sperm in the form of long white threads at intervals of two to ten minutes. After some hours fertilized eggs were found on the bottom of the box. Edwards adopted Slenka's live-box method and obtained fertilized eggs within four to ten hours.

7. Newly-shed Eggs.

The egg is slightly flattened, especially so on the side of the animal pole (Pl. 8, fig. 4), as is known to be the case in C. normani from Newth's observation (**36,** p. 633). Along the axis through the poles it measures about 300–35 μ, and the greatest diameter as measured along the equatorial plane is about 340–400 μ, most commonly 400 μ. Externally the egg is covered with a radially striated gelatinous layer which is 50–72 μ thick. At the centre of the more flattened surface, the animal pole, the jelly canal can be distinctly seen. The egg is heavier than sea-water.

According to Kowalewsky (**17,** pp. 2, 6) the egg of C. kirchsbergii is opaque with a greenish yolk, and is heavier than sea-water. The egg of C. planci is said to be four to five times larger than that of the former species, and, according to Selenka (**45,** p. 167), it is lighter than sea-water and floats immediately below the surface of the water. The egg of C. frondosa is, as observed by Des Arts (**2,** p. 3), intransparent and of a red colour, with a distinct micropyle. Newth (**36,** p. 633) observed that the egg of C. normani tends to float with its animal pole directed upwards, and though no definite micropyle could be found the 'umbilicus' of the follicle seemed to act instead.

Remarkable records of large eggs are known among deep-sea forms, e.g. Enypniastes eximia has an ovarian egg of 3·0–3·5 mm. diameter, and in both Benthodytes gotoi and Euphronides depressa the ovarian egg measures 2·5 mm. in diameter (Ohshima, **38,** 1915, p. 214). Besides these cases, large eggs are met with in Cucumariidae, especially in those forms which are accustomed to care for their brood.

The following table shows the sizes of eggs observed in the family by various writers:

TABLE I.

	Diameter of egg (mm.).		
Species.	*Ovarian Egg.*	*Egg newly shed or found in brood-pouch.*	*Observer.*
Cucumaria parva	0·2	—	Ludwig (**23**, 1898)
C. echinata	—	0·44	Ohshima (**40**, 1918)
C. frondosa	—	0·46	Des Arts (**2**, 1910)
Psolus granulosus	—	0·5	Vancy (**48**, 1906)
Cucumaria ijimai	0·5–0·55	—	Ohshima (**38**, 1915)
C. crocea	0·6–0·65	0·7	Ludwig (**20**, 1887; **23**, 1898)
C. lamperti	0·8	—	Ohshima (**38**, 1915)
C. glacialis	—	1·0	Mortensen (**32**, 1894)
C. lateralis	—	1·0	Vancy (**48**, 1906)
C. curata	—	1·0	Cowles (**10**, 1907)
C. laevigata	1·0	—	Lampert (**18**, 1889)
,,	—	1·34–1·5	Ackermann (**1**, 1901)
Thyone imbricata	1·2	—	Ohshima (**38**, 1915)

Among the twelve species in the table, there are only two which have no brooding habit and lay eggs freely in water, namely C. echinata and C. frondosa; all the others have the brooding habit.

Maturation.—Examination of sections of the egg fixed immediately after being shed show that the egg has just extruded the first polar body (Pl. 8, fig. 4, *pb*), and that the second maturation spindle (*ps*) can be seen orientated either obliquely or vertically with reference to the circumference of the egg, while the sperm head (*sp*) has in most cases just entered. When the spindle is perpendicular to the surface a conical cytoplasmic process is formed projecting into the canal through the jelly, through which the first polar body may have been expelled. According to Boveri (**4**, 1901, p. 147) the canal through the jelly of the egg of a sea-urchin, Strongylocentrotus lividus, is widened at the maturation period and serves as the way through which the polar bodies are given out. Selenka (**45**, p. 167) noticed for the first time a polar body in the egg of C. planci and described it as 'der Koth des Eies'. The egg of C. normani when taken from among

the tentacles of the mother is, according to Newth (**36**, p. 633), undergoing or has just completed the second maturation division. In the egg of Holothuria floridana Edwards (**12**) saw three polar bodies, one of which was remarkably larger than the others.

Fertilization.—The sperm head is still minute and stains intensely; it measures about 2·5 μ in diameter.[1] It is found situated rather peripherally and quite distant from the animal pole, and rather near the equator (Pl. 8, fig. 4, *sp*), so that it is doubtful whether it is at the animal pole that the spermatozoon penetrates into the egg. It is highly probable that the spermatozoon enters the egg after, or at the same time as, the protrusion of the first polar body. In some sections the sperm nucleus is seen approaching the centre of the egg and becoming somewhat vesicular. In an egg fixed at fifty minutes after being shed the sperm nucleus is found lying close to the egg nucleus; it is of the same size as the latter and encircled with astral rays.

8. Cleavage.

Among the eggs fixed fifty minutes after being shed were found some showing the first cleavage spindle lying horizontally at the centre of the egg. Thus the first cleavage seems to begin in about an hour. The amount of the material which I was fortunate to rear was so limited that, from fear of destroying the whole culture or in any case of losing much before any further development could be observed, I was unable to examine closely the living embryos during cleavage, &c. The following statements are given from preserved materials.

The eggs fixed within two and a half to three hours after being shed show various stages between four-cell and thirty-two-cell stages.

Four-Cell stage.—The blastomeres are equal in size, elongated along the egg-axis, and flattened or even slightly concave on the axial surface. Usually the blastomeres inter-

[1] The sperm head before entering the egg measures about 2μ in diameter.

lock, i. e. a pair situated diagonally do not lie parallel to each other but their ends approach at one pole, while the other pair approach at the other pole. In an extreme case, these two pairs come to lie in different planes, one pair being high above the other.

Eight-Cell stage.—In consequence of the interlocking of the blastomeres in the preceding stage, the two tiers of blastomeres in the eight-cell stage tend to lie shifting 45° above the other. Much irregularity in regard to the size and position of blastomeres is often met with.

Sixteen-Cell stage.—Here eight blastomeres in a tier lie above the other set consisting of eight. Very frequently, however, each tier shows a zigzag arrangement, thus the alternating four are slightly above the remaining four, and in an extreme case they form a tier of themselves at each pole.

Thirty-two-Cell stage.—Now the embryo is globular in shape leaving a spacious blastocoele inside it. In the most regular cases there are four cells in a tier on each pole, and between these there are three tiers of eight cells arranged in zigzag rows. No remarkable difference in size among the blastomeres can usually be discerned.

Above Sixty-four-Cell stage.—Now it is hardly possible to recognize any special arrangement of blastomeres. Hereafter those at and near the vegetative pole are found to be a little larger than those of the opposite pole. No coagulable matter is as yet found in the blastocoele. The blastula is found still wrapped within the egg-membrane.

According to Kowalewsky (**17**), Selenka (**45**), Edwards (**12**), and Des Arts (**2**), the cleavage of the eggs of C. kirchsbergii, C. planci, C. frondosa, Holothuria tubulosa, and H. floridana is total and equal or approximately equal. Selenka noticed that in C. planci the difference of size among blastomeres is evident only after the thirty-two-cell stage, and that the cleavage ends at the beginning of the second day. Edwards stated that in H. floridana the four-cell stage is reached within three hours and the sixteen-cell stage within four hours. Des Arts observed in the egg

of C. frondosa various regular stages of cleavage on the second day. In C. normani and C. saxicola, as examined by Newth (**36**), the cleavage is not absolutely regular, in that the four blastomeres may rearrange themselves diagonally, and no orderly scheme could be detected in the cleavage later than the sixteen-cell stage. Only in a few individuals of the latter-named species perfect symmetry up to the sixteen-cell stage was met with. A very curious feature is seen in C. glacialis as reported by Mortensen (**32**, pp. 722–3). As a remarkable exception among echinoderms, the cleavage here is said to be superficial in that the divided nuclei migrate towards the periphery, increasing in size, and at last there is formed an epithelium, each nucleus being separated by a cell wall.

9. Blastula.

The blastula when free from egg-membrane floats at about the middle layer of the water, rotating actively by means of cilia. Its diameter as measured in life is about 335 μ. Though I was unable to observe its emergence from the egg-membrane, the presence of a wrinkled stage inside the membrane is hardly conceivable in view of the fact that no remarkable increase in size of the free-swimming blastula as compared with the embryonic blastula is to be found. The wall consists of a layer of very high slender cells, the vegetative pole being indicated by a thicker wall. In the blastocoele a coagulable fluid now appears, known as blastocoele jelly or ' Gallertkern ' (Pl. 8, figs. 5, 6, *bj*), which increases in density with the growth of the embryo.

The blastula of C. kirchsbergii is said to be still covered with egg-membrane (Kowalewsky, **17**). In C. planci the blastula is formed at the end of the first day (Ludwig, **22**, p. 605) or in ten hours (Kowalewsky, **17**, p. 3), and the cleavage ends early on the second day (Selenka, **45**, p. 168). According to Selenka cilia arise here and there at the end of the first day, and when the cleavage is ended every cell is beset with a cilium; the embryo then gets out of the egg-membrane, and swims usually near the surface of the water. During the course of twelve hours the

blastula diminishes in size by one-fifth of its diameter, and the internal cavity becomes filled with blastocoele jelly. Ludwig (**22,** p. 605) denied the diminution of size in the blastula. For my part, I should think an increase of size would seem more probable in such a form where the blastula is wrinkled while inside the egg-membrane. Des Arts (**2,** p. 5) observed that in C. frondosa the blastula is formed on the third day, and that on the fourth day the cells are so multiplied that many folds appear on the surface and an irregularly formed internal cavity makes its appearance. As late as on the sixth day it is still covered with egg-membrane, but it then acquires cilia and rotates actively inside the membrane. On the seventh day it emerges from the membrane and is then 405 μ in diameter, and on the next day a thickening at the vegetative pole occurs. The same author gives further the results of the influence of the temperature upon the embryo. Besides the syncytium-formation which usually results by its being put in a warm place, the blastula, being accelerated by warmth, begins to rotate on the fifth day, and on the next day it casts off the membrane and the vegetative pole thickens. The discrepancy found between my culture and those of Mitsukuri with regard to the growth-rate, as will be stated later on, seems to be due largely, if not exclusively, to the influence of temperature. I cannot therefore lay much stress upon the time-record.

Similarly wrinkled blastulae are reported by Newth (**36,** p. 633) in C. normani and C. saxicola. In these species the morula is solid, and the blastocoele first appears during the formation of a wrinkled blastula. At the latter stage cilia appear and the embryo soon emerges from the egg-membrane and begins to rotate slowly at the bottom. The rotation in C. normani is counter-clockwise in direction as seen from above, while in C. saxicola it is clockwise. The wrinkled surface smoothes out before invagination occurs. According to Selenka (**45,** p. 160) the blastula of H. tubulosa acquires cilia near the end of cleavage, and at the twentieth hour it comes out of the membrane. The blastula of H. floridana

is, as observed by Edwards (**12,** p. 213), reached at the fourteenth hour.

Before invagination begins mesenchyme cells are formed by the active proliferation of the cells at the vegetative pole (Pl. 8, fig. 5). Having become free from the wall, these cells wander into the blastocoele, some lying attached to the wall near the animal pole (Pl. 8, fig. 6, *me*).

The mesenchyme-formation begins, according to the species, either before or after the invagination, or sometimes at the same time as the latter. In C. frondosa and C. echinata the mesenchyme-formation precedes the invagination. The same is true for C. planci in normal cases (Selenka, **45**) but it may occur afterwards (Ludwig, **22**). In H. tubulosa and H. floridana both the processes occur at the same time, while in Synaptids the mesenchyme cells are formed from the tip of the already formed archenteron. Ludwig (**21,** 1889–92, p. 258) noticed this fact and concluded that these differences are proportional to the rapidity of development. Thus in a form whose development is rapid mesenchyme is formed later, and vice versa. I may point out further that in those forms where the mesenchyme-formation takes place early the cells are generally very numerous and they readily fill up the blastocoele, while in those where invagination precedes the mesenchyme-formation the cells are generally few.

As to the origin of the mesenchyme Ludwig (**21,** p. 258) surmised that some mesenchyme cells may arise from the blastoderm in other places than that where the future endoderm is situated, and from his study in C. planci (**22,** p. 605) he claimed to have proved this statement. His view could not be confirmed by Newth (**36,** p. 635), while Clark (**7,** p. 61), in his observations on Synaptula hydriformis, felt 'no hesitation in affirming that the mesenchyme arises exclusively from the endodermal cells'. It is highly probable that Ludwig saw those cells attached to the future ectoderm, as I have mentioned above. I could not, however, find any positive evidence to support his view, and in contrast to the vegetative part where many mitotic figures

are to be met with, no such thing is found in the ectoderm. Newth observed some enucleated cytoplasmic droplets attached to the ectoderm. All I have seen were nucleated cells showing no notable difference from other mesenchyme cells suspended in the jelly. However, I cannot deny the rôle that the ectoderm plays in mesenchyme-formation in a later stage of the gastrula, followed by the appearance of stomodaeum, as will be described below.

Selenka (**45,** pp. 160–1, 168) observed some peculiar cells consisting partly of those detached from the blastoderm and partly of those which arose from subdivisions of the former, and called them ' Mesodermkeim '. Every subsequent observer, however, denies their presence.

10. Gastrula.

Invagination begins early in the morning of the next day, i.e. about at the fifteenth hour. The larva gradually increases in length in accordance with the growth of the invaginated archenteron and the multiplication of mesenchyme cells. It swims with the apical end forwards, at the same time rotating around its longitudinal axis. Cilia usually beat towards the oral pole. According to Ludwig (**22,** p. 605) the gastrula of C. planci is complete at the end of the second day, while Des Arts (**2,** p. 8) records that in C. frondosa the gastrula is formed as late as on the tenth day. Newth (**36,** p. 633) noticed in C. saxicola that the direction of rotation mostly changes, and at the gastrulation is the reverse of that seen in the blastula.

The invaginated pit is beset with especially long marked cilia (Pl. 8, figs. 6, 7, *c*), which remain forming a bundle attached to the end of the archenteron for some period, still being visible even when a slight twisting has occurred in the archenteron (Pl. 8, fig. 8, *c*). The cells of the archenteron increase very actively, which fact is shown by many mitotic figures lying always near the surface and parallel to it (Pl. 8, fig. 7). The top of the archenteron shows no definite cell boundaries on the side towards the blastocoele; it here assumes the

appearance of a syncytium. Mitotic figures are found here and, as a result of rapid proliferation, the cells of the distal part detach themselves and move into the blastocoele. These detached cells continue to multiply after being free in the blastocoele. While the free mesenchyme cells are amoeboid in shape, the dividing ones are readily distinguishable by their rounded shape (Pl. 8, fig. 10).

While rapidly increasing in body length, no mitotic figures are found in the ectoderm. The cells here seem simply to decrease in height and to extend in surface. It is thickest at the hind end near the blastopore, gradually thinning out as it approaches the apical end. The nuclei lie near the internal surface in the hinder half, while in the apical half they are nearer to the outer surface. Only in abnormal embryos, which grow to an enormous size without developing beyond the gastrula, were many mitotic figures found in the ectoderm.

When the gastrula reaches its full length the archenteron almost exceeds half the length of the whole body continuing active cell-division. Selenka (**45,** p. 164) noticed that the archenteron lies in H. tubulosa near the future ventral side, and Ludwig (**22,** p. 605) also found in C. planci that it bends slightly ventrad. I have noticed no such feature in the case of C. echinata. At about this stage the archenteron begins to flatten in the anterior portion, and then an unequal growth of the wall occurs, resulting in the characteristic twisting of the hind part.

The above-mentioned bundle of long cilia at the bottom of the archenteron remains until about this stage. It then seems to disappear, and in the same place the archenteric wall now begins to bud off cells into the lumen of the archenteron (Pl. 8, fig. 9, *bl*). The cells thus liberated into the archenteron, sometimes called ' blood corpuscles ', vary in amount according to different individuals, in some being tolerably numerous while there are none at all in others.

Archenteron.—The archenteron of the fully developed gastrula is very characteristically twisted in the sinistrorse direction, and may be described in three parts : the flat expanded

free end, the transverse ring-shaped middle part, and the longitudinal tubular end opening at the blastopore (Pl. 8, figs. 8, 11, 12, ar_{1-3}).

The first part lies perpendicularly to the frontal plane (Pl. 8, fig. 11 A, ar_1), and its posterior end approaches the left side (Pl. 8, fig. 12 A, ar_1). The frontal plane is determined from the position of the stomodaeum, which soon afterwards makes its appearance. This part is round in outline, with thickened walls near the centre, thinning out towards the periphery, making the internal lumen appear usually as a slender dumb-bell shape in transverse section. This resembles the feature found by Newth in a younger gastrula of C. normani (**36,** p. 635). It is continuous at its postero-ventral end with the second part.

The second part runs transversely round the dorsal side, across the mid-dorsal line, and bends ventrad on the right side of the body, slightly turning anteriorly (Pl. 8, figs. 11 B, 12 B, ar_2). The wall is not very thick, the lumen being somewhat compressed, with the greater diameter along the body-axis.

The third part is directly continuous with the second at the ventral end of the right limb of the latter. It runs posteriorly, and is slightly oblique to the left (Pl. 8, fig. 12 A, ar_3). In transverse section it is round, containing a narrow, often almost obliterated, internal lumen (Pl. 8, fig. 11 c). The posterior end is continuous with the blastopore.

The internal surface of the archenteron is probably lined with cilia all over, though I could not demonstrate their presence in sections.

Selenka (**45,** p. 170) observed in C. planci that when the tip of the archenteron reaches the centre of the blastocoele it begins to bifurcate. The dorsal branch increases in size very rapidly, lying obliquely forward and ventrad, while the other ventrally situated branch remains short. This stage is said to have been met with on the fourth day. According to Newth (**36,** p. 635 ; Pl. 8, fig. 8) the circular flat archenteron of the gastrula of C. normani turns to bend in an S-shape at right angles to its plane of flattening, and the anterior flattened sac lies obliquely to the body-axis.

My own observation on the specimens of C. planci brought back from Naples by the late Professor Mitsukuri shows clearly that the archenteron is twisted exactly in the same manner as in C. echinata. It seems to me highly probable that Selenka's figure (Pl. xl, fig. 21) was obtained from a thick section, as he was apparently unable to get a good series of well-orientated sections. His figure is said to represent a sagittal section, but really it is a frontal one. From Newth's figure of a longitudinal section of a forty-fourth-hour gastrula of C. normani (Pl. 8, fig. 8) it is obvious that the archenteron is not simply folded in an S-shape, but is twisted in a spiral. The figure, too, is a frontal section, I believe, not a sagittal one as he supposed.

It was found by Edwards (**12,** p. 213) in Holothuria floridana that by the twenty-second hour a plug of cells grows out from the blind end of the archenteron towards the blastopore, and that by this plug the archenteron is divided into the dorsal and ventral branches. No such changes were observed by Selenka in H. tubulosa.

In C. planci the position of the blastopore changes, accord- to Selenka, slightly towards the future dorsal side, but according to Ludwig it is said to shift ventrad. I could not decide which of the two holds true in my case. In most cases the blastopore opens at the hind end.

Stomodaeum. The stomodaeum makes its first appearance in the quite old gastrula, where the archenteron begins to divide into hydro-enterocoele and gut (Pl. 8, figs. 13, 14 A, *st*). It is preceded by a thickening of ectoderm on the ventral side at about the middle of the body (Pl. 8, fig. 11 A, *sy*). This is partly due to a sinking down of the ectodermal cells and partly to an accumulation of the multiplying mesenchyme cells. Here a syncytium is formed, the internal surface of the ectoderm not being a definite one, touching the ventral edge of the flattened part of the archenteron. The surface of the latter is still clearly cut, no mitotic figures being found on this side.

The stomodaeal depression then comes in sight a little on the left side of the plane in which the flattened part of the archen-

teron has been lying (Pl. 8, fig. 14 A, *st*). These changes very much resemble the mesenchyme-formation and the invagination process occurring in the late blastula. Only in this place does Ludwig's opinion, that the ectoderm shares in the mesenchyme-formation, seem to be true.

This author observed in the third-day larva of C. planci that the stomodaeum appeared on the ventral side immediately behind the pre-oral hood (**22,** p. 606). According to Newth (**36,** p. 634), the stomodaeum appears in C. saxicola and C. normani in forty-eight hours, i.e. on the middle of the third day, as a crescentic invagination at the junction of the opaque and less opaque regions. The horns of the crescent extend backwards and ultimately fuse up and the enclosed area sinks in. It lies very obviously to the left of the mid-ventral line as determined by the pedicels. Similarly a crescentic depression appears in the second-day embryo of H. floridana, according to Edwards (**12,** p. 213), but it gradually deepens and straightens, growing out to either side until it extends entirely across the ventral surface. The plane in which the groove lies is at an angle of 50° with the sagittal plane of the adult holothurian.

11. Dipleurula.

Under the term 'dipleurula' I mean the stage which connects the gastrula with the barrel-shaped larva or doliolaria. This stage is characterized by remarkable changes occurring in the archenteron accompanied by a rapid increase of the mesenchyme cells. As seen from the exterior the larva has become slightly shorter than in the foregoing stage, the stomodaeum has appeared, and it differs from the next stage in having no ciliary bands. This stage is passed during the thirtieth to fortieth hours, i.e. from the end of the second day till early in the morning of the third.

Ludwig (**21,** pp. 274–5) suggested that there might be a stage, reminding us of the auricularia, during the changes which take place between the gastrula and the barrel-shaped stage. In his study of C. planci (**22,** p. 606) he pointed out the fact that the buccal cavity has at the beginning a garland-

shaped thickening on its edge, which he believed to be homologus with the ciliary band of the auricularia. I was unable to verify Ludwig's opinion, but the stage which I call dipleurula is homologous with the auricularia in respect to the arrangement of the internal vesicles.

The arrangement of the ciliary bands as well as the degrees of development of the alimentary tract cannot, I believe, help us in discussing homologies among different forms, because they vary in degree according to different modes of living. In the free-living auricularia of Labidoplax digitata the alimentary canal is well differentiated into fore-, mid-, and hind-gut, and the ciliary band is typically developed, as is well known from the records of many observers. The embryo of Synaptula hydriformis developing inside the mother's body-cavity retains an elliptical shape of body, showing no trace of any ciliary band, the gut being quite rudimentary (Clark, **7**, p. 62). Another viviparous form, Chiridota rotifera, shows an intermediate feature between the above two (Clark, **9**, p. 501).

The division of the twisted archenteron in the old gastrula occurs first at the postero-ventral end of the second part, where a solid cell-mass with obliterated lumen connects the divided portions for a while (Pl. 8, fig. 13). The larger vesicular portion, consisting of the first and second parts of the archenteron is now to be called hydro-enterocoele or vaso-peritoneal vesicle, while the smaller one which was the third part is the future gut. The latter has a very narrow lumen, in most cases being still continuous with the exterior through the blastopore.

The next change occurring in the larva is the displacement, change of shape, and division of the hydro-enterocoele, and the enormous multiplication of the mesenchyme cells which fill up the blastocoele, so that no external examination of the internal structure on clarified material is now possible.

The anterior part of the hydro-enterocoele, which in the late gastrula was concave on the right side (Pl. 8, fig. 14 A, ar_1), now moves round to the right across the dorsal side and becomes narrow in breadth (Pl. 8, fig. 16 A, *hy*). The posterior part

of the same vesicle, on the contrary, moves to the left through the dorsal side (fig. 16 c, *en*), and, as the two parts thus move in opposite directions, they gradually begin to be cut off from each other a little on the left side (Pl. 8, fig. 15).

The anterior part, which will give rise to hydrocoele (*hy*), gives out from the postero-dorsal margin obliquely backwards a conical process which finally unites with the dorsal ectoderm (*pc*). This is the rudiment of the pore-canal. The posterior part, which is the future enterocoele (*en*), is a little smaller than the anterior part and lies on the left side, extending round the body-axis and stretching from the antero-ventral to the postero-dorsal side.

The walls of the hydrocoele and enterocoele consist of a single layer of cells, clearly distinct from the free mesenchyme cells, and the latter do not yet attach themselves to the surface of the former.

The first observer who traced the fate of the archenteron was Selenka (**45,** p. 170). He noticed that in C. planci the archenteron bifurcated at the top, and that the dorsal branch increased rapidly in size, bending obliquely antero-ventrad, and at last becomes separated as a vaso-peritoneal vesicle from the other branch, which latter was stunted and later gave rise to the gut ('Körperdarm'). In my opinion, his two branches are a complete vaso-peritoneal vesicle, and he seems to have overlooked the separation of the gut from that vesicle. He further stated that after the separation of the two vesicles the vaso-peritoneal vesicle shifted to the left side of the gut, while the latter rapidly grew forwards and at last united with a ventral invagination ('Munddarm'). He is right in saying that the first part then lies on the left side, but that the gut breaks through to the stomodaeum is improbable at such an early stage, and moreover, the part which he called 'Körperdarm' is, I think, to be identified with the enterocoele, which should never have any communication with the stomodaeum at all. A careful comparison of his figs. 21, Pl. xi, and 22 B, Pl. xii, leads one to conclude that the part he denoted P (enterocoele) in the fig. 22 B is derived from that part denoted B in the fig. 21.

His fig. 22 B resembles very much what I observed in C. echinata in a corresponding stage.

Ludwig (**22,** p. 605) observed in the third-day larva of C. planci that the hydro-enterocoele had separated from the rest of the archenteron, and again divided into the hydrocoele and two enterocoele vesicles. Some larvae were somewhat younger, and in them the hydro-enterocoele was still in connexion with the archenteron. In these statements he seems to have been unable to give the time and sequence of the separations of those vesicles. Selenka was of the opinion that the hydro-enterocoele, at first as long as it is connected with the gut, lies on the dorsal side of the latter, but shifts to the left side about the time when the separation sets in. Ludwig observed, contrary to Selenka, that it had been lying on this side from the beginning. I agree with Ludwig on this point.

Newth (**36,** p. 635) could not be sure about the breaking off of the archenteron in C. saxicola and C. normani, being only able to say that the water-vascular system, the posterior (perivisceral) coelom, and the gut are separated from successive regions of the archenteron in the order named, beginning at the anterior end. In C. normani the separation of the enterocoele was complete by the middle of the third day, though in some individuals the hydro-enterocoele connexion was not then broken.

The hydrocoele then increases in breadth again, stretching round the right side of the body, and its free anterior margin begins to divide into lobes, which are indistinct at first but rapidly become distinct processes. These changes as well as those of the enterocoele to be mentioned below vary very much according to different individuals. The following statements seem, however, to represent what is most frequently met with.

Hydrocoele.—In the beginning three lobes are formed (Pl. 9, fig. 17, *hy*). The first is narrow, situated at the left anterior corner, the second is broad, formed of the greater part of the anterior margin of the hydrocoele, and the third is again narrow, situated on the right ventral edge of the hydrocoele

directed transversely. The second broad part then divides into three lobes almost equal in breadth, while another lobe arises on the left edge, behind the first lobe (Pl. 9, fig. 19). Thus the hydrocoele is now fan-shaped, with a narrow conical process directed postero-dorsally, which is the future pore-canal, and an expanded anterior margin, wavy with six lobes as just described. From that transverse lobe, at first numbered third, is formed the mid-ventral radial canal (*mv*), while the other five lobes are rudiments of the five primary tentacles (*t*). Except the mid-ventral one no other radial canals appear at this stage. The pore-canal opens to the exterior through the dorsal pore about the end of these changes (*dp*).

Kowalewsky (**17,** p. 4) and Selenka (**45,** p. 171) are of the same opinion that, in C. kirchsbergii and C. planci respectively, there are first formed only three tentacles situated dorsally, and the remaining two appear after the hydrocoele ring has closed. Ludwig (**22,** p. 608) found, on the contrary, that in C. planci the five primary tentacles appear simultaneously as outgrowths of the radial canals. This divergence in view from other observers results probably from the fact that he originally dealt with eighth-day larvae without examining any earlier stages. I agree with Kowalewsky and Selenka in assuming the appearance of the tentacles to be earlier than that of the radial canals, but those three lobes which first appeared are, in my opinion, not the dorsal three of the primary tentacles. Ludwig's account of the early features of the hydrocoele is very incomplete owing to the lack of any intermediate stages. According to him the hydrocoele is of an irregular horseshoe shape, whose arched part lies dorsally, the right limb is short, stretching obliquely antero-ventrad ('nach vorn und unten'), and the left limb is longer, stretching postero-ventrad ('nach unten und hinten'). I never observed such a condition, and am convinced that he was in error in these statements, from which he drew an incorrect conclusion that the hydrocoele ring probably closed on the right side.

Enterocoele.—Soon after being separated from the hydrocoele the enterocoele divides into two vesicles, one larger and

antero-ventral in position, the other smaller and on the left dorsal side stretching posteriorly (Pl. 9, figs. 17, 18, *le*, *re*). The former corresponds with the left enterocoele of other echinoderms, while the latter, although situated at first on the left dorsal side, is the right enterocoele. Selenka (**45**, p. 171) noticed in C. planci that the peritoneal vesicle (enterocoele) divides, immediately after being separated from the vascular vesicle (hydrocoele), into two ellipsoid vesicles lying on the right and left sides of the gut respectively. Likewise in H. tubulosa the enterocoele which stretches behind and below the gut divides into two vesicles which lie symmetrically on each side of the gut.

Stomodaeum.—The stomodaeum is formed by an encircling of the slit-like depression and a sinking down of the included area. It contains a thin lumen, extending parallel to and below the external surface, which opens through a narrow orifice to the exterior (Pl. 8, fig. 16 A; Pl. 9, fig. 18 A, *st*). The syncytium (*sy*) extending over the stomodaeum grows between the hydrocoele and enterocoeles to form a solid cell-mass running backwards to join with the gut. The gut is of a single layer of cells but very thick, leaving a narrow lumen inside (Pl. 9, fig. 18 B, *g*).

12. Doliolaria.

The doliolaria, or barrel-shaped stage, is reached about at the fortieth to the fiftieth hour, i.e. on the third day. This is characterized by the acquisition of three transverse ciliary bands on the posterior half of the body, the appearance of rudiments of the pedicels, and the further development of the five primary tentacles and radial canals. This stage lasts until the fourth day or even the eighth day or more.[1]

The larva measures above 500 μ in length, and swims usually immediately beneath the surface of the water, being either vertical or oblique in position. Cilia beat usually towards the posterior

[1] My own culture showed no evidence of changing into the pentactula-stage even on the eighth day, when I had to leave Misaki and could not follow any further changes.

end but often reverse, which latter movement makes the larva sink to the deeper part of the water. Besides these two kinds of locomotion, rotation around the body-axis is observed at the same time. In no case is the pre-oral hood directed downwards.

Although no marked change is visible externally, the latter half of the stage had better be treated under a distinct heading, Metadoliolaria, owing to its internal changes. Here in the present chapter I will confine myself to the earlier part, doliolaria in the narrow sense.

In the corresponding stage of C. frondosa, Danielssen and Koren (**11**) found that rudiments of the tentacles appear on the tenth day and a pair of the primary pedicels on the twentieth. Des Arts (**2**, p. 9) observed in the same species that the larva measures on the fifteenth day 510 μ by 375 μ, and that the tentacles are visible in section on the twenty-first day, but are observable externally so late as on the twenty-fourth day, and the pedicels make their first appearance on the thirty-seventh day. The internal structure of the doliolaria of C. kirchsbergii was described and figured by Kowalewsky (**17**, fig. 12). The same author gave an external view of the larva of C. planci (figs. 16, 17), while Selenka (**45**) and Ludwig (**22**) made much closer observations. From the observations of Newth (**36**), we gather that the corresponding stage in C. saxicola and C. normani is not distinguishable externally from lack of the ciliary bands which are so characteristic of the stage in other species.

Ciliation of the Ectoderm.—The presence of three, very rarely four, transversely-running bands of cilia is a very marked character of doliolaria. They seem to appear simultaneously. The most anterior band lies about on the middle of the body (Pl. 9, fig. 25, c_1), the second and third run parallel to the former and in such a way that they divide the posterior half of the body into three equal divisions, or, as is often the case, the hindermost third is a little broader than the other two (c_2–$_3$). In preserved specimens the cilia are extremely difficult to make out, but they can easily be found in the living state.

The length, as roughly measured, is about 25 μ. Much weaker cilia are found uniformly covering both the parts anterior to the first ciliary band and that posterior to the third. The areas lying between the bands seem to be devoid of them.

In C. planci the ciliary bands present are four (Kowalewsky) or very rarely five (Selenka) in number, besides the uniform ciliation all over the pre-oral hood and anal field. After the appearance of pedicels and tentacles these uniform weaker cilia disappear (Selenka, **45,** p. 172). Mortensen (**33,** pp. 23–4; Pl. i, fig. 8, *a*, *b*, *c*) could not ascertain the presence of ciliary bands in preserved specimens of doliolaria which are about 1 mm. long, of a light reddish colour, and which were found in the Southern Kattegat. He referred them to Psolus phantapus and suspected the presence of three ciliary bands. I am much inclined to believe that there are four bands running along the circular spaces free from calcareous bodies (fig. 8, *c*). In C. frondosa we have no record of ciliary bands (Danielssen and Koren, **11**; Des Arts, **2**), and in C. kirchsbergii the bands seem to be really absent (Kowalewsky, **17**). Doliolariae of C. saxicola and C. normani are ciliated uniformly all over as in other stages, no segregation of cilia into bands being found (Newth, **36**). The larva of Phyllophorus urna, too, shows no zonary distribution of cilia while actively swimming inside the mother's body-cavity (Kowalewsky, **17,** p. 7).

When examined in section, the cilia are very obscure and markedly short, due to shrinkage. The ectoderm is thickened at the band, being about twice as thick as other parts, and of a lens shape in transverse section (Pl. 9, fig. 25, c_{1-3}). The nuclei are situated near the base of the cells. As to these cells I could find no distinction between 'Wimperzellen' and 'Reservezellen' as Reimers (**43,** 1912, p. 270) did in his observations on the larva of Labidoplax digitata. Further, I could not clearly make out either 'Binnenfaser' or 'Basalstäbchen' as clearly figured by him (Pl. ii, figs. 5–9).

Hydrocoele.—The lobe no. 6 of the hydrocoele, as numbered from the dorsal one towards the ventrum, stretches

out ventrad, bringing together with it the lobes nos. 4 and 5, and on reaching the mid-ventral line it turns posteriorly to give rise to the mid-ventral radial canal (Pl. 9, fig. 21 A, *mv*). The other lobes except no. 1 are now differentiated into cylindrical tubes—primary tentacles (t_{2-5})—connected at the base by a rather narrow canal, which forms a horseshoe-shaped rudiment of the ring canal. The lobe no. 1 eventually gives rise both to the remaining one of the primary tentacles and to the free end of the dorsal limb of the open hydrocoele ring. It remains for a while as an inconspicuous outgrowth.

Radial Canals.—The free end of the rudimentary mid-ventral radial canal then dilates laterally to form a rhombic vesicle in ventral view, and then takes on a cross shape (Pl. 9, fig. 23 A, *mv*). The transverse branches thus formed are the primary pedicel canals (*rpc*, *lpc*), and in correspondence with each of them a rudiment of the primary pair of pedicels (*rp*, *lp*) is formed.

The four radial canals, other than the mid-ventral one, are formed comparatively late, especially the ventral pair (*rd*, *ld*, *rv*, *lv*). They arise at first as small knobs on the anterior margin of the ring canal, one in each interval of the primary tentacles. The knobs then bend outwards and immediately turn posteriorly, and they are remarkably thin as compared with the mid-ventral one.

Kowalewsky (**17**, p. 4) first described in C. kirchsbergii the first appearance of the mid-ventral radial canal, which soon divided into two, pushing the body-wall outwards to form a pair of pedicels. In C. planci Selenka (**45**, p. 171) ascribed the development of the mid-ventral radial canal to too late a period, stating that it made its first appearance soon after the closure of the hydrocoele ring, and that four other radial canals and the Polian vesicle followed it. As to the fact that the four radial canals, other than the mid-ventral one, appear later than the latter, all observers are unanimous. Ludwig (**22**, p. 181) further noticed that among those four the ventral pair are shorter and narrower than the dorsal pair, the difference being observable throughout the life of the young; they grow to be equal much later on.

Primary Pedicels.—A pair of the primary pedicels are first indicated by circular pits formed on the ectoderm corresponding to the pedicel canals branching from the mid-ventral radial canal (Pl. 9, fig. 22 D, *p* ; fig. 24 F, *lp*, *rp*). These I may call pedal pits. The formation of the pedal pits a good deal resembles that of the stomodaeum, the rudiment of the pedicel being formed by the syncytium below the pit and arising from the bottom of the pit covered by the ectoderm. Finally it projects from the pit, the latter being soon flattened out. These changes strikingly resemble those found in the primary tentacles. The pits are situated between the second and third ciliary bands and at an angle of about 40° on each side of the sagittal plane. Specimens are often found in which only one of the pair has just appeared. Of six cases of such specimens I observed all had only the left pedicel (Pl. 9, figs. 21, 22, *p*). Out of seventeen cases where both the pedicels had appeared, eight cases showed that the left pedicel lies more or less anteriorly to the right one, while seven cases were the reverse, and in the remaining two cases the two lay on the same level. Thus we can find no constant feature as to the relative position of the two primary pedicels.

According to Ludwig (**22**, pp. 185, 607), in C. planci the pedal pits appear in most cases near the end of the fourth day, and the pair of pedicel canals appear from the mid-ventral radial canal either at the end of that day or early on the next. Of the pair of pedal pits the right one always lies a little anterior to the left one, and the same holds true in Phyllophorus urna as observed by the same author (**24**, p. 97). Newth (**36**, p. 637) found in the third-day larvae of C. saxicola and C. normani that the posterior end of the mid-ventral radial canal formed a rhombic dilation representing the rudiments of pedicel canals. Here, he says, the left pedicel lies further forward than the right, just contrary to the feature seen in C. planci and Ph. urna.

From Edwards's observation (**12**, pp. 222–3) we learn that in H. floridana the first pedicel is unpaired and appears at the posterior end of the mid-ventral radial canal. It is said

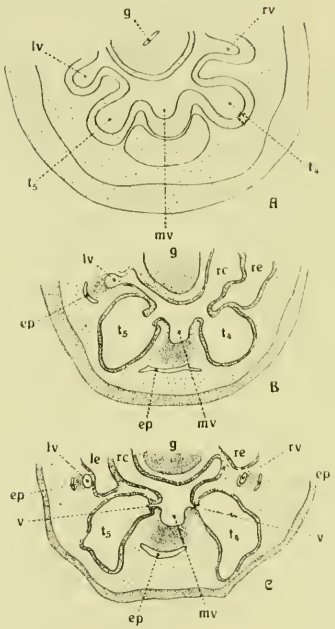
that J. Müller had observed in the young developed from 'Auricularia mit Kugeln' that the first pedicel was put forth on the right from the mid-ventral radial canal (Ludwig, **21,** p. 291).

Primary Tentacles.—Now the five rudiments of primary tentacles can be referred to the respective interradius (Pl. 9, figs. 23 A, B ; 24 A–D ; t_{1-5}). No. 2 (t_2) lies in the mid-dorsal interradius, no. 3 (t_3) in the right dorsal, no. 4 (t_4) in the right ventral, no. 5 (t_5) in the left ventral, while the remaining one, no. 1 (t_1), which should arise from the dorsalmost lobe, and is the last to appear, is in the left dorsal interradius.

In their early stages the five primary tentacles arise directly from the ring canal, or more properly speaking, the hydrocoele differentiates into the tentacle-rudiments and the ring canal. In the course of growth a peculiar grouping of tentacles begins to appear in relation to the radial canals (Text-fig. 4, A–C). It seems very probable that the rudiments of the radial canals develop at the expense of the adjoining parts of the hydrocoele ring, attracting towards them the bases of the tentacle-rudiments, so that the latter seemingly become branches given out from the radial canals, totally independent of the ring canal. The grouping of tentacles is precisely the same as that found by Ludwig (**22,** pp. 183, 608) in C. planci, i.e. the ventral pair gather at the mid-ventral radius, that of the right dorsal interradius moves towards the right dorsal radius, and the remaining two meet with the left dorsal radial canal (Text-fig. 9 A, I, I′). Ludwig insisted upon the opinion that all the five primary tentacles appeared simultaneously as branches of the radial canals, not directly from the ring canal. One must keep in mind that Ludwig's first observations were made on the eighth-day larvae, which are quite well established doliolariae, and that in his second report he tried to trace the fact as far back as to the fourth-day larvae. Newth (**36,** p. 637–8), confirming Selenka's view, claims that the primary tentacles are originally interradial in position, arising from the ring canal directly and alternating with the radial canals. He found that some individuals had shown on the third day that curious grouping

of the three dorsal tentacles, while still on the fourth day the ventral two retain their original interradial position, their bases, however, beginning to approach the mid-ventral radial canal. According to Edwards (**12,** p. 216) the feature is quite different in H. floridana. Here on the fourth day each

TEXT-FIG. 4.



Cross-sections cut at the height of the ring canal, to show the gradual displacement of primary tentacles. A. Doliolaria, reconstructed from several sections. B. Metadoliolaria. C. Still advanced metadoliolaria. ×200. *ep* = epineural canal; *g* = gut; *le* = left enterocoele; *lv* = left ventral radial canal; *mv* = mid-ventral radial canal; *rc* = ring canal; *re* = right enterocoele; *rv* = right ventral radial canal; $t_{4,5}$ = primary tentacles; *v* = ventilating apparatus.

of the mid-ventral and the left dorsal radial canals produces a tentacle on the right side, and each of the paired ventral radial canals sends out one on the dorsal side. Of these four, the former two seem to be the first to develop. The fifth appears

last on the left side of the mid-ventral radial canal (Text-fig. 9B, I, I′); this attains a size equal to the other four as late as the seventh day. Becher (**3**, 1908, p. 4) attributes this difference between Cucumaria and Holothuria to the higher tentacle number of the latter in the adult. This must not be rashly concluded since the feature in question has not yet been recorded in other many-tentacled forms such as Phyllophorus, Pseudocucumis, &c. Apart from the difference in the sequence of their appearance, the distribution of the primary tentacles with regard to the radii does not differ very much in Cucumaria and Holothuria. Indeed, the two agree in the circumstance that the tentacles alternate with the radial canals as pointed out by Newth (p. 639). The only notable difference is that in Cucumaria each of the pair lying in the dorso-lateral interradii is supplied by a tentacular canal sent ventrad from the dorso-lateral radial canal of each side, while in Holothuria the corresponding tentacles are supplied by branches sent dorsad from the ventro-lateral radial canals. Now, if we are right to admit that the primary tentacles all originate directly from the ring canal, but not from radial canals, such difference as found between these two genera does not seem to me so great and fundamental. As will be shown later, in Cucumaria the dorsal pair of radial canals grow faster than the ventral pair, so that the bases of the tentacles in question shift dorsally and at last become branches of the dorsal pair. In Holothuria, on the contrary, the ventral pair of radial canals, being more vigorous in growth than the dorsal pair, might have conquered in pulling together the bases of those tentacles. As a matter of fact, in Holothuria the ventro-lateral radial canals are in adult state generally more strongly developed than the dorsal pair, while in Cucumaria these two pairs differ very little. Another common feature in both cases is that two of the primary tentacles belong to the mid-ventral radial canal. It is a noteworthy fact that even in such a form as C. echinata, whose ventral pair of tentacles are markedly smaller than the remaining eight in the adult stage, the former are represented

in the five primary tentacles (compare Becher, **3**, p. 4). It is an open question how the mid-ventral radial canal behaves in the early stages of Sphaerothuria bitentaculata, which possesses only eight tentacles when adult, the mid-ventral radial canal supplying no tentacles at all.

Polian Vesicle.—The Polian vesicle appears at the free end of the ventral limb of the open hydrocoele ring, directed posteriorly and lying inside the enterocoele vesicle (Pl. 9, figs. 23 A, B ; 24 D ; *pv*). It may often appear after the closure of the ring.

Stone-Canal.—About at the same time as the appearance of the Polian vesicle the pore-canal swells out dorsally at its middle part, and as a result of it the canal slightly bends at this point (Pl. 9, fig. 25, *as*). In transverse section this swelled part shows a very characteristic feature in that the wall of the axial side is of a very high epithelium, while along the dorsal side the wall is very thin. Bury (**5**, 1889, p. 427 ; Pl. xxxix, fig. 26) first noticed this structure in a Cucumaria, and considered it as a vestigial anterior enterocoele, the presence of which he had proved in auricularia. Ludwig (**22**, p. 609) observed it in the fifth-day larva of C. planci and called it the madreporic vesicle, with an assumption that it is only a secondary outgrowth. In his later paper Bury (**6**, 1895, pp. 53–4) insisted upon his former view, and suggested that future and closer examinations would reveal changes similar to those in auricularia, proving its origin from the enterocoele. According to Newth (**36**, p. 637) this enlargement occurs by an up-pushing of the antero-dorsal wall of the canal on the third day in C. saxicola and C. normani. On the next day the cells of the antero-dorsal wall of this vesicle become large and clear. This swelling up of the cells seemed to him to be a preliminary stage in the thinning out of the part as seen by Bury, Ludwig, &c. I was unable to find either the change which Bury suggested to be present or the swelling up of the cells in the early stages of this structure. The same structure has further been proved to be present in Phyllophorus urna by Ludwig (**24**, p. 98) and by Russo

(**44**, p. 45; Pl. iii, fig. 52), in Holothuria floridana by Edwards (**12**, p. 214), in Cucumaria crocea and another antarctic Cucumaria by MacBride (**25**, pp. 7, 8; **27**, p. 4). The last-named author called this vesicle the axial sinus.

The distal portion of the canal, which should now properly be called the pore-canal, runs through the dorsal body-wall and opens to the exterior. The opening, or dorsal pore, is situated between the second and third ciliary bands, and is in most cases slightly on the right of the mid-dorsal line (Pl. 9, fig. 22 B, *dp*; fig. 24 E, *pc*). Ludwig (**22**, p. 186) also found that the pore opens on the right.

Closure of the Ring Canal.—From the fact that the rudiment of the left ventral radial canal appears on the ventral limb of the open hydrocoele ring, while that of the left dorsal radial canal belongs to the dorsal limb of the same, it is clear that the closure of the ring occurs on the left dorsal interradius. The Polian vesicle lies at first very near to the left ventral radius, but later it moves towards the middle of the dorsal interradius, which is its normal position as found in adult individuals.

As to the time and position of the closure of the ring no entirely satisfactory observations have been given. Kowalewsky (**17**, p. 4) and Selenka (**45**, p. 171) were in agreement in the opinion that the ring closed after the formation of three dorsal tentacles, while the remaining two developed from the closed ring. Ludwig (**22**, p. 607) observed in C. planci that the ring was complete at the end of the fourth day, and the closure seemed to have taken place on the right side of the body. From this incorrect view he concluded that the Polian vesicle which lay on the left dorsal interradius could not be an indication of the point of closure. Newth (**37**, p. 637) is quite right in concluding that in C. saxicola the ring closed in the left dorsal interradius on the third day, when the radii can be identified. He could not determine to which limb of the free ends of the unclosed ring the rudiment of the Polian vesicle belonged, being only able to say that it

was found as a small blunt outgrowth produced at the point of closure.

From the table on p. 215 the following features may be summarized :

1. Of the five primary tentacles the one situated in the left dorsal interradius appears last (nos. 1–4).

2. To the four tentacles and one mid-ventral radial canal the right dorsal radial canal is first added (nos. 2–4).

3. The left dorsal radial canal appears at about the same time as the appearance of the fifth tentacle, after which the right ventral radial canal follows immediately (nos. 5–7).

4. The appearance of the left ventral radial canal is still later (no. 10).

5. The appearance of the Polian vesicle in some cases precedes the closure of the ring canal (nos. 8, 9, and 12), and in others it is later (nos. 6, 11, 13, and 15).

6. The formation of the axial sinus also in some cases precedes the closure of the ring (nos. 8, 9) and in others it is later (nos. 6, 11).

7. The closure of the ring takes place in most cases after five radial canals have all appeared.

Stomodaeum.—Now the position of the stomodaeum can be determined by the establishment of the mid-ventral radial canal. It lies in front of the first ciliary band, and at about 30° to the left of the sagittal plane. The ectoderm covering the interior of the atrial cavity is pushed up by the growing tentacles, forming an epidermal covering for the latter (Pl. 9, fig. 25, *at*). The orifice is often found plugged up by the left ventral tentacle which lies nearest to the stomodaeum (Text-fig. 5, t_5). Newth (**36,** p. 634) noticed the asymmetrical position of the stomodaeum, while Ludwig (**22,** p. 610) observed the same fact but interpreted it erroneously. He was of the opinion that the larval symmetry plane is not coincident with that of the adult, and thought that the left ventral tentacle stands nearest to the mid-ventral line.

Simultaneously with the growth of the primary tentacles and the diminution of the pre-oral hood, the stomodaeum

Table II.

To show the development of the hydrocoele appendages and the time of closure of the ring canal.

In the table are indicated whether the tentacles, Polian vesicle, and axial sinus are formed (×) or not yet (–); whether the ring canal is still open (*o*) or closed (*c*); whether the rudiments of radial canals are only buds (*b*), or directed anteriorly (*a*), or laterally outwards (*l*), or posteriorly (*p*); whether the mid-ventral radial canal ends simply in the form of a knob (*k*), or is slightly expanded into a rhombic vesicle (*r*), or has formed a pair of lateral branches, pedicel canals (*pd*).

			Primary tentacles.					*Radial canals.*						
No.	*Age in days.*	*Ring canal.*	1 (*ld.*)	2 (*md.*)	3 (*rd.*)	4 (*rv.*)	5 (*lv.*)	*LD.*	*RD.*	*RV.*	*MV.*	*LV.*	*Polian vesicle.*	*Axial sinus.*[1]
1	2	*o.*	—	×	×	×	×	—	—	—	*k.*	—	—	—
2[2]	1·5	*o.*	—	×	×	×	×	—	*a.*	—	*k.*	—	—	—
3	2	*o.*	—	×	×	×	×	—	*a.*	—	*r.*	—	—	—
4	2	*o.*	—	×	×	×	×	—	*l.*	—	*k.*	—	—	—
5	2	*o.*	×	×	×	×	×	*b.*	*a.*	*l.*	*k.*	—	—	—
6	2	*c.*	×	×	×	×	×	*a.*	*l.*	—	*k.*	—	—	—
7	2	*o.*	×	×	×	×	×	*l.*	*l.*	*a.*	*r.*	—	—	—
8[3]	2	*o.*	×	×	×	×	×	*p.*	*p.*	*l.*	*pd.*	—	×	×
9	2	*o.*	×	×	×	×	×	*p.*	*p.*	*l.*	*pd.*	—	×	×
10	2	?	×	×	×	×	×	*p.*	*p.*	*l.*	*pd.*	*b.*	×	×
11	2	*c.*	×	×	×	×	×	*l.*	*p.*	*l.*	*pd.*	*a.*	—	—
12[4]	2	*o.*	×	×	×	×	×	*l.*	*p.*	*l.*	*pd.*	*l.*	×	—
13	2	*c.*	×	×	×	×	×	*p.*	*p.*	*l.*	*pd.*	*a.*	—	×
14	2	*c.*	×	×	×	×	×	*p.*	*p.*	*l.*	*pd.*	*a.*	×	×
15	3	*c.*	×	×	×	×	×	*p.*	*p.*	*p.*	*pd.*	*p.*	—	×
16	3	*c.*	×	×	×	×	×	*p.*	*p.*	*p.*	*pd.*	*p.*	×	×

[1] The pore-canal is not yet open dorsally in no. 1, but no. 2 and all subsequent specimens have the dorsal pore open.

[2] This specimen is shown in Pl. 9, figs. 21, 22.

[3] This specimen is shown in Pl. 9, fig. 25.

[4] This specimen is shown in Pl. 9, figs. 23, 24.

gradually widens and at last flattens out, so that the tentacles freely protrude above the body-surface.

Alimentary Canal.—The rudiment of the gut has been growing both in length and diameter by rapid cell-division and by increase of the cells in height. Its anterior end extends beyond the ring canal by which it is encircled (Pl. 9, fig. 25, *g*), and while both ends remain solid its middle portion has a distinctly discernible flat lumen lying parallel to the frontal plane (Pl. 9, figs. 24 E, F ; *g*).

Enterocoeles.—Early in the doliolaria stage, where four of the primary tentacles have become apparent, the right and left enterocoeles come into contact with each other at their free margins. Both the enterocoeles have been rapidly growing in size, extending across the median line and encircling the gut. The fusion of their ends takes place on the right side, beginning either at the anterior part or at the posterior part of the line of contact, leaving for a while an oblique incision at either end of the line (Pl. 9, figs. 21, 23 ; *re, le*).

The other ends of the two vesicles approach each other but are separated by a narrow interval. This intervening part gives rise to the dorsal mesentery in the end, and lies at first obliquely on the left side, beginning anteriorly near the mid-dorsal line to end near the mid-ventral line. It, however, gradually bends into an S-shape, indicating the three sections as found in the future mesentery—the first, mid-dorsal and descending section ; the second, oblique and ascending section on the left ; and the third, descending section running along the mid-ventral line.

I failed to find any ' finger-like process ' as seen by Bury (**6,** p. 48) in Synaptids and verified by others. Though very often there appears a process on the antero-dorsal end of the enterocoele, stretching beyond the primary stone-canal to the left, I could not follow its fate, and am uncertain whether the peripharyngeal sinus originates from it or not.

The behaviour of the enterocoeles in C. planci was first observed by Selenka (**45,** p. 171), according to whom the union of the right and left vesicles takes place on the ventral

side so soon after the separation into two from the original single vesicle that he at first overlooked this separation. Ludwig (**22,** pp. 609, 611) observed in the fourth-day larva that the right and left enterocoeles extended around the gut so as to meet and break through on the ventral side, while they remain separate on the dorsal side. At the end of the sixth day the rudimentary mesentery begins to bend, the last section lying on the right ventral side of the body. From Östergren's comparative study in the Dendrochirotae (**41,** 1898) it has been shown that the last section of the mesentery does not lie on the right side of the mid-ventral radius, but with the exception of the Psolinae always on the left side. Ludwig was wrong in this respect.

The above feature is essentially the same in the Synaptids. I may only point out that the pointed ends of the two enterocoeles unite on the right side of the mid-ventral line (Reimers, **43,** p. 280).

Blastocoele Jelly and Mesenchyme.—By the time that the dipleurula is reached the pre-oral hood is filled up with blastocoele jelly. It consists of a structureless gelatinous substance and a few sparsely arranged mesenchyme cells suspended in it. The former stains with plasma-dyes and often shows a netted appearance in some fixatives. This substance is seen most developed in the doliolaria stage, while near the end of the late doliolaria it gradually diminishes, probably being absorbed as nourishment.

Most of the other mesenchyme cells gather thickly around the hydrocoele, enterocoele, and gut, without, however, forming a definite cell-layer of any kind. Others lying below the ectoderm form a loose connective tissue of cutis. Metschnikoff (**29,** p. 4) showed in a Synaptid the origin of the cutis from mesenchyme. Selenka (**45,** p. 169) opposed this view, claiming that mesenchyme gives rise to musculature only. Later, he (**46,** p. 57) corrected his former view admitting that mesenchyme gives rise to connective tissues, and on the other hand that the musculature of some parts originates from other sources than the mesenchyme.

13. Metadoliolaria.

Near the end of the doliolaria stage many important changes occur internally, though but few changes are seen from the outside. When seen externally, the pre-oral hood gradually diminishes in size, and in consequence the tentacular crown shifts anteriorly, calcareous deposits appear while the ciliary bands degenerate, and the tentacles and pedicels become prominent and visible from the outside. The internal changes are: the further development of the hydrocoele appendages into the adult water-vascular system, the differentiation of musculature and nervous tissue, the widening of the enterocoele, &c. This I may call the metadoliolaria stage. The larva now very often sinks to the bottom from its increased specific gravity and degenerated ciliary function.

Water-vascular System.—As was stated by Ludwig and Newth, the ring canal lies a little obliquely in such a direction that its dorsal half approaches the anterior end of the body rather more than the ventral, but I could not find any lateral inclination such as was observed by Ludwig, who stated that the left half is slightly more posterior than the right (**22**, p. 181).

In my culture the tentacles begin to protrude a very little above the surface of the body at the end of the fourth day, and early in the morning of the next day a slow movement was observed, obviously owing to the differentiation of muscle fibres in their wall. The tip is found covered with minute hyaline papillae as known to Selenka, Ludwig, and others (Text-fig. 5, *p*). The ramification occurs on the seventh day. The primary pedicels now protrude as short cylindrical prominences, as clearly seen in the sixth-day larva.

Musculature.—Longitudinal muscle fibres are now to be found below the hydrocoele epithelium in the tentacles and pedicels, and along the radial canals. They appear in the tentacular wall first along the internal (axial) side and then spread around the cavity. Those of the radial canals lie along the

internal (axial) side between the hydrocoele and peritoneal epithelia.

In C. planci Ludwig (**22**) observed the first appearance of muscle fibres in tentacles on the seventh day (p. 612), in pedicels on the tenth day (p. 185), and along the mid-ventral radial canal on the thirteenth day (p. 182). According to him, all these are derived from the hydrocoele epithelium.

Nervous System.—The nervous tissue is well marked in this stage. Immediately below the atrial cavity the ring nerve is formed, encircling the still closed anterior end of the gut. Anteriorly a branch, the tentacular nerve, is put forth in each interradius to run along the oral side of the tentacle. Posteriorly the five radial nerves appear, of which the mid ventral is the strongest. The latter gives out a pair of branches to the primary pedicels.

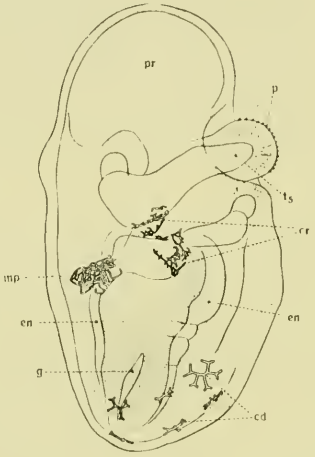
Along the oral side of each tentacle, a part of the atrial cavity extends backwards as a thin flat canal. On reaching the ring nerve, these canals unite with one another to form a circular canal above the former. This is the epineural ring. From this the epineural canal is sent out along each radial nerve (Text-fig. 4, B, C, *ep* ; Pl. 9, figs. 24, A–E, *cnc*).

I can give no further account, and will refer to Ludwig's detailed descriptions on the origin, differentiation, and development of the nervous system given for C. planci (**22**). According to him, the rudiments of the nervous system first appear on the fourth day (p. 608), the epineural ring and canals are formed on the fifth day (p. 609), differentiation of fibrous structure takes place on the sixth day (p. 611), the tentacular nerves are formed on the ninth day, and the pedal nerves are given off on the seventeenth day (p. 188). In C. echinata I could make out all these features even in the fifth-day larva.

Calcareous Deposits.—In my culture calcareous deposits made their first appearance on the sixth day. They occur at three places, i.e. in the wall of the axial sinus (Text-fig. 5, *mp*), at the bases of the tentacles (*cr*), and in the integument of the posterior part (*cd*).

The deposits formed in the wall of the axial sinus consist of a loose basket-work, which forms a short tube opening on both ends surrounding the stone-canal. There is another opening which is directed dorsad, corresponding to the thin-walled part of the vesicle. Ludwig (**23**, p. 27) noticed a similar structure in the pentactula of Cucumaria parva, with a wide opening directed anteriorly.

Text-fig. 5.



Seventh-day metadoliolaria. Right-side view to show calcareous deposits. × 100. *cd* = deposit of integument; *cr* = rudiment of calcareous ring; *en* = enterocoele; *g* = gut; *mp* = axial sinus; *p* = papilla on the tip of tentacle; *pr* = pre-oral hood; t_5 = tentacle.

Those which appear at the bases of the tentacles are a delicate netted ring, giving off a pair of anteriorly-directed pointed processes at each radius. These represent the rudiments of the radial segments of the calcareous ring. It has been shown by Ludwig (**22**, p. 611; **23**, p. 27) and Clark (**7**, p. 67) that the calcareous ring is first represented by five radial segments. In C. echinata it does not consist of five separate pieces but of a continuous ring, as stated above.

Those which appear in the integument increase and develop rapidly and soon cover the body on its posterior half. Their shape is not quite regular, but is commonly a delicate lattice plate formed of successive dichotomous branchings of the original primary cross. They lie parallel to the surface embedded in the dermal connective tissue formed below the ectoderm.

According to Kowalewsky (**17,** p. 6), in C. kirchsbergii the calcareous body first appears in the wall of the stone-canal. Ludwig (**22,** p. 610) found in C. planci that deposits appear on the sixth day at three different places, i.e. the stone-canal, ring canal, and pedicel canal. I failed to notice the last-mentioned part in C. echinata, in which the deposits in the integument are most marked among the three kinds. Mortensen's figure (**33,** Pl. i, fig. 8, *c*) of the larva of Psolus phantapus represents a similar feature, where delicate lattice plates in the integument and the rudiment of the calcareous ring are shown.

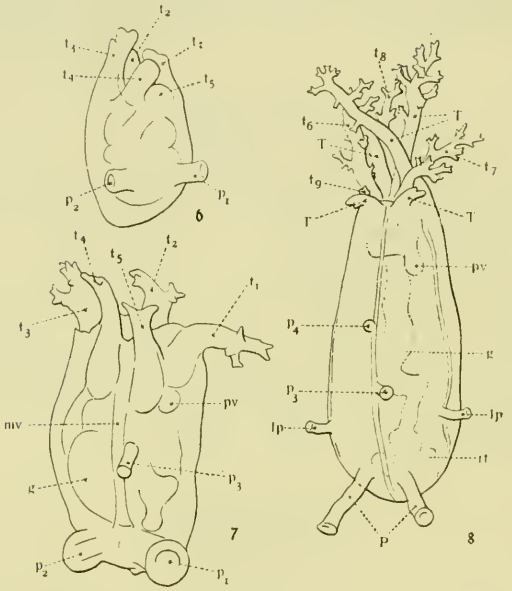
14. Pentactula.

In this stage the ciliary bands have disappeared, the tentacular crown has assumed its terminal position from the diminution of the pre-oral hood, and at the centre of the tentacular crown the mouth is opened while the anus has appeared posterodorsally. This stage is reached as early as on the seventh day, as found among the Mitsukuri material. The larva now begins to creep on the bottom and to feed itself.

The internal changes taking place at this stage may be described as the further completion of all the systems and organs which were roughly established in the preceding stage. A very conspicuous feature of this stage as found in sections is the large space which the body-cavity occupies and the thinning out of every epithelium lining the water-vascular system and body-cavity.

Water-vascular System.—No marked change is found in the water-vascular system. The tentacles have some

TEXT-FIGS. 6–8.



TEXT-FIG. 6.
Pentactula viewed from ventral side. × 60.

TEXT-FIG. 7.
Same, but still advanced, being beset with branched tentacles and the third pedicel. × 60.

TEXT-FIG. 8.
Nine-tentacled young, 1·3 mm. long. Ventral view to show the order of appearance of tentacles and pedicels (no. 2 represented in Table III in the text). × 30. *g* = gut; *lp* = lateral pedicel; *mv* = mid-ventral radial canal; $P, p_{1,2}$ = primary pedicels; $p_{3,4}$ = secondary pedicels; *pv* = Polian vesicle; *rt* = rudiment of respiratory tree; T, t_{1-5} = primary tentacles; t_{6-9} = secondary tentacles.

simple branches and stand at the anteriormost end of the body (Text-fig. 6, t_{1-5}), while the primary pair of pedicels are growing

longer and have removed near to the posterior end ($p_1, _2$). Thus, as compared with the doliolaria, the ventral surface has very much extended. The mid-ventral radial canal is still the largest of the five radial canals ; the other four do not as yet reach the posterior end of the body. Muscular layers of the ring, radial, tentacular, and pedicel canals have much developed and are well distinguishable, but no fibres are as yet visible in the Polian vesicle. The pore-canal still opens to the exterior through the body-wall.

Almost at the end of the stage, on the tenth day, the third pedicel appears on the left side of the mid-ventral radial canal at about the middle of the body (Text-fig. 7, p_3). It is much smaller than the primary pair, and, like the subsequent members, develops directly above the body-surface without forming at first any sort of pedal pit as met with in the primary pair. Ludwig (**22**, p. 186) found a similar condition in the forty-fifth-day young of C. planci, and described a rudiment of the ampulla projecting into the body-cavity. I could not make out any ampulla in the early stage.

Alimentary Canal.—The gut has now become an open canal beginning at the mouth to end in the anus. The pharynx seems to originate from the endoderm, the atrial wall forming only a very beginning part of the canal. The wall has become quite thin, and the internal lumen widened remarkably. Circular muscle fibres are found only at the pharyngeal part, the other part forming no such structure as yet. The intestine now shows a characteristic coil in accordance with the peculiar arrangement of the mesentery.

The corresponding stage was observed by Danielssen and Koren in C. frondosa, and by Kowalewsky in Phyllophorus urna. The larvae in both forms had five tentacles and a pair of the primary pedicels. Ludwig (**23**, p. 26) observed the pentactula of C. parva found in the brood-pouches, measuring 0·5–0·6 mm. by 0·28–0·31 mm. The five tentacles showing no trace of ramification, a pair of the primary pedicels, gut, stone-canal, calcareous ring, and calcareous deposits of integument are described. A very interesting

case was reported by Clark (**8,** 1901, pp. 168–70) in another brooding form, Psolidium nutriens. The young had the five primary tentacles just indicated and a pair of pedicels, which latter were very remarkable in size and apparently served to attach them to the inner skin of the mother's back. It is interesting to note that in such a form characterized by the degenerated state of the mid-ventral radial canal and its appendages in contrast to a comparatively stronger development of the lateral ventral ones, the first appearing pedicels still belong to the former and attain such a remarkable degree of development.

15. Young.

In the post-larval stage which I call young, five more tentacles are added to the primary five, the pedicels increase by degrees, and, moreover, retractor muscles, respiratory trees, genital organs, &c., appear, so that a miniature adult Cucumaria is now formed.

This stage has been known in many cases. Danielssen and Koren (**11**) first described and figured the young of C. frondosa. Among others the following instances may be enumerated: C. glacialis by Mortensen (**32**), C. crocea by Ludwig (**23**), MacBride and Simpson (**27**), Thyone rubra by Clark (**8**), C. saxicola by MacBride (**25,** 1912, Pl. i, fig. 4[1]; **26,** Text-fig. 402), C. ijimai, C. lamperti and Thyone imbricata by the present writer (**38,** 1915). Besides these, young referable to Cucumaria were reported from the Antarctic Seas by MacBride (**25,** pp. 3–7; Pl. i, fig. 3; Pl. ii, figs. 5–8) and Mortensen (**34,** 1913, p. 87; Pl. xii, figs. 6, 7).

From want of materials in consecutive series, I am compelled to leave untouched many important problems in connexion with the origin of several organs. I give here only some points of my observations.

Stone-Canal.—The pore-canal which has in the preceding stage been distinctly seen lying in the dorsal body-wall has

[1] Identified doubtfully with C. lactea.

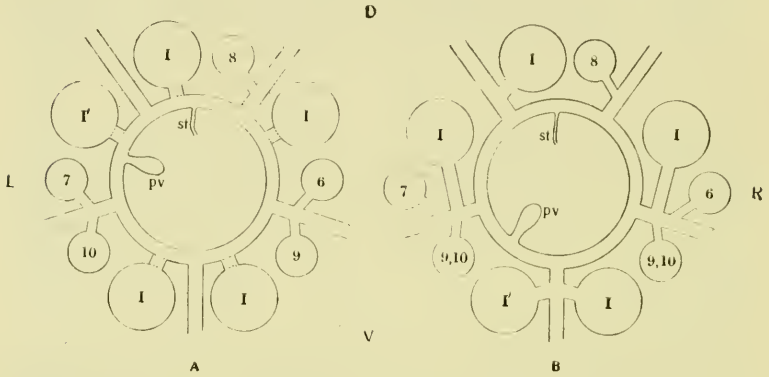
now utterly disappeared. The axial sinus has given rise to the internal madreporite shaped like a folded leaf. A very peculiar feature is seen in the young of C. ijimai. The ten-tentacled young of this species found in the mother's brood-pouch measure about 5 mm. in length. No well-marked madreporic body can here be found, but the distal end of the stone-canal is dilated at reaching the dorsal body-wall into a flat cavity. The cavity extends posteriorly and ramifies like a root, each of these branches opening to the exterior. Delicate calcareous deposits are found at the junction of the canal and the flattened cavity, as well as in the wall of the canal. In the young of C. crocea MacBride and Simpson were able to find the opening of the pore-canal. According to Ludwig (**22,** p. 186) the pore-canal of C. planci loses its opening on the eighteenth to twenty-fourth days, and until the ninety-eighth day the axial sinus opens to the body-cavity through its thin-walled side. The canal of Phyllophorus urna remains longer than in C. planci (Ludwig, **24,** p. 98; see also Russo, **44,** p. 42).

Secondary Tentacles. Ludwig (**22,** p. 184) found two more tentacles added to the primary five by the one hundred and sixteenth day. These were sent out dorsad from each of the lateral ventral radial canals. He was, however, unable to observe actually the successive appearance of the remaining three. He only assumed that the eighth should appear dorsad from the right dorsal radial canal, the ninth and tenth ventrad from each of the lateral ventral radial canals. According to Mitsukuri (ante, p. 175) the first to appear among the secondaries in C. echinata is that given out from the right ventral radial canal.

From observations on some specimens at my disposal I can corroborate Ludwig's view. In some specimens, as is seen in no. 10 of Table III, there are only eight tentacles where the sixth and seventh have attained a size equal to the primary five, but the eighth, which appears dorsad from the right dorsal radial canal, is distinctly smaller. Thus Mitsukuri's second statement, contradicting his first one, is obviously a mistake. Among

ten-tentacled specimens some are often found having a pair of small and bud-like tentacles given out ventrad from each of the lateral radial canals, as seen in nos. 3 and 8 in Table III. These are the ninth and tenth. As to which of these two should appear first, a specimen represented in Text-fig. 8 and no. 2 in Table III gives an indication. Here the right one of them only is present, and thus the young is nine-tentacled (Text-

TEXT-FIG. 9.



Diagrams showing the sequence of appearing of tentacles in Cucumaria echinata (A) and Holothuria floridana (B). Viewed from behind anteriorly. I = primary tentacles; I′ = same appearing last (dotted lines indicate the later position of tentacular canals); 6–10 = secondary tentacles numbered according to the order of appearing; *pv* = Polian vesicle; *st* = stone-canal.

fig. 9, A). There seems to be a considerable period before the appearance of the last two, as noticed by Mitsukuri.

It is very interesting to find that this order of appearance of the secondary tentacles in Cucumaria coincides precisely with that observed by Edwards in Holothuria floridana (**12,** pp. 217–20; Diagram I). As stated above, the five primary tentacles of H. floridana arise in a manner quite different from those of Cucumaria. But the sixth arises dorsad from the right ventral radial canal, and the seventh, in opposition to it, dorsad from the left ventral radial canal

The eighth is given out dorsad from the right dorsal radial canal, and the ninth and tenth arise ventrad from either the right or left ventral radial canal. Thus the ten-tentacled young of Holothuria has two tentacles on each interradius, but the dorsal paired radii have each only one, while the ventral paired radii have each three (Text-fig. 9, B).

Pseudocucumis africanus, which is a twenty-tentacled form, remains while young in the ten-tentacled stage for a considerable period (Ohshima, **39**, 1916). Here in this stage each radial canal sends out a tentacular canal on each side, just as in Cucumaria and different from Holothuria. According to Ludwig (**24**, p. 97), in Phyllophorus urna, another twenty-tentacled form, the sixth and seventh tentacles appear between the dorsal and ventral pairs of the primary five, just as was known in C. planci. In the ten-tentacled stage of Ps. africanus of about 6·5 mm. in length, the relative sizes of the tentacles indicate, to a certain extent, their order of appearance, presumably agreeing with C. planci and C. echinata.

Manner of Branching of the Tentacles. In one of my former papers (**37**, 1914) I described the manner of branching seen in the adult Cucumaria. Some passages may here be translated.

'Living specimens of C. echinata measure, in their fully extended state, up to 10 cm. in length and 2 cm. in diameter, and the tentacles attain about 4 cm. in length. The pair of tentacles belonging to the mid-ventral radius are markedly smaller than the others.

'Each of the eight tentacles, other than the ventral pair, gives out twenty-five to thirty side branches (first order), arranged in a dextrorse spiral, or turning "with the sun", with an angular divergence of one-quarter or 90°. The first branch (no. 1) stands at about 5 mm. above the base of the stem and on the right of the outside (as seen from outside). The second branch (no. 2) is the largest, standing on the left of the outside. No. 3 is markedly smaller, standing on the left of the inner side, and no. 4, also small, on the right of the inner side. No. 5,

again, is larger and stands just above no. 1. The same relationships are to be seen in the corresponding parts in the following. The angular divergence may often vary as much as two-sevenths (*ca.* 102° 51′ 25″), but rarely to three-elevenths (*ca.* 98° 19′ 5″). In the former case the branch no. 8 comes above no. 1 with two spiral turns between them, while in the latter no. 12 comes above no. 1 after three turns.

'No. 1 of the first order gives out smaller branches about fifteen in number, arranged in a dextrorse spiral, with an angular divergence of one-quarter or 90°, or rarely one-third or 120°. These I may call branches of the second order. Among them no. 1 is the largest. Each of these branches of the second order again gives out smaller branches, the third order, in a sinistrorse spiral or turning "against the sun", with an angular divergence of one-quarter or one-third. These of the third order produce still smaller branches, the fourth order, in a dextrorse spiral, and these latter once more give out the smallest branches, the fifth order, in a sinistrorse spiral.

'No. 2 and subsequent branches of the first order give out a series of smaller branches in a manner quite contrary to that found in no. 1. Here the branches of the second and fourth orders are arranged in the sinistrorse direction, those of the third and fifth orders in the dextrorse direction.

'The two ventral tentacles differ in appearance from the other eight. But a closer examination reveals the fact that they are only modified in the relative sizes of branches. Here no. 2 of the first order[1] is of a length almost equal to the main stem, giving the tentacle the appearance of being bifurcated. Further, no. 1 of the second order given out from no. 1 of the first order is relatively large. Just as in the other eight tentacles the arrangement of the smaller branches of no. 1 of the first order is the reverse of that found in no. 2 and subsequent branches.

'Thus the tentacles of C. echinata branch according to a definite plan like the phyllotaxis among plants. The angular

[1] In the preliminary paper (**40**, 1918, p. 387) I was in error in stating that this was the first branch.

divergences, one-third, one-quarter, two-sevenths, three-elevenths, &c., show a gradual approximation to the angle of about 99° 30′. The angles seem to undergo no variation from different degrees of contraction, for only longitudinal muscle fibres are present in the wall of the tentacle.

' Hand in hand with the regular spiral arrangement of branches,

TEXT-FIG. 10.

A. Tentacle of young, viewed from external side to show the manner of branching. B. One of the ventral pair. ×40. P_{1-13} = branches of the first order; S_{1-18} = same of the second order; T_{1-3} = same of the third order.

supporting calcareous bodies lie in spiral distribution, always on the side where a branch is given out.'

In the young, whose length exclusive of tentacles measures 2·5–4·5 mm., the regular manner of branching as referred to above is plainly visible. In the eight tentacles, other than the ventral pair (Text-fig. 10, A), there are about a dozen branches

of the first order ($P_{1\text{-}13}$) arranged in a dextrorse spiral, and with an angular divergence of one-quarter or two-sevenths. Of these, no. 2 (P_2) is the largest, being beset with eight to ten branches of the second order ($S_{1\ 8}$). The latter are arranged in a sinistrorse spiral except on no. 1 of the first order (P_1), where the arrangement is dextrorse. In some comparatively larger ones of the second order, one can distinguish two to three branches of the third order ($T_{1\text{-}3}$).

In the two ventral tentacles the features are quite different (Text-fig. 10, B). These keep for a considerable period a very simple appearance, in that the tip is branched twice dichotomously. This may probably be an adaptive change. The left branch undoubtedly gives rise to no. 2 of the first order, which grows as large as the main stem rising from the right branch. They later give out branches along their whole length as seen in the adult state. No. 1 of the first order appears later on the outer side immediately below the bifurcated point. In none of the other Cucumarids does such a peculiar feature seem to have been noticed.

Mitsukuri (*ante*, p. 175) first noticed the regularity of branching of the tentacles in that the 'pinnules' stand in a spiral arrangement (sinistrorse as judged from his figure), with an angular divergence of one-quarter, and that the second pinnule is the largest. But as regards the direction of the spiral his statement does not agree with my observations. Ludwig (**22**, p. 185; **24**, p. 97) stated that both in *C. planci* and *Phyllophorus urna* the five primary tentacles first bifurcate at the tip, and then each branch produces side branches. In *C. echinata* I observed no such terminal bifurcation except in the ventral pair (Text-fig. 7). Kowalewsky (**17**, p. 6) was of the opinion that the branching of the tentacles in *C. kirchsbergii* occurs, not simply from terminal bifurcation, but from producing a bud near the apex of the tentacle.

In the ten-tentacled stage of *Pseudocucumis africanus* of about 6·5 mm. in length, no such differentiation of the ventral pair is found, all being beset with several side branches.

Increase of Pedicels.—The order of the appearance

of the pedicels may deserve a special notice. In the late pentactula stage we have met with the third pedicel appearing on the left side of the mid-ventral radial canal in front of the first primary pair. Now the fourth makes its appearance on the right side of the same radius but still in front of the third (Text-fig. 8, p_4). According to Mitsukuri (ante, p. 175), previous to this, a pedicel appears on the ventral side of each lateral ventral radii, between the height of the primary pair and the third (*lp*). Further on from this condition the appearance of new pedicels takes place, as will be seen in the following table.

Table III.

To show the number of pedicels with reference to the radii in young of different stages.

No.	*Length of body in mm.*[1]	*Date of collection.*	*LD.* *d.*	*v.*	*LV.* *d.*	*v*	*MV.* *l.*	*r.*	*RV.* *v.*	*d.*	*RD.* *v.*	*d.*	*Total.*
1	1·1	July 20, 1916			1	2	2	1					6
2	1·3	Latter part of July, 1897		1	1	2	2	1		1			8
3	1·4	,, ,,		1	2	2	2	1		1			9
4	1·1	,, ,,		1	2	2	2	2					9
5	1·5	,, ,,		1	2	2	2	2					9
6	1·3	,, ,,		1	2	2	2	2		1			10
7	2·2	August 1, 1916		2	3	2	2	2		2			13
8	1·4	Latter part of July, 1897		1	2	2	3	1		1			10
9	1·4	,, ,,		1	2	2	3	2		1			11
10	1·3	,, ,,		1	2	2	3	2		1			11
11	1·3	July 20, 1916		2	2	2	3	2		2			13
12	1·5	Latter part of July, 1897		1	3	2	3	3		1			13
13	1·5	,, ,,		2	3	2	3	3		2			15
14	1·5	,, ,,		1	4	2	3	4		1			15
15	1·5	July 20, 1916		3	6	2	3	4		3			21
16	1·3	Latter part of July, 1897		1	2	3	3	2		1			12
17	1·9	,, ,,		2	3	3	3	3		2			16
18	1·5	,, ,,		2	3	3	3	4		2			17
19	1·6	,, ,,		2	3	3	3	4		2			17
20	1·6	,, ,,		3	3	3	3	4		2			18
21	1·7	,, ,,		3	4	3	3	5		3			21
22	2·4	July 25, 1916		5	5	3	3	5		5			26

[1] The length of body refers to the preserved state and is measured exclusively of tentacles.

Table III (*continued*).

No.	Length of body in mm.[1]	Date of collection.	LD. d.	v.	LV. d.	v.	MV. l.	r.	RV. v.	d.	RD. v.	d.	Total.
23	2·0	Latter part of July, 1897	3		4		2	4	5		3		21
24	1·4	,, ,,	1		4		4	3	3		1		16
25	1·3	,, ,,	1		3		4	3	4		1		16
26	1·6	,, ,,	2		3		4	3	3		1		16
27	1·7	,, ,,	3		4		4	3	4		2		20
28	1·5	,, ,,	3		4		4	3	4		2		20
29	1·7	July 20, 1916	4		4		4	3	5		4		24
30	1·5	Latter part of July, 1897	2		3		3	4	3		3		18
31	1·6	,, ,,	3		5		3	4	5		3		23
32	2·6	August 1, 1916	4		6		4	3	6		6		29
33	1·7	Latter part of July, 1897	3		5		5	2	4		3		22
34	1·6	,, ,,	3		4		5	2	5		4		23
35	1·8	,, ,,	2		3		4	4	4		2		19
36	1·7	,, ,,	2		4		4	4	4		2		20
37	1·7	,, ,,	2		4		4	4	4		3		21
38	1·6	,, ,,	3		5		4	4	5		3		24
39	2·4	August 1, 1916	3		5		5	3	5		3		24
40	2·3	,, ,,	4		6		5	3	6		5		29
41	3·0	,, ,,	5		7		5	3	5		6		31
42	3·8	,, ,,	8		8		5	4	8		7		40
43	3·0	,, ,,	3		5		5	5	6		6		33
44	2·8	,, ,,	3		7		5	5	7		5		32
45	3·2	,, ,,	6		8		5	6	9		7		41
46	4·5	,, ,,	10		9		5	7	8		8		47

From nos. 1–15 given in the above table we get the number of pedicels belonging to each radius as follows :

Table IV.

	LD.	LV.	MV.	RV.	RD.
Total . .	19	37	68	32	17
Average .	1·3	2·5	4·5	2·1	1·1
Percentage .	11·0	21·4	39·3	18·5	9·8

[1] The length of body refers to the preserved state and is measured exclusively of tentacles.

Let us further examine the more advanced individuals :

Table V.

No.	*Length of body in mm.*	*Date of collection.*	*LD.*	*LV.*	*MV.*	*RV.*	*RD.*	*Total.*
1	3·3	August 1, 1916	5	7	8	6	6	32
2	3·7	,, ,,	4	6	9	7	4	30
3	3·2	,, ,,	5	7	9	6	6	33
4	3·0	,, ,,	5	8	9	6	6	34
5	3·7	,, ,,	5	8	10	7	5	35
6	2·5	,, ,,	9	8	10	7	7	41
7	3·9	July 20, 1916	6	8	11	8	7	40
8	3·5	July 25, 1916	9	11	11	8	8	47
9	3·9	August 1, 1916	10	12	13	14	9	58
10	4·8	,, ,,	10	14	14	12	10	60
11	4·8	,, ,,	12	16	15	16	12	71
12	4·8	,, ,,	10	13	16	13	10	62

The summarized result of these twelve specimens is as follows :

Table VI.

	LD.	*LV.*	*MV.*	*RV.*	*RD.*
Total . .	90	118	135	110	90
Average . .	7·5	9·8	11·3	9·2	7·5
Percentage .	16·6	21·7	24·9	20·3	16·6

Of adult individuals of different sizes the number of pedicels with reference to the radii is as follows :

Table VII.

No.	*Length of body in mm.*	*LD.*	*LV.*	*MV.*	*RV.*	*RD.*	*Total.*
1	9·0	29	38	42	35	33	177
2	8·0	36	40	43	40	39	198
3	9·0	41	53	55	49	47	245
4	10·0	53	60	62	62	55	292
5	18·0	64	75	79	80	63	361
6	16·0	72	76	80	78	73	379
7	16·0	66	76	83	74	70	369
8	29·0	108	120	132	126	105	591
9	30·0	112	133	140	129	111	625
10	32·0	121	140	148	136	123	668

From these ten specimens the following summary can be derived :

Table VIII.

	LD.	*LV.*	*MV.*	*RV.*	*RD.*
Total . .	702	811	864	809	719
Average . .	70·2	81·1	86·4	80·9	71·9
Percentage .	18·0	20·8	22·1	20·7	18·4

From comparison of the Tables IV, VI, and VIII we may draw the following conclusions :

1. The numbers of pedicels in each pair of lateral radii are approximately equal, showing no asymmetrical features.

2. The pedicels of the mid-ventral radius develop early, whereas those of the dorsal paired radii increase later. Those of the lateral ventral radii remain almost constant throughout in regard to the ratio to the total number of pedicels.

Order of appearance of Mid-ventral Pedicels. Of special interest is the examination of the order of the appearance of pedicels from the mid-ventral radial canal.

As mentioned above, the fourth pedicel develops on the right side of the radius in front of the third (Text-fig. 11, 4). This condition is seen in the specimens nos. 1–7 of Table III. The fifth (5) appears again on the right side and in front of the primary pair. This is observed in the specimens nos. 8–15. The sixth (6) appears on the left side behind the primary pair, as seen in the specimens nos. 19–22. The seventh (7) appears far forwards, on the left side and in front of the fourth, as seen in the specimens nos. 24–9. The eighth (8) appears again on the left side, immediately in front of the primary pair, as seen in the specimens nos. 39–41.

Among some specimens variations are found in the order and position of newly-appearing pedicels. The specimen no. 23 has the sixth on the right side instead of on the left, while the specimens nos. 17 and 18 have the sixth in front of the fourth on the left side. The specimen no. 16 has the fourth on the left side instead of on the right, and the sixth on the right in front of the fourth. Nos. 30 and 31 have the seventh on the right instead of on the left. Nos. 33 and 34 have the fifth on the left instead of on the right. In no. 32 the seventh appeared on the left, immediately in front of the primary pair. In nos. 36–8 the eighth stands on the right side assuming the anteriormost position.

Increase in numbers above the nine pedicels is represented by a few specimens. In no. 42 the ninth (9) appeared on the right side between the fourth and fifth. In nos. 43 and 44 the tenth (10) appeared again on the right side between the ninth and

fifth. No. 45 has added the eleventh (11) on the right side behind the primary pair. In no. 46 the twelfth (12) is seen on the right side in front of the fourth, and the two behind the primary pair stand in the reverse order to the preceding specimen, in that the right side one stands far behind the left.

TEXT-FIG. 11.

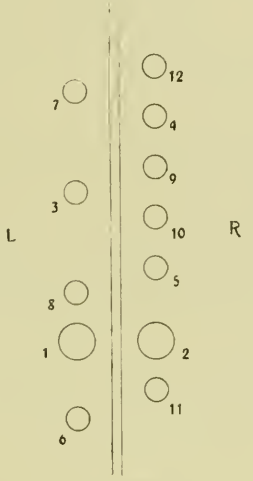


Diagram showing the position and order of appearing of pedicels belonging to the mid-ventral radius.

The stage figured by Mitsukuri (ante, Text-fig. 3) corresponds with my specimens nos. 4–6 of Table III. According to him, of the two pairs produced from the lateral ventral radii the right always precedes the left. In contradiction to his statements, in my specimens nos. 3 and 8, a new pedicel is formed only on the left ventral radius in front of the old one. In C. frondosa Danielssen and Koren (**11**) described simply that on the thirty-fourth day a pair of pedicels are added to, and in front of, the primary pair, and on the fifty-sixth day the third pair are added still anteriorly. In the latter stage papillae appeared here and there on the dorsal side. A similar stage was figured by MacBride (**25,** Pl. i, fig. 4; **26,**

Text-fig. 402) for C. saxicola having developed the second pair in front of the primaries. According to Ludwig (**22**, p. 186), in C. planci the third pedicel is distinctly seen on the forty-fifth day, constantly on the left side of the mid-ventral radius and in front of the primary pair. The fourth makes its appearance on the eighty-fourth day, on the right side of the radius and further anteriorly to the third. Thus far the order and position agree with my observations. But he differs from me in that the fifth appears ventrad from the left dorsal radial canal near the anterior end of the body. The same author (**23**, pp. 21–2) traced the order in the young of C. crocea. The youngest stage he examined had eight pedicels corresponding to that figured by Mitsukuri (loc. cit.). The ninth and tenth appear from the mid-ventral radial canal, intervening between the anterior and posterior pairs. Subsequently new pedicels increase very rapidly on the ventral side of both the lateral ventral radii. Up to the stage where the body length attains *ca.* 8 mm. the dorsal paired radii are free from pedicels, while ten or more have appeared in each of the ventral radii. These facts differ very much from the case of C. echinata, where the dorsal radii share in the pedicel-formation quite early when each of the ventral radii has only one (specimen no. 2). MacBride and Simpson's statement (**27**, p. 8) referring to C. crocea differs from Ludwig's in that there are four pedicels arising from each radial canal. Probably the observers overlooked some others in the ventral radii from their 'not having reached the surface'.

Edwards's laborious task of elucidating the order of the appearance of the pedicels in Holothuria floridana (**12**, pp. 222–6) shows that that species is totally different in this respect from that seen in Cucumaria. Here in Holothuria an unpaired pedicel first appears at the posterior end of the mid-ventral radial canal on the fourth day. The second appears to the left of the same canal on the seventh day. The third and fourth follow equally on the left side. As late as the fortieth day a pedicel appears for the first time to the right of the radius. It seems to me highly probable that

a similar feature occurs in Stichopus japonicus also, judging from the figure given by Mitsukuri (**30,** 1903, p. 12, fig. 3). Here the posteriormost unpaired one seems to be the first to appear, and besides it the mid-ventral canal seems to be provided with three pedicels to the left and one to the right, whereas each of the ventral radii has three pedicels.

16. Summary.

1. The breeding season of Cucumaria echinata seems to begin in the middle of June and to last until the early part of August. During that season the wall of the genital tubes is thin, but in an inactive period it is very thick. No muscle layer could be made out in the wall. The genital papilla is subdivided, the branches being more numerous in males than in females. Both sexes occur in almost equal numbers.

2. The ovarian egg is attached to the wall of the genital tube by its broad vegetative half. At the animal pole which is directed towards the internal lumen of the tube a short rod-like cytoplasmic process is found. This structure develops near the end of the growth of the egg, and probably has some significance in relation to future changes of the egg.

3. Freshly captured mature animals spawn in the evening. At first the males shed out spermatic fluid, and after some minutes the females begin to lay eggs. During these acts no special movements of tentacles are observed in either sexes.

4. The newly-shed egg is slightly flattened and measures about 390–400 μ in diameter. It is covered with a gelatinous layer, through which a canal opens at the animal pole. The egg is heavier than sea-water.

5. The first polar body has been formed by the time it is shed, when the second maturation spindle is to be seen. The spermatozoon enters the egg before the second maturation division, and probably at the point near to, but not precisely identical with, the animal pole.

6. The first cleavage spindle is formed within an hour. The cleavage is total and equal, proceeding quite regularly up to about the thirty-two-cell stage. Very often an interlocking

of blastomeres occurs. Inequality in size of the blastomeres is met with above the thirty-two-cell stage, and the embryo is wrapped up within the egg-membrane until the blastula stage has been attained.

7. The blastula is spherical but not wrinkled, and is now free from egg-membrane. It swims about by means of cilia. The mesenchyme-formation precedes invagination, occurring exclusively at the vegetative pole. The invagination begins the next morning.

8. In a fully-formed gastrula the archenteron shows a peculiar twisting, enabling one to distinguish in it three parts. The most anterior flat part is the future hydrocoele, the second transverse part is the future enterocoele, and the hindermost tubular part is the future gut.

9. Very late in the gastrula stage the stomodaeum makes its first appearance, being preceded by a thickening of the ectoderm at about the middle of the ventral side. Some mesenchyme cells seem to be formed here by the proliferation of ectodermal cells. The position of the stomodaeum is, as can be shown in later stages, a little on the left of the median line.

10. The dipleurula stage begins late on the second day. In this stage the hydro-enterocoele first becomes separated from the gut. The former then divides into the hydrocoele and enterocoele. The hydrocoele produces the rudiment of the pore-canal directed postero-dorsad, and six lobes on the anterior expanded margin. These latter are rudiments of the five primary tentacles and of the mid-ventral radial canal. The enterocoele divides into right and left vesicles, situated on the left dorsal and antero-ventral sides respectively.

11. On the third day doliolaria is formed, which is characterized by the possession of three ciliary bands around the posterior half of the body besides the weaker uniform ciliation over the pre-oral hood and on the anal field. From the hydrocoele are first differentiated the mid-ventral radial canal and four of the primary tentacles.

12. The primary pair of pedicels make their appearance as ectodermal depressions (pedal pits) situated between the second

and third ciliary bands. The left pedicel is a little earlier in appearing than the right, while neither of the two can be said definitely to be anterior to the other in position.

13. The original position of the primary tentacles is decidedly interradial, but their bases gradually shift towards the respective radial canal according to a definite asymmetrical feature. The one in the left dorsal interradius appears last.

14. The Polian vesicle appears at the free end of the ventral limb of the hydrocoele ring, while about the same time the axial sinus is formed as a secondary dilatation of the middle part of the pore-canal. The dorsal pore has now opened between the second and third ciliary bands.

15. The hydrocoele ring closes in the left dorsal interradius. This is clearly shown by the position of the rudiments of the dorsal and ventral radial canals of the left side, appearing usually before the closure of the ring. Of the four paired radial canals the right dorsal appears first, while the left ventral is the last to appear.

16. Fusion of the right and left enterocoeles occurs on the right side, while on the other side the two vesicles lie close but separated. This intervening portion gives rise to the mesentery, which at last bends in an S-shape in agreement with the coil of the gut in the future. The gut is almost solid, leaving but very narrow lumen. Blastocoele jelly is most massive in the doliolaria stage, and mesenchymé cells thickly cover all the internal vesicles, without, however, forming any definite cell-layer.

17. The latter half of the doliolaria stage may be distinguished by calling it metadoliolaria. Here degeneration of the pre-oral hood and ciliary bands sets in, while muscles and nerves are differentiated, besides the further completion of hydrocoele and enterocoele. Calcareous deposits, too, make their first appearance in this stage. They appear in three places : the wall of the axial sinus, the bases of the tentacles, and the integument of the posterior part of the body.

18. In the course of a week or more the larva changes into a creeping stage, pentactula. The five tentacles have now a few branches and the third pedicel appears at last. The gut

is now open throughout, both at the mouth and anus, the lumen becoming quite spacious.

19. During the transformation of the pentactula into the ten-tentacled young, the pore-canal becomes obliterated. Of the secondary tentacles those given out dorsad from the paired ventral radial canals appear first, while those given out ventrad from the same canals are completed very late. Among the respective pair the right one appears slightly earlier than the left.

20. In the young, the branches of the tentacle can be classified in three orders, and are sent out either in dextrorse or sinistrorse spiral according to a definite arrangement. The angular divergence of branches is about one-quarter or two-sevenths. The ventral pair remain for a long while in a twice dichotomously branched condition, and further branching usually takes place very late.

21. The increase of pedicels takes place faster in the mid-ventral radius than in the others, while those of the dorsal radii increase slowly. In none of the stages is any asymmetrical feature found as concerns the numbers of pedicels between right and left.

22. Along the mid-ventral radius I could ascertain that the pedicels up to the twelfth appear according to an almost definite order. But pedicels above the fourth may undergo some variations with respect to the order of appearance or the position on the right and left.

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EXPLANATION OF PLATES 8 AND 9.

List of Abbreviations.

an = anus. *ar* = archenteron. ar_1 = the anteriormost part of archenteron, the future hydrocoele. ar_2 = the middle part of the same, the future enterocoele. ar_3 = the last part of the same, the future gut. *as* = axial sinus, or 'madreporic vesicle'. *at* = atrial cavity. *bc* = blastocoele. *bj* = blastocoele jelly. *bl* = free cell in the archenteron, hydrocoele, or enterocoele, so-called 'blood corpuscle'. *bp* = blastopore. *c* = cilia. c_{1-3} = ectodermal thickenings at ciliary bands. *dp* = dorsal pore. *en* = enterocoele. *enc* = epineural canal. *ep* = ovarial wall. *f* = follicular epithelium. *g* = gut. *gs* = germinal spot. *hy* = hydrocoele. *j* = the

space probably occupied by a jelly layer. *ld* = left dorsal radial canal. *le* = left enterocoele. *lp* = left pedal pit. *lpc* = left pedicel canal. *lv* = left ventral radial canal. *ma* = micropyle appendage. *me* = mesenchyme. *mv* = mid-ventral radial canal. *n* = germinal vesicle. *p* = pedal pit. *pb* = first polar body. *pc* = pore-canal. *ps* = second maturation spindle. *pv* = Polian vesicle. *rc* = ring canal. *rd* = right dorsal radial canal. *re* = right enterocoele. *rp* = right pedal pit. *rpc* = right pedicel canal. *rv* = right ventral radial canal. *sp* = sperm nucleus. *st* = stomodaeum. *sy* = syncytium. *t* = primary tentacle. t_1 = primary tentacle in left dorsal interradius. t_2 = same in mid-dorsal interradius. t_3 = same in right dorsal interradius. t_4 = same in right ventral interradius. t_5 = same in left ventral interradius.

PLATE 8.

Fig. 1.—Very young ovarian egg, fixed on August 1, 1916. ×500.

Fig. 2.—Immature ovarian egg cut meridionally, fixed on March 27, 1914. ×200.

Fig. 3.—Same as seen in the breeding season, fixed on August 1, 1916. ×200.

Fig. 4.—Freshly laid egg in meridional section, showing the first polar body and sperm nucleus. ×200.

Fig. 5.—Longitudinal section of blastula in which mesenchyme-formation has begun. ×150.

Fig. 6.—Same in which invagination has begun. ×150.

Fig. 7.—Gastrula with still straight archenteron. Longitudinal section. ×200.

Fig. 8.—Gastrula, whose archenteron has begun to bend. Longitudinal section. ×200.

Fig. 9.—Tip of the archenteron to show the origin of mesenchyme cells and free cells in the archenteron. ×500.

Fig. 10.—Mesenchyme cells in division. ×1,000.

Fig. 11A.—Fully-formed gastrula, whose archenteron is typically twisted. Cross-section to show ectodermal thickening towards the ventral edge of the flattened archenteron. ×200.

Fig. 11B.—The ninth section below the former in the same series, to show the second transverse part of archenteron. ×200.

Fig. 11C.—The fifth section below the former in the same series, to show the third tubular part of archenteron. ×200.

Fig. 12A.—Gastrula of the same age as the former. Dorsal view of the frontal section, to show the first flat and the last tubular parts of archenteron. ×200.

Fig. 12B.—The seventh section dorsad from the former, to show the second transverse part of archenteron. ×200.

Fig. 13.—Very old gastrula to show the internal feature. Viewed from the right side. The archenteron has divided into hydro-enterocoele and gut, and the stomodaeum has appeared. ×200.

Fig. 14A.—Posterior view of the cross-section cut along the plane 1 in fig. 13 to show the stomodaeum. ×200.

Fig. 14B.—Fifteenth section below the former in the same series, cut along the plane 2 in fig. 13. To show the posterior part of hydro-enterocoele and the gut separated from it. ×200.

Fig. 15.—Early dipleurula viewed from the left side. The internal cavity as a solid body; gut not represented. ×200.

Fig. 16A.—Posterior view of the cross-section cut along the plane 1 in fig. 15. ×200.

Fig. 16B.—Eleventh section below the former in the same series, cut along the plane 2 in fig. 15. ×200.

Fig. 16C.—Fourth section below the former, cut along the plane 3 in fig. 15. ×200.

Fig. 16D.—Seventh section below the former, cut along the plane 4 in fig. 15. +200.

Plate 9.

Fig. 17.—Dipleurula viewed from the left side. The internal cavities shown as solid bodies; gut not represented. ×200.

Fig. 18A.—Posterior view of the cross-section cut along the plane 1 in fig. 17. ×200.

Fig. 18B.—Sixteenth section below the former in the same series, cut along the plane 2 in fig. 17. ×200.

Fig. 19.—Late dipleurula viewed from the left side. The internal cavities shown as solid bodies; gut not represented. ×200.

Fig. 20A.—Posterior view of the cross-section cut along the plane 1 in fig. 19. ×200.

Fig. 20B.—Twelfth section below the former in the same series, cut along the plane 2 in fig. 19. ×200.

Fig. 21A.—Early doliolaria (no. 2 represented in Table II in the text) viewed from the ventral side. The internal cavities shown as solid bodies; gut not represented. ×200.

Fig. 21B.—Same viewed from the left side. ×200.

Fig. 22A.—Posterior view of the cross-section cut along the plane 1 in fig. 21. ×200.

Fig. 22B.—Fourth section below the former in the same series, cut along the plane 2 in fig. 21. ×200.

Fig. 22C.—Third section below the former, cut along the plane 3 in fig. 21. ×200.

Fig. 22D. Sixth section below the former, cut along the plane 4 in fig. 21. ×200.

Fig. 23A.—Ventral view of doliolaria in which the ring canal is not yet closed (no. 12 represented in Table II in the text). The internal cavities shown as solid bodies ; gut not represented. ×200.

Fig. 23B.—Left-side view of the same. ×200.

Fig. 24A.—Cross-section cut along the plane 1 in fig. 23. Seen from behind anteriorly. ×200.

Fig. 24B.—Section immediately next to the former. ×200.

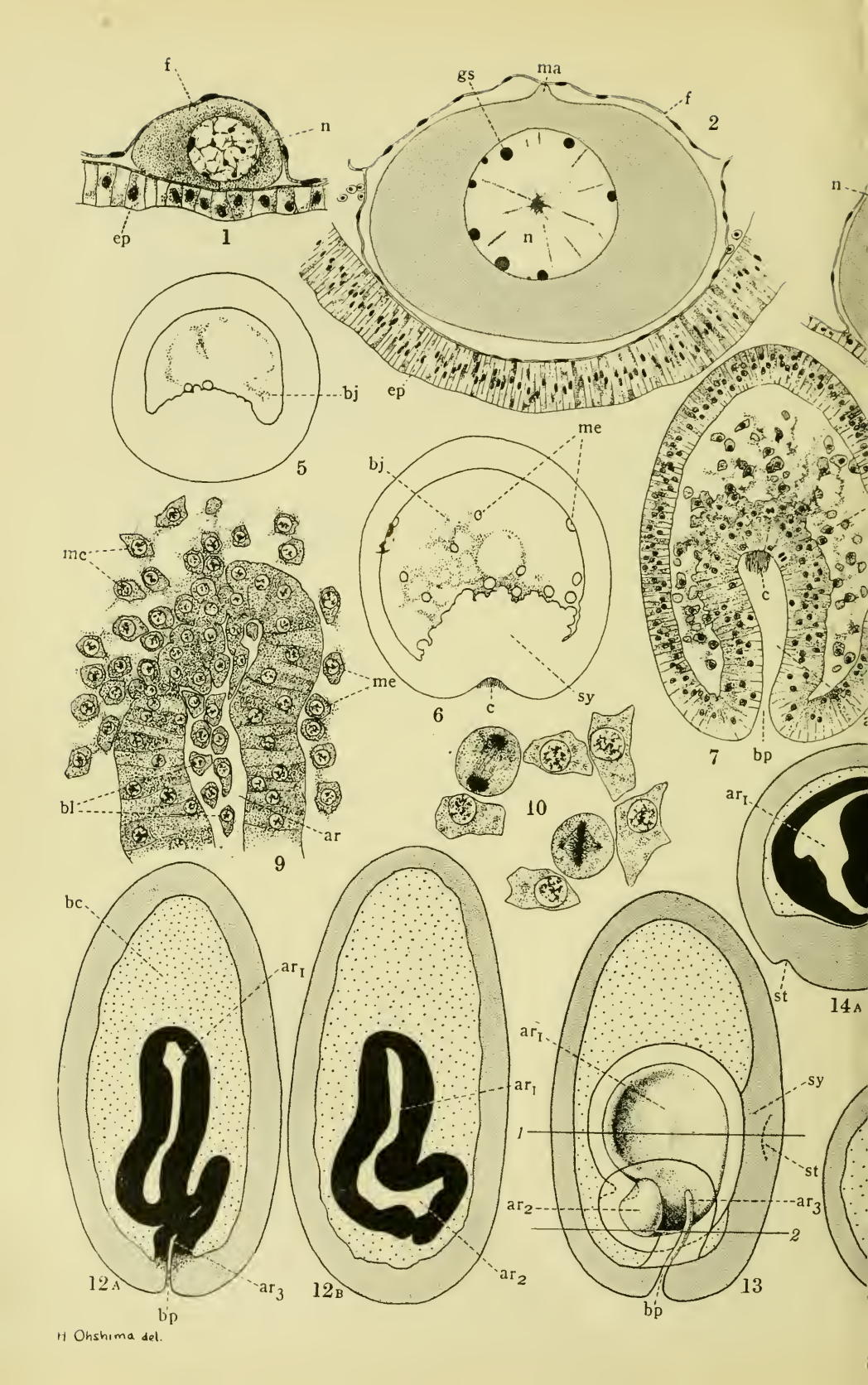
Fig. 24C.—Section immediately next to the former, cut along the plane 2 in fig. 23. ×200.

Fig. 24D.—Section immediately next to the former. ×200.

Fig. 24E.—Third section below the former, cut along the plane 3 in fig. 23. ×200.

Fig. 24F.—Fifth section below the former, cut along the plane 4 in fig. 23. ×200.

Fig. 25.—Sagittal section of doliolaria cut through the pore-canal (no. 8 represented in Table II in the text). ×200.



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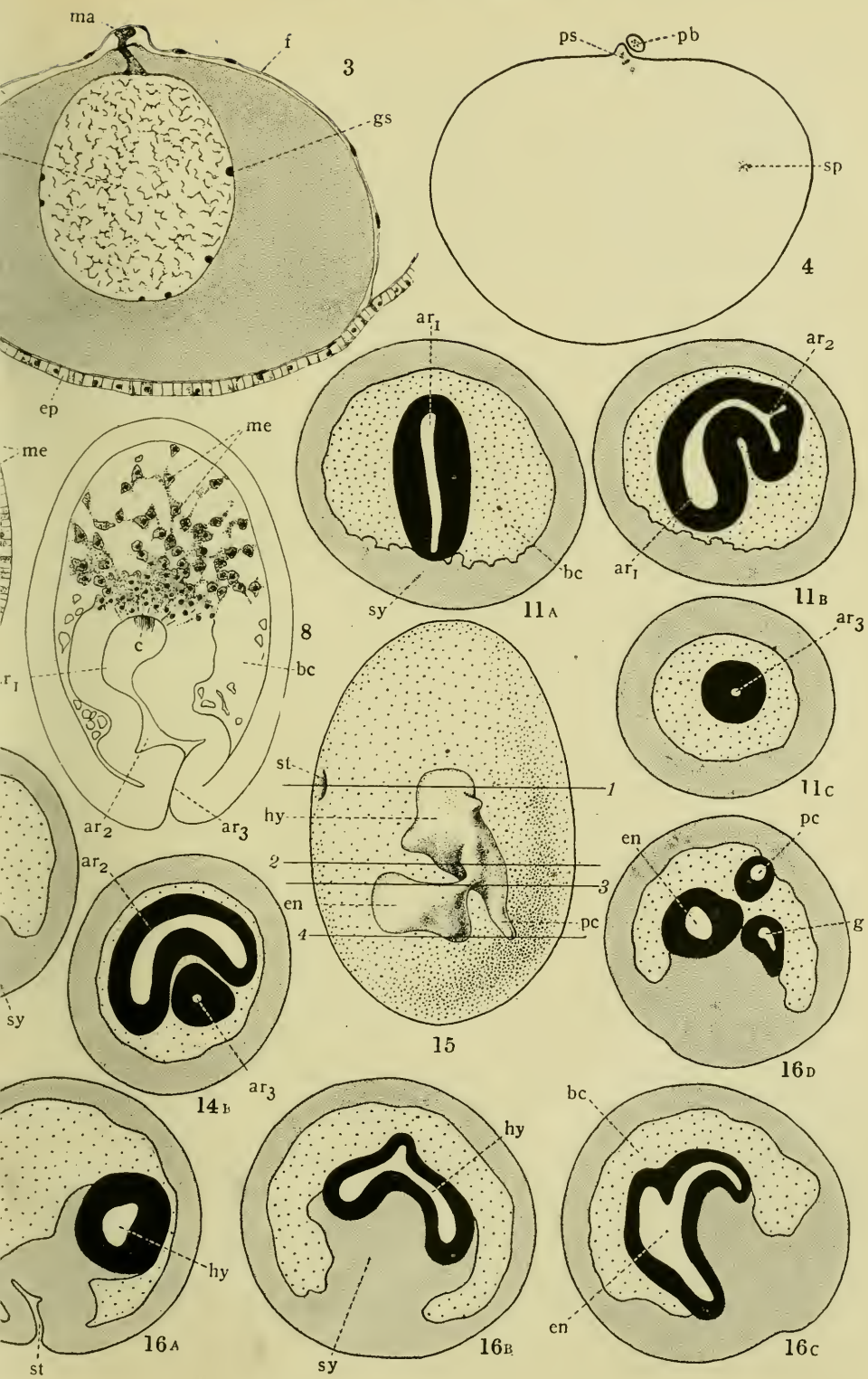
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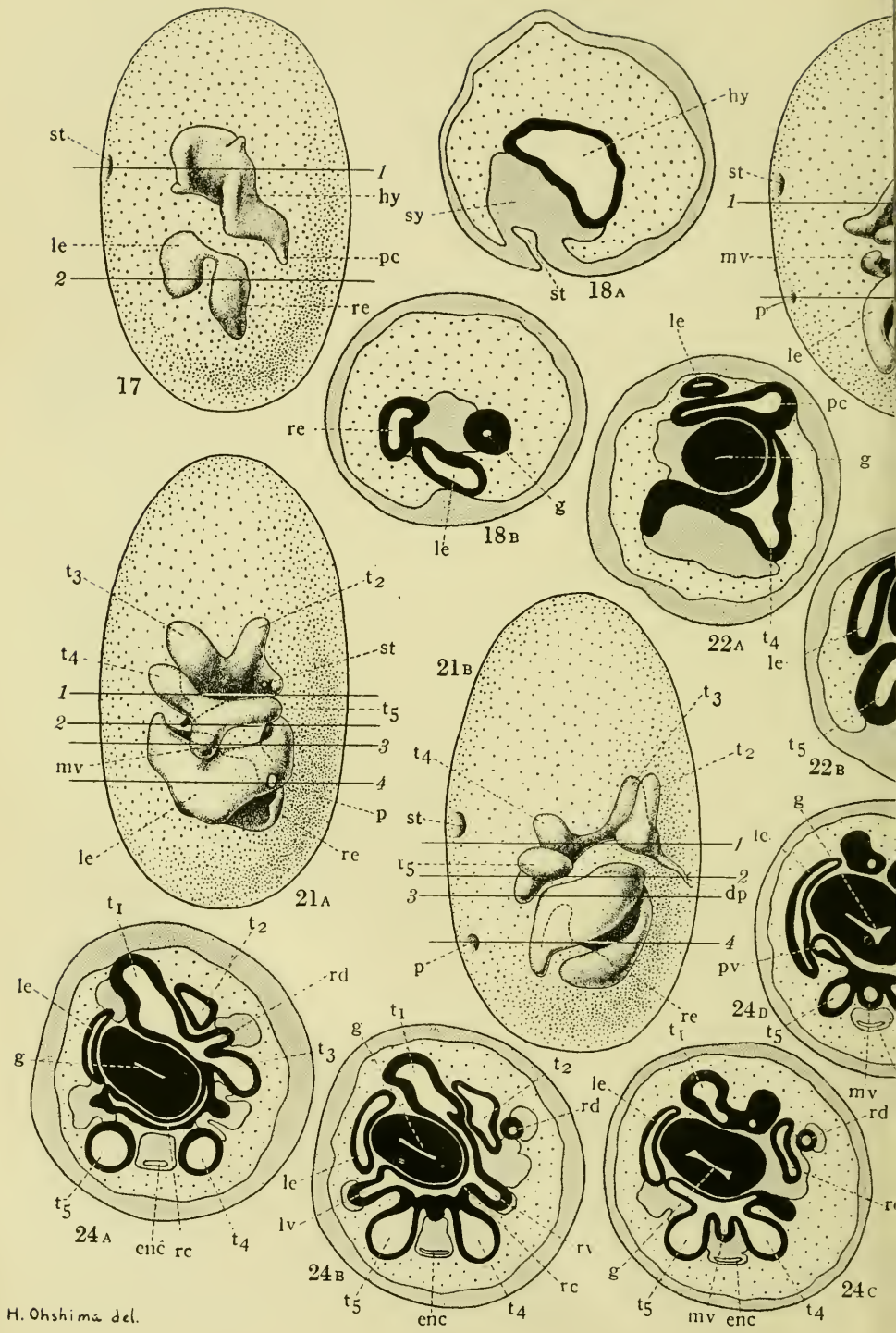

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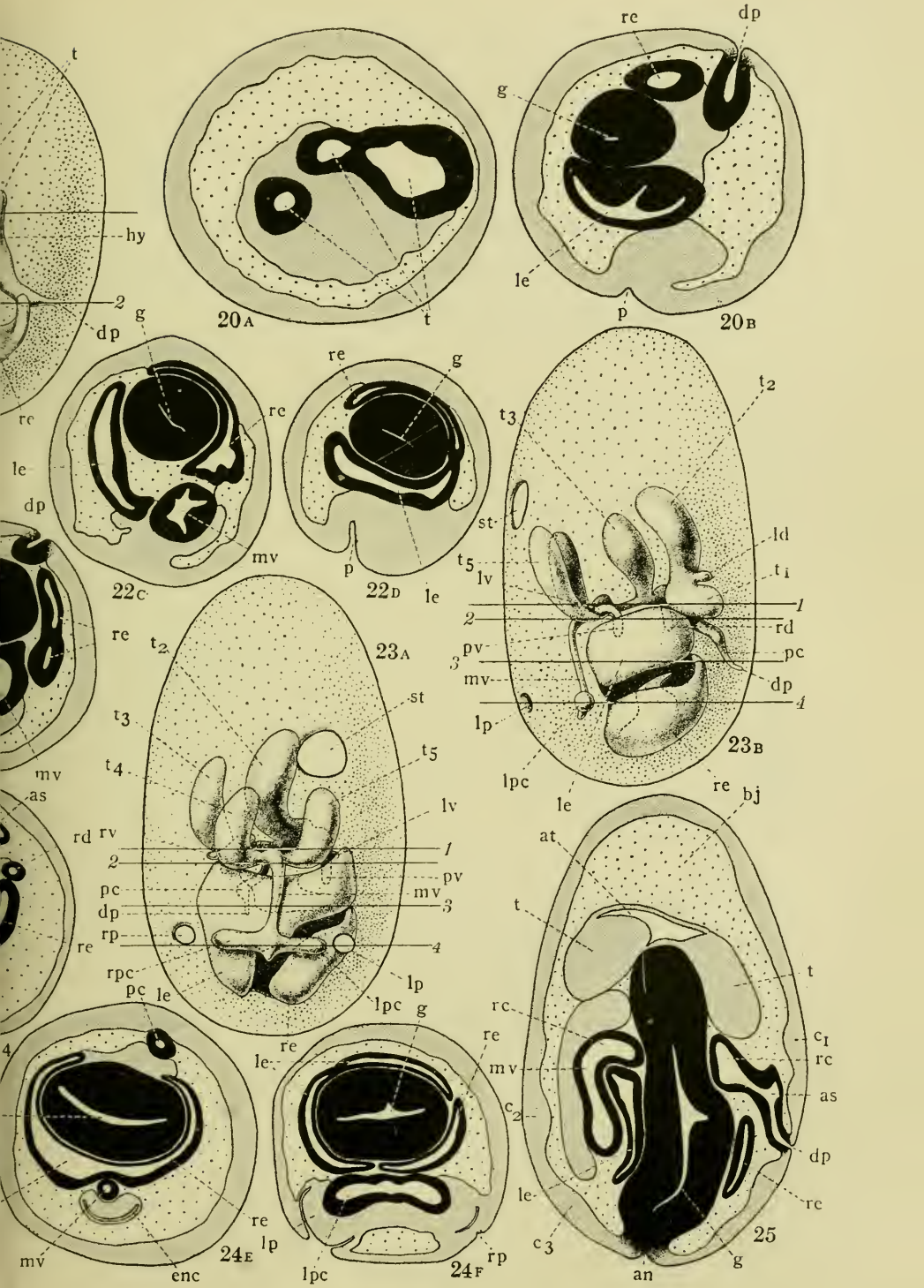
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Observations on the Protozoa parasitic in Archotermopsis wroughtoni Desn.

Part III. Pseudotrichonympha pristina.

By

D. Ward Cutler, M.A., Cantab.

With Plate 10 and 8 Text-figures.

Contents.

Introduction.

In previous papers are described species of Protozoa resident in the hind gut of the Indian termite Archotermopsis wroughtoni Desn. It is my purpose here to give an account of a fourth species of these unicellular organisms, already described by Imms (**11**) under the term Trichonympha (Holomastigotoides) pristina.

The true name for the animal is undoubtedly Pseudotrichonympha pristina, but owing to an unfortunate mistake made by Grassi in his earlier papers a good deal of

confusion has arisen around the nomenclature of these forms. In 1910 Hartmann (**9**) gave an account of a flagellate which he named Trichonympha hertwigi, and described male and female forms from which gametes were produced. Conjugation between these gametes was supposed to occur, and the resulting young forms were figured. Hartmann's observations, however, did not bear out these assumptions, and it is certain that they have no foundation in fact. His conclusions were attacked in 1911 by Grassi (**6**), who pointed out that 'Trichonympha hertwigi' was in reality a mixture of two or more genera, the male form belonging to the genus Holomastigotoides, the female form to the genus Pseudotrichonympha, and the 'young form' was referred to Pyrsonympha. The 'gametes' were undoubtedly minute oval flagellates abundant in the intestines of many termites. The confusion arose round the 'male' and 'female' forms of Hartmann, for Grassi's description of the genera, to which he referred them, did not appear to agree with Hartmann's account, as Franca pointed out in 1916. In a later paper Grassi (**7**) rectified his error, referring the 'male' form to the genus Pseudotrichonympha and the 'female' form to Holomastigotoides, thus reversing his earlier statement. Unfortunately, however, the mistake received a wide acceptance, and even in Doflein's latest edition of his text-book (**3**) it is still perpetrated. Kofoid and Swezy (**18**) also in their recent paper on Trichonympha campanula adhere to Grassi's first classification.

The organism described in the present paper is undoubtedly closely related to the 'male' form of T. hertwigi, and should therefore be named Pseudotrichonympha pristina and not Trichonympha (Holomastigotoides) pristina, as Imms has called it.

Methods.

The methods used for the study of P. pristina are those already described in my previous papers (**2**), to which I would refer those interested.

General Considerations.

Systematic Position.

That P. pristina is a flagellate belonging to the order Hypermastigina (Grassi) is indubitable. The Trichonymphidae have suffered much at the hands of systematists. Stein (**26**) in 1878 correctly placed them among the flagellates, though Leidy (**21, 22**) himself considered them as intermediate between the gregarines and ciliates. Kent (**14**) in 1882 founded the family Trichonymphidae and placed it among the holotrichous ciliates, a view supported by Butschli in 1889. Senn (**25**) in 1900 added these forms, as an appendix to the Flagellata ; while Hickson (**10**) allocated them to an appendix of the Ciliata.

In the 1911 edition of Doflein's text-book the classification of Senn was followed ; but in the last edition of 1916 Grassi's correct classification is given.

Finally, in 1913 Poche (**23**) added his quota to the existing confusion by creating the new order Trichonympha, which was placed among the Euflagellata. Kofoid and Swezy (**17, 18**) have recently published papers dealing with the flagellate affinities of these organisms, to which those interested are referred. One point which appears to have escaped notice is the complete absence of a micronucleus in any of the Hypermastigina, a fact which in itself is suggestive of their flagellate affinities, for with a few doubtful exceptions the ciliates are all heterokaryote, as Hickson pointed out in 1903.

P. pristina so differs from Hartmann's male form of T. hertwigi that the two forms cannot be regarded as one species. Grassi distinguishes four species of Pseudotrichonympha, none of which appear to be identical with P. pristina. The descriptions given of the species, however, are so scanty that it is impossible adequately to compare them with the animal described here.

Living Condition.

Movement and General Appearance.

P. pristina is at once striking because of its great swimming power, exceeding that of any other protozoon of this termite.

In living preparations it is a very pleasing sight to observe these animals gliding across the field of view, thrusting away with their anterior flagella the numerous wood particles and other protozoa impeding their progress. This gliding movement, too, is characteristic, resembling that of many of the large ciliates, and doubtless is due to the whole body being supplied with flagella, the anterior of which are probably the main propelling organs, as in Trichonympha campanula described by Kofoid and Swezy (**18**). During progression the whole of the animal's body revolves on its longitudinal axis, but the direction of revolution is not constant, sometimes occurring clockwise, at others counter-clockwise.

The whole of the body with the exception of the extreme anterior and posterior extremities is covered with flagella, very little differentiated, except that those arising from the peculiar tube-like organ at the anterior end—to be described later—are a little longer than the rest, being 14–16 μ in length, while the remainder are about 12 μ. Also these anterior flagella are much more active during progression. When the animal is stationary, however, the flagella still show movement, the majority independently, but the anterior ones in such harmony that they appear as paired thick bands in whip-like undulation. I was unable to find any indication of a prehensible function in the posterior flagella as described by Kent (**15**) and Porter (**24**). The continuous movement of the flagella, even though the animal is at 'rest', has been described in T. campanula by Kofoid; doubtless the function is to keep the body bathed in the intestinal fluid of the termites. In shape the animal is almost oval, but there is a gradual tapering from the anterior to the rounded posterior extremity. There is no sharp demarcation into ectoplasm and endoplasm except at the anterior end, where the proto-

plasm is clearer than that of the rest of the body. The large food particles are aggregated at the posterior two-thirds of the body, and are always found behind the nucleus, as in Trichonympha. This is, however, in sharp contrast to Grassi's experience, for, in his last paper (**7**), he states that in the Pseudotrichonympha the food particles are not limited to the posterior extremity, but on occasion may be seen in the region of the anterior organ 'mamella'. Buscalione and Comes (**1**), in their paper, state that when treated with iodine dissolved in iodide of potassium, the region, near to the nucleus, in Trichonympha, gives the characteristic reaction of glycogen, and that this reacting region is sharply defined from the rest of the body. In P. pristina, however, the glycogenic reaction is diffused through the whole body, being greatest behind the nucleus. This reaction and the results of other microchemical tests will be fully discussed in a forthcoming paper. As regards the method of food ingestion I can supply no evidence beyond the fact that I have been unable to find any trace of the peculiar process described by Porter (**24**) in T. agilis. Kofoid and Swezy (**18**)—apparently with reluctance—conclude that in Trichonympha campanula the anterior organ (centroblepharoplast) may function also as a cytopharynx; a view also held by Buscalione and Comes. A grave objection to this conclusion is that food particles are never found in the anterior region of the body; Kofoid and Swezy themselves say, 'the anterior region of endoplasm has, in all individuals observed, been entirely free from food bodies or vacuoles, with the exception of small darkly-staining rodlets which may be bacteria or possibly chromidia'. This has been the experience of all workers on Trichonympha, and Pseudotrichonympha pristina offers no exception to this rule. As Porter says, 'it seems highly improbable—to say nothing of the absence of any trace of a permanent oral structure—that solid food should pass through this anterior region so quickly that not a single case of its passage, or of its presence in this part, should have been discovered by any of those who have

studied these parasites'. One is thus driven to the belief that the food is incorporated into the body at the posterior region, though the method is still unknown.

Morphology.

P. pristina is a relatively large animal, its length varying from 133·9–259·2 μ with a breadth of 60·5–111·2 μ. The average size may then be taken as 226·3–99·9 μ. In stained preparations it is evident that the whole of the body flagella are arranged in longitudinal series (Pl. 10, fig. 1). The extreme posterior end is, however, naked, and in many preparations there can be seen a collection of darkly stained bodies, triangularly arranged with the apex directed anteriorly (Pl. 10, fig. 2). These granules are not to be found in every specimen and are irregular as regards size, never attaining, however, to that of the numerous food particles formed in other regions of the body. From their general appearance and from the fact that they are always confined to the naked posterior region of the body, it seems possible that they are of an excretory nature and that this naked region may be regarded as the physiological anus of the animal. This is, however, a pure conjecture, as I have found no evidence of granules being ejected from this region of the body.

Cell Inclusions.

In preparations fixed by Fleming, as modified by Gatenby (**5**) and then stained by Heidenhain's iron haematoxylin, there are seen, scattered through the entire plasma, numerous short deeply-stained rods of a fairly uniform size and thickness (Pl. 10, fig. 6). In appearance these bodies are very similar to those found in Ditrichomonas termites and described in a previous paper (**2**). On the other hand they in no way resemble the cytoplasmic inclusions found in the various animals investigated by Gatenby (**5**). As I have been unable to carry out any of the tests requisite for an accurate determination of the various cell inclusions, I shall content myself with simply recording their presence in Pseudotrichonympha pristina.

Anterior Organ (Centroblepharoplast).

Pseudotrichonympha pristina terminates at the anterior end in the curious organ found in the Trichonymphidae and described under various names by different observers: thus the Italian workers designate it as 'la bottiglia', 'il cappuccio', or 'il mammillare'; to it Hartmann has applied the term 'Kopforgan', and Porter 'the nipple-like part'. Recently, however, Kofoid and Swezy have identified it as a centroblepharoplast, the name which I prefer to adopt. In P. pristina it is composed of two portions, an inner tube-like one surrounded by a sheath which appears to cover it completely (Pl. 10, figs. 3, 5). This ectoplasmic sheath at its distal extremity becomes continuous with the rest of the body, and this is the only region where differentiated ectoplasm is found. I have been unable to detect any trace of a break in the tip of the sheath such as one would expect were the inner region in reality a tube capable of expelling or taking in liquids as some observers would have us believe. Each anterior flagellum takes origin from a granule situated on the inner surface of the ectoplasmic layer of the centroblepharoplast. These granules are difficult to detect, but in a few suitable preparations they are unmistakably demonstrated (Pl. 10, fig. 5, B.G.). Finally, from the extreme end of the organ there arise two fine threads, which, taking a parallel course down the centre of the endoplasm, diverge at their distal ends to reach the nuclear membrane where they are attached (Pl. 10, figs. 3, 5, S.T.). It seems indubitable that there is such attachment, for in specimens whose nuclei have been thrust out of position the threads are still seen running to the membrane. Thus the nucleus is more or less fixed in position by these threads, in contrast to the 'free' nucleus described by Grassi.

Striations and Granules.

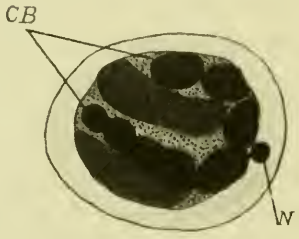
The striations that are seen crossing the body in a longitudinal series arise from the centroblepharoplast. They consist of ridges in the body surface, and thus broadly agree with those

found in Joenopsis polytricha. Just beneath the surface of these ridges numerous granules are located, from each one of which a body flagellum has its origin (Pl. 10, figs. 1, 4, 6, s.r., b.g.). The flagella origins are in the main similar to those described by Kofoid and Swezy in T. campanula and Leidyopsis sphaerica, except that I can find no trace of oblique fibres running to the granules.

Nucleus.

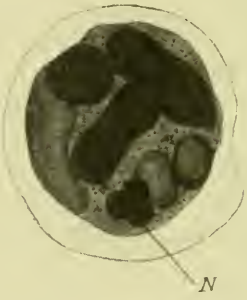
This body is a large structure situated at the anterior end of the body and possessing a well-developed membrane, always

Text-fig. 1.



'Resting' nucleus of P. pristina showing chromatin blocks embedded in the plastin matrix. Note the clear peripheral space with the nucleolus-like body. × 1,880; s.a., h.i.h.[1]

Text-fig. 2.



Similar to Text-fig. 1, but showing the tripartite nucleolus-like body. × 1,800; s.a., d.h.

clearly visible (Pl. 10, figs. 1, 3, and Text-fig. 1). Inside the membrane there is constantly present a clear space, while the centre of the nucleus is filled with chromatin, in the form of large irregularly-shaped masses lying in a matrix of what is probably plastin. The number of chromatin blocks appear to be quite indefinite (Text-figs. 1 and 2, c.b.). Lying amongst them there is commonly seen a large body, staining very deeply with iron haematoxylin, which is sometimes distinctly tripartite in nature (Text-fig. 2, n.). Unfortunately I have been

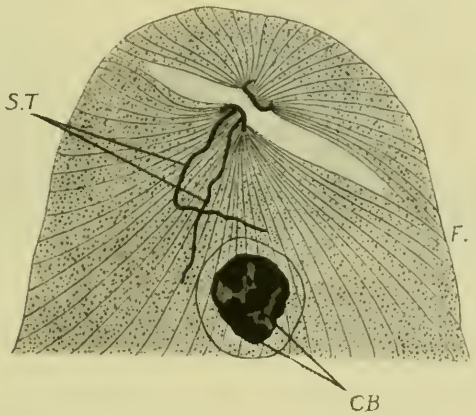
[1] For explanation of lettering of text-figures see pp. 263–4.

unable to trace its origin and fate, but that it plays no part in division is shown by its absence in dividing nuclei. Probably it is cast out of the nucleus before division takes place. It appears to have no relation with the curious 'heterochromosome' described by Kofoid in T. campanula.

Division.

As in Joenopsis polytricha the reproductive phases of P. pristina are difficult to find, and I have had to

Text-fig. 3.

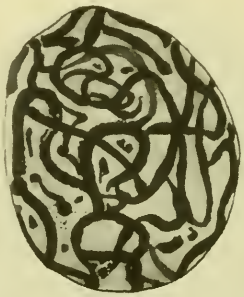


Early stage in the division of P. pristina; the centroblepharoplast has separated into two, leaving a split in the protoplasm. From one of the centroblepharoplasts the threads still persist, but with their distal ends free from the nuclear membrane. ×1,000; S.A., H.I.H.

examine a large number of preparations to obtain those here described. Division is initiated by the splitting into two of the centroblepharoplast. This condition is rarely seen, partly because it is rare to find an animal so orientated as to render visible the split blepharoplast. Commonly it becomes inflected on to the body plasma, thus rendering it very difficult to obtain a clear picture. In the first stage of the process the two suspensory filaments become detached from the nuclear

membrane, thus rendering their distal ends free in the plasma ; subsequently they are absorbed into the body (Text-fig. 3). The actual divisions of the centroblepharoplast takes place exceedingly rapidly, and I have not seen the intermediate phases. It seems probable, however, that the splitting originates at the posterior end and travels forwards, for in a good many animals the basal region is double, but the anterior one still single, though obviously much thicker than normal. At the completion of division the plasma lying between the two centroblepharoplasts splits, leaving a clear space which is

Text-fig. 4.

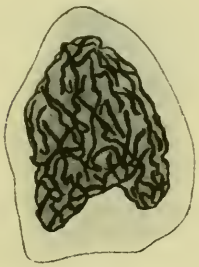


Dividing nucleus with the chromatin in the form of a loose spireme. × 1,250 ; S.A., H.I.H.

probably the initiation of division of the animal into two (Text-fig. 3). The whole process recalls that described by Kofoid and Swezy in T. campanula, and the incomplete description given by Hartmann for his male form of T. hertwigi ; a paradesmose, however, is not formed between the daughter centroblepharoplasts in P. pristina. As already mentioned, the resting—non-dividing—nucleus is composed of large irregular clumps of chromatin. At the onset of division these chromatin blocks break up into a number of small rounded granules embedded in a matrix (Text-fig. 8). Soon the granules become arranged to form a long spireme, and at this stage the clear space between the membrane and the chromatin disappears (Text-fig. 4). The nuclear membrane, however, remains

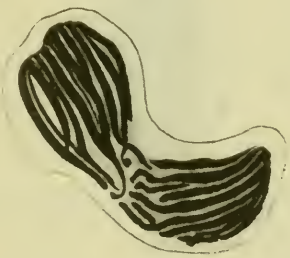
intact, and can be seen throughout the whole process of division. This is contrary to the statement made by Imms. Directly after its formation the spireme is loosely packed together, but subsequently its component parts become more closely aggregated. Finally, it breaks up into a number of long threads, which separate one from the other to form the so-called chromosomes (Text-figs. 5 and 7), and the clear space once more arises. These threads, however, do not appear to split longitudinally, nor can they be seen to be lying together in pairs previous to their separation. During the process just described the nucleus elongates, becoming

TEXT-FIG. 5.



Nucleus in which the spireme is breaking into individual threads. ×950; S.A., H.I.H.

TEXT-FIG. 6.



Dividing nucleus with the 'chromosomes' passing to each pole. Spindle fibres or paradesmose not present. ×1,250; S.A. (D.J.), H.I.H.

oval in shape, with the poles somewhat pointed. The long chromosome-like threads now separate into approximately two equal groups, one of which passes to either pole of the elongate nucleus (Text-fig. 6). Further elongation occurs, and at the same time the threads begin to aggregate to form a compact mass, which finally breaks up into irregular chromatin masses to form the daughter nuclei (Pl. 10, fig. 7). Finally, the membrane constricts, dividing in the middle.

This process must take place rapidly, for it is common to find bi-nucleate animals and animals in which the division phase is being initiated, but it is exceedingly rare to encounter the intermediate stages.

As will have been noted, throughout the whole of the division there is no development of spindle fibres, centrioles, or paradesmose.

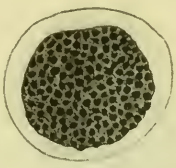
During the formation of the daughter nuclei the centroblepharoplasts migrate from each other, carrying with them some of the flagella (Pl. 10, fig. 7).

I have been unable to discover the origin of the remaining flagella or that of the suspensory threads to the nucleus.

Text-fig. 7.

A slightly more advanced stage than the one shown in Text-fig. 5. ×950; S.A., H.I.H.

Text-fig. 8.



Early stage of nuclear division with chromatin blocks resolved into numerous small granules. ×900; S.A. (D.J.), D.H.

The actual division of the animal into two probably does not occur immediately after the formation of the daughter nuclei, for binucleate animals are commonly encountered in which the plasma shows no obvious sign of splitting.

The division is, however, longitudinal, for the daughter centroblepharoplasts and nuclei always lie in a plane transverse to the axis of the body. This longitudinal division is a further indication of the flagellate relationship of P. pristina.

General Considerations.

Comparing P. pristina with the other species of Pseudotrichonympha it is evident that, in many respects, it differs markedly from them. The species described by Hart-

mann is larger than P. pristina, measuring 760–330 μ by 60–40 μ, and in shape it is more elongated, with well-defined ectoplasm and endoplasm, the latter divided into internal and external zones. As in P. pristina the body is traversed with longitudinal ridges from which the flagella takes origin, but basal granules are not definitely described, though Hartmann thinks that they may occur. The chief point of difference, however, is the centroblepharoplast. In Hartmann's organism it is composed of three distinct regions: (*a*) a cylindrical tube starting in the ectoplasm and extending to the endoplasm; (*b*) cap, covering the tube; (*c*) a second semicircular cap covering the whole of the anterior ectoplasm. Hartmann suggests that the cap represents the true blepharoplast, and that the tube is formed of fused basal granules. Obviously this 'Kopforgan' is of a more complicated structure than its homologue in P. pristina, and the location of the basal granules in the ectoplasm and not in 'the tube' in this latter organism indicates that Hartmann's suggestion as to the origin of the 'tube' is not correct. Grassi's latest description of the Pseudotrichonympha is as follows: 'Body much elongated and sharpened, with the flagella extending over the whole of the body, leaving the posterior region naked. The striations from which the flagella arise are seen running longitudinally. The nucleus is found in various positions of the body, and in its 'resting' stage is composed of a membrane, peripheral clear zone, and a central mass. The food, consisting of wood, is not limited to the posterior region of the body, but is sometimes found in the region of the 'mamella".

'The four rods, characteristic of the suspension of the nucleus in Trichonympha, are not found, and consequently the position of the nucleus is not fixed.'

Grassi distinguishes four species, P. hertwigi var. minor in Coptotermes Sjosteddi, P. hertwigi var. major in Coptotermes lacteus, P. magnipapillosa in Schedorhinotermes putorius, and P. parripapillosa in S. intermedius.

The above is sufficient to show that the organism described

in this paper is undoubtedly a member of the Pseudotrichonympha.

The two threads in P. pristina, arising from the centroblepharoplast and distally connected with the nucleus, have not been described in any of the other species, though Hartmann believes that he saw them on one occasion. In P. pristina, however, they are conspicuous elements in practically every animal observed, and undoubtedly function as suspensory or supporting structures of the nucleus. Rods and threads, often complicated in their arrangement, have been described as supporting the nucleus in the Trichonymplia, and it is reasonable to believe that the two threads found in P. pristina are the homologues of this nuclear 'basket' described by the Italian workers.

Foa (4) has suggested that the threads of the Trichonymphidae can be regarded as homologous with the collar of Joenia, which Janicki regards as the parabasal body of this animal. There seems to be little justification for so homologizing the threads of Trichonymphidae, but until our knowledge of these bodies is greatly extended it is unprofitable to discuss their possible homologies. It may well be that future research will show that many of the so-called parabasal bodies are totally unrelated one to another. As far as the evidence goes the Trichonympha and Pseudotrichonympha do not possess such bodies.

The nucleus of P. pristina is substantially like that described by Hartmann. As Imms states in his paper, there is not the slightest evidence of it being of a poly-energid nature; nor have I found any trace of secondary nuclei scattered through the cytoplasms. It is surprising that such a wonderful cycle of events as that described by Hartmann could have been found in such a relatively simple nucleus as that of the Pseudotrichonympha!

P. pristina is, I think, the first species in which the reproductive phases have been followed: Hartmann describes a few phases, which agree with some described here. Thus he states that

the blepharoplast (centroblepharoplast) first divides, followed by a split in the protoplasm. The chromatin blocks of the nucleus become resolved into granules, which aggregate to form a spireme. These phases have been found in P. pristina. Hartmann's further account, however, of the degeneration of the primary nucleus and the formation of secondary nuclei, with the final appearance of gametes, finds no counterpart in the animal I have investigated. In one important respect the nuclear division described by Hartmann differs from that of P. pristina. In this species there is no trace of paradesmose or spindle fibres, whereas Hartmann figures both these structures. This is a point of interest, for in all the protozoa of Archotermopsis, which I have investigated, the division centres of the nucleus are either absent or poorly developed.

Thus in Ditrichomonas termites (**2**) a paradesmose is formed, but no spindle fibres, centrioles, &c., whereas in other Trichomonads they are described by Kuczynski (**19**) and Kofoid and Swezy (**16**). In Joenopsis polytricha (**2**) nuclear division occurs without any obvious centre, which is not the case in any of the related animals; for in Joenia (**18**) and Parajoenia (**13**) a spindle is formed. Finally, as already noted, the Pseudotrichonymphid described by Hartmann has a paradesmose and spindle fibres; as is also the case in Trichonympha major and minor described by Foa (**4**). In P. pristina such structures are entirely lacking.

Thus in all the protozoa examined from the gut of Archotermopsis wroughtoni the nuclear division is very different from that found in related species.

Further, in D. termites the nuclear division and the locomotor complex is of a more primitive nature than that described for other Trichonomads; a statement probably true for Joenopsis polytricha and Pseudotrichonympha pristina. It appears that the protozoa to which A. wroughtoni is host are in general more primitive than those inhabiting other species of termites. Imms

describes A. wroughtoni as 'one of the most primitive of living Termites'. The association, therefore, of primitive parasites or 'guests', whichever the case may be, with a primitive host is extremely interesting, and is suggestive that the two groups of organisms have remained associated together for a long period, neither having developed into more complex species, as has occurred with other termites and their associated protozoa.

In conclusion, I wish to express my thanks to Mr. J. B. Robinson for re-drawing for publication, with the exception of figs. 4, 5, 6, and 7, the figures illustrating this paper.

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EXPLANATION OF PLATE 10 AND TEXT-FIGURES.

All the figures are drawn from fixed and stained preparations. The optical apparatus employed was as follows: Zeiss apochromatic oil-immersion objective 2 mm. (N.A. 1·3), and compensating oculars 4, 6, 12, 18. Critical illumination was always employed. The method of fixing and staining, and the approximate magnification is given below in the case of each figure. The following abbreviations are employed: S.A. = Schaudinn's sublimate-alcohol mixture. S.A. (D.J.) = Schaudinn's sublimate alcohol as modified by Dobell and Jepps. Fl. (Gat.) = Fleming's

strong fluid as modified by Gatenby. H.I.H. = Heidenhain's iron-alum haematoxylin. D.H. = Dobell's iron-alum haematein. The lettering of the figures is as follows: B.G. = basal granules. C.B. = chromatin blocks. C.BL. = centroblepharoplast. C.I. = cell inclusions. F.B. = food bodies. N. = nucleolus-like body. S.R. = striations. S.T. = suspensory threads.

Fig. 1.—Stained preparation of Pseudotrichonympha pristina showing 'resting' nucleus, striations with basal granules, food particles behind the nucleus. ×300; S.A. (D.J.), H.I.H.

Fig. 2.—Posterior region of animal with triangular-shaped collection of granules. Note the region without flagella. ×950; S.A., H.I.H.

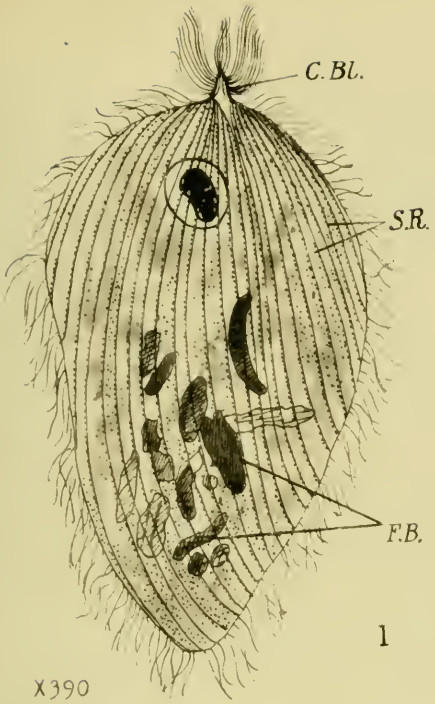
Fig. 3.—Anterior region of P. pristina with centroblepharoplast, from which arise the two threads running to the nuclear membrane. ×950; S.A. (D.J.), H.I.H.

Fig. 4.—Portion of a section through body of the animal, showing the ridges (striations) under which are situated the basal granules, the origin of the flagella. ×300; Fl. (Gat.), H.I.H.

Fig. 5.—Centroblepharoplast of P. pristina with the threads and basal granules from which the anterior flagella spring. ×1,000; S.A. (D.J.), H.I.H.

Fig. 6.—Posterior region of the body, showing the basal granules and flagella. The endoplasm contains unidentified cell inclusions. ×1,000; Fl. (Gat.), H.I.H.

Fig. 7.—Top view of a late phase in the division of P. pristina, the centroblepharoplasts are situated at either side of the body with the dividing nucleus between them. Note the absence of any division centre. ×950; S.A. (D.J.), D.H.



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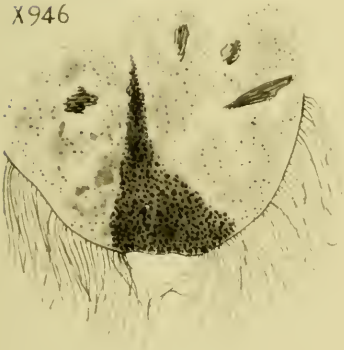

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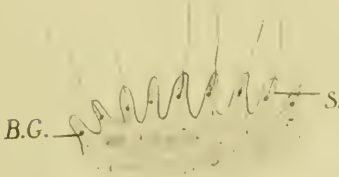

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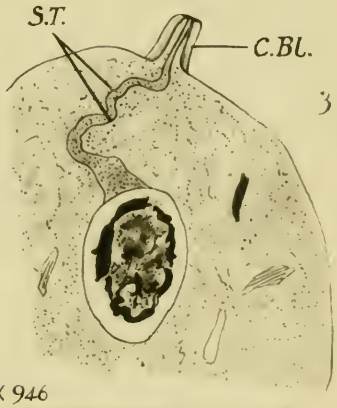

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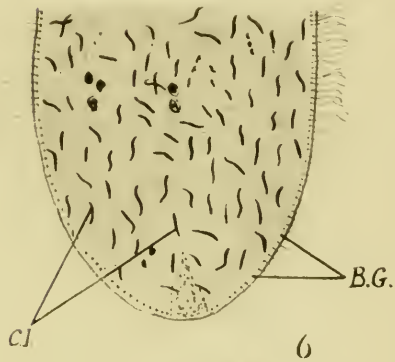

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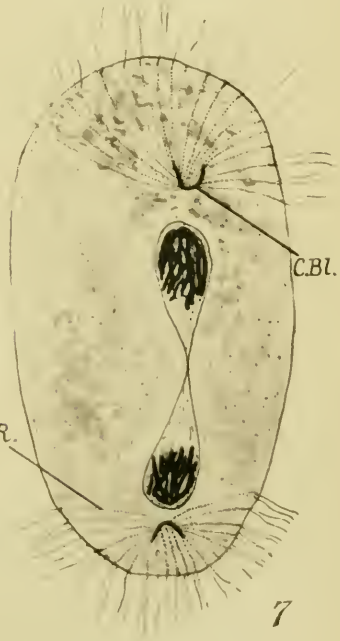

7

D. W. Cutler.

The Cytoplasmic Inclusions of the Germ-Cells.[1] Part IX. On the Origin of the Golgi Apparatus on the Middle-piece of the Ripe Sperm of Cavia, and the Development of the Acrosome.

By

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With Plates 11 and 12 and 2 Text-figures.

Contents.

[1] Part of the materials used for this research was provided by a Government Grant of the Royal Society, for which thanks are expressed.

1. Introduction.

It is well known from the work of Retzius that the middle-piece of the ripe spermatozoa of many mammals bears around itself a small clear bead of protoplasmic material which can be easily recognized in the fresh sperm.

In 1912 Weigl (**32**) published some comparative studies on the Golgi apparatus of the somatic- and germ-cells of different animals, in which he showed that the protoplasmic bead on the middle-piece of the spermatozoon of the guinea-pig contained structures possessing all the microchemical characteristics of true Golgi elements.

The work out of which the present paper arose was primarily undertaken with a view to discovering the mode of origin of these argentophile structures from the Golgi apparatus of the spermatid and spermatocyte.

The first part of this paper consists, therefore, of a description of our results in this field.

The study of the Golgi apparatus of the spermatocytes and spermatid naturally led, however, to the investigation of the relations of this structure to other cell constituents, especially to the acrosome.

The development of the acrosome in Cavia has been the object of repeated study by Niessing, Moore, Meves, and others, and quite recently by Papanicolaou and Stockard, but the exact relation of this body to the Golgi apparatus has not hitherto been described.

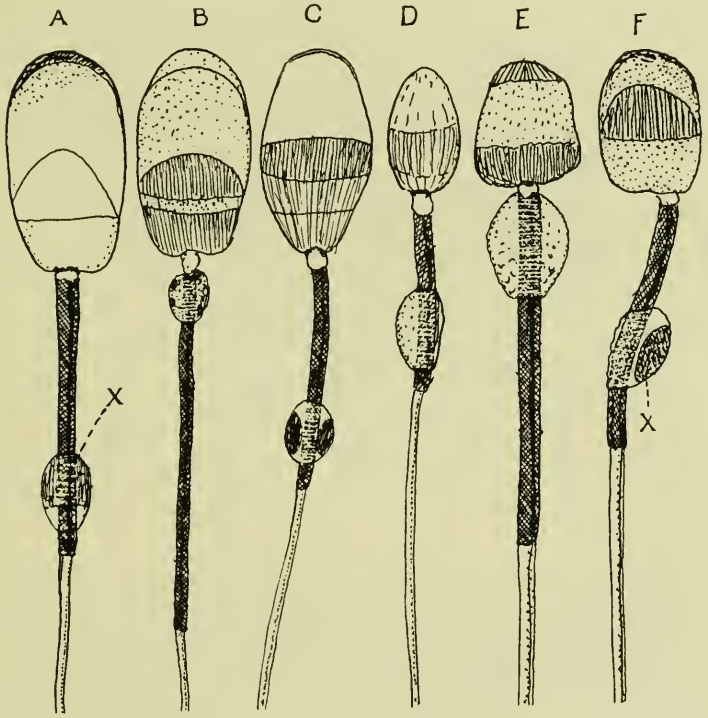
Our observations upon this point form the second part of the present paper, and we have also attempted to give a general account of the spermatogenesis of Cavia based upon the confirmed results of modern workers, together with certain suggestions for a revised and simplified English nomenclature of the subject.

2. Part I. The Development of the Definitive Middle-piece Golgi Apparatus.

Retzius, as is well known, has published a large number of drawings of various mammalian and other spermatozoa. If

we examine his figures (**29**), we find, as has already been mentioned, that Retzius has represented in many mammalian spermatozoa a small bead of protoplasm on some part of the middle-piece. In our Text-fig. 1 are reproduced six of this observer's figures, showing at x the bead of the middle-piece.

TEXT-FIG. 1.

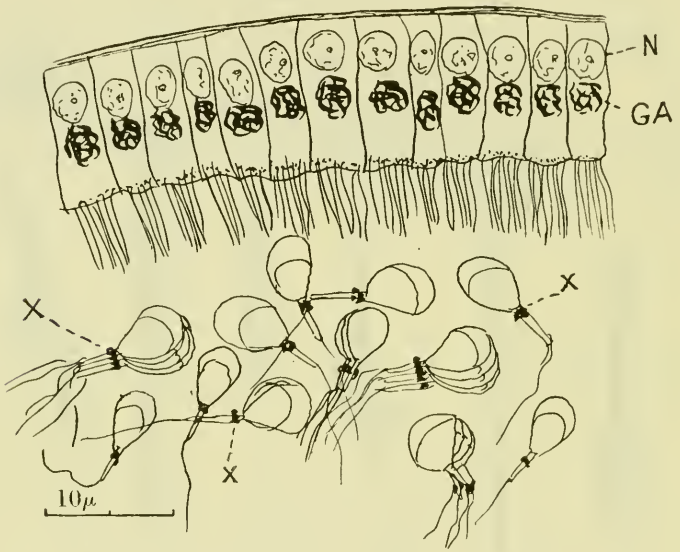


Ripe spermatozoa after Retzius (**29**). A = pig. B = sheep. C = rabbit. D = cat. E = lemur. F = hedgehog; showing at X the protoplasmic bead associated with the middle-piece.

In fig. 1, A is the spermatozoon of the pig; fig. B that of the sheep; fig. C, the rabbit; fig. D, the cat; fig. E, the lemur (*Lemur catta*); and fig. F, the hedgehog. A glance through the work of Retzius shows that this peculiar bead has been figured by him in several other mammals, namely: *Sciurus*

vulgaris, Cynomys, Myoxus glis, Cavia, Equus, Capra, Alces, Bos, Canis, and doubtfully in Dicotyles. In the spermatozoa of the following the bead does not appear in Retzius's figures: Homo, Didelphys, Talpa, Bradypus, Dipus, Hystrix, Lemmus, Mus, Myopotamus, Cervus, Rangifer, Globicephalus, Vulpes, Meles, Halichaerus, Hapale, and Innus. Some of

Text-fig. 2.



A Da Fano (8) preparation of the epididymis of Cavia. At N is the nucleus, and at GA the Golgi apparatus of the cells of the epididymis. At X are the middle-piece Golgi apparatus of the ripe spermatozoa impregnated like the Golgi apparatus of the epididymis cells. (Original.)

these are, however, doubtful, and may possess the bead in a very reduced and atypical condition.

If now, as Weigl (32) has shown, the epididymis of Cavia be prepared by one of the Golgi apparatus techniques (Golgi, Cajal, or Da Fano), the protoplasmic beads of the free spermatozoa lying within the tubules are all found to contain a number of little rodlets or elongate platelets as shown in Text-fig. 2 at x. In this figure, drawn from a preparation by Da Fano's

cobalt-nitrate-silver method (**8**), the magnification is too low to show the minute structure of the bead ; at N is the nucleus of the cells of the epididymis wall ; and at GA the Golgi apparatus of these cells. In all preparations we possess, the Golgi apparatus of the epididymis wall and of the bead-contents of the middle-piece are the only objects which go black with the reduced silver. In Pl. 11, fig. 3, a nearly ripe Cavia spermatozoon is drawn to illustrate the more minute structure of the bead (GAX) after treatment with Cajal's method.

The question now arises: What relation does the impregnating middle-piece bead (GAX in Pl. 11, fig. 3) bear to the Golgi apparatus of the spermatid cell (GA in Pl. 11, fig. 3, and GE in Pl. 12, figs. 7, &c.) ?

Extensive trials were made with Golgi apparatus techniques, and our best preparations were examined independently by both of us. We believe that the conclusion which each of us has arrived at independently is the correct one, but at the same time it is recognized that to come to a definite conclusion is difficult.

In Pl. 11, fig. 2, is drawn a ripening spermatid in which the Golgi apparatus (GA) lies in the hinder part of the cell. It is from a preparation made by Cajal's unmodified Golgi apparatus method, and the mitochondria appeared as light golden spheres (M). The most striking point to be noted is the undoubted double structure of the Golgi apparatus, which has a distinct bead projecting from its surface on one side (GAX). At this stage in the development it is possible to find pockets of cells within the testis in which every Golgi apparatus has this double appearance. If the spermatid be examined at earlier stages such as in Pl. 11, fig. 13, the bead (GAX) can still be seen as a swelling on the surface of the Golgi apparatus.

With Cajal, Da Fano, or Kopsch methods, it is found that this outgrowing bead is not homogeneous—its centre being formed of a more lightly impregnating material, closely resembling archoplasm in its appearance. If, moreover, ripe spermatozoa are fixed in some such mixture as Flemming or Hermann, and stained in acid fuchsin, it will be noted that the

middle-piece bead stains like the archoplasm of the spermatid, i. e. a deep pink or reddish. We consider, therefore, that the outgrowing bead figured by us in Pl. 11, fig. 2, and Pl. 12, figs. 13 and 14, probably consists of detached portions of both archoplasm as well as Golgi apparatus elements.

Tracing now the history of the bead after the stage at which it still adheres to the main Golgi apparatus, we next find that it has become separated from the latter in the manner shown in Pl. 12, fig. 14. In a large number of cases the bead has been observed lying in a position intermediate between the main Golgi apparatus and the nucleus, that is, near the letter M in Pl. 11, fig. 2.

In the majority of cases the Golgi apparatus bead of the ripe sperm of Cavia lies in the position shown in Pl. 11, fig. 3, and less commonly in the position indicated in Pl. 12, fig. 16. Reference to Text-fig. 1 shows that the middle-piece beads in other animal sperms vary a good deal in position.

It seems probable that the small Gogli apparatus bead moves up from its position in Pl. 11, fig. 2, or Pl. 12, fig. 14, to its definitive position near the head centrosome-complex (Pl. 12, fig. 15), the bead becoming applied to the 'skeleton' of the middle-piece (MD in Pl. 12, fig. 14) at a time when the mitochondrial granules (M) are themselves becoming grouped around the skeleton.

3. Part II. Literature.

Meves (**20**), in his classical paper on the spermatogenesis, has given a detailed review of previous work on Cavia. To this the reader may be referred. More recently Papanicolaou and Stockard (**26**) have gone over the same ground, and also given a comprehensive review of the results of previous observers. The work of Papanicolaou and Stockard is chiefly concerned with the fate of the archoplasm (their 'idiosome') and its contents based on a study of material stained with a new methylene-blue-acid fuchsin combination after Zenker's fixation. The following is a brief résumé of their account, using their new and elaborate terminology.

(1) In the Primary Spermatocyte the idiosome is differentiated into an outer blue-staining 'idioectosome' and an inner purple-staining 'idioendosome'. (2) During the preparation for the First Maturation Division the idioectosome disappears and, during the division, the substance of the idioendosome becomes scattered through the cytoplasm in the form of minute granules called 'idiogranulomes'. (3) In the Secondary Spermatocyte a new idioectosome is re-formed, containing the idiogranulomes. (4) During the Second Maturation Division the idiogranulomes are again scattered through the cytoplasm. (5) In the re-formed idioectosome of the spermatid each idiogranulome is seen to be surrounded by a clear vacuole—the 'idiogranulotheca'. (6) The idiogranulomes rapidly fuse to form a single large red-staining 'idiospherosome' enclosed in a large vacuole, the 'idiosphaerotheca' formed by the fusion of the idiogranulothecae. (7) The idioectosome now begins to move away to one side and is re-named the 'idiophthartosome'. Meanwhile the idiosphaerosome secretes a crescentic blue-staining 'idiocalyptosome', and is itself known henceforth as the 'idiocryptosome'. (8) In the ripe spermatozoon the idiophthartosome disappears with the cytoplasm which is lost during metamorphosis. The idiocrypto- and idiocalypto-somes together form a double cap to the sperm-head called the 'spermiocalyptra', and the idiosphaerotheca 'persists through all later stages and develops into a membranous cover for the cap and head of the sperm', and is then known as the 'spermiocalyptrotheca'.

As we shall mention below, we have not been able to confirm the statement of these observers as to the scattering of the 'idiogranulomes' during the maturation divisions, but we have adopted their account for several reasons.

We cannot, however, feel that Papanicolaou and Stockard have really improved the nomenclature of the subject by the introduction of these cumbersome new terms.

In the following table we have placed side by side the new terms of these authors and the corresponding synonyms used by previous workers. In the third column we have put forward

suggested English equivalents based upon those used by previous English authors, wherever these do not involve any ambiguity.

We object to the term 'idiosome' because it has already been used by Whitman (**33**) to mean 'an ultimate hereditary unit'. The term 'archoplasm' has been used by Moore (**22**), and we have adhered to it. We have avoided the 'archoplasmic vesicle' of Moore because it has sometimes been applied to the whole of the archoplasm, but we have substituted 'archoplasmic vacuole' instead. The only new term we have introduced is 'Proacrosomic granules' for the minute granules (idiogranulomes) of Papanicolaou and Stockard, which ultimately fuse to form one large 'Proacrosome', from which the acrosome is later differentiated. No one can object to this word for it is self-explanatory. It will be noted that we have explained all the complicated processes leading to the formation of the acrosome, without having recourse to the invention or adoption of a terminology of the type introduced by Papanicolaou and Stockard.

Papanicolaou and Stockard.	*Older Authors.*	*Suggested English Equivalent.*
Idiosome.	Idiozome (Meves). Sphäre (Niessing and Meves). Accessory corpuscle (Brown). Nebenkern (Hermann). Archiplasm (Benda). Archoplasm (Moore).	Archoplasm (AR).
Idioendosome.	Markschicht der Sphäre (Niessing).	Inner region of archoplasm.
Idioectosome.	Rinderschicht (Niessing).	Outer region of archoplasm.
Idiogranulomes.	Archosomes (Moore). Körnchen (Meves). Microsomenstrata (Niessing).	Proacrosomic granules (APG).
Idiogranulothecae.	Archoplasmic vesicles (Moore). Bläschen (Meves).	Archoplasmic vacuoles (VV).
Idiosphaerosome (becomes idiocryptosome).	The archosome (Moore). Das Korn (Meves), Die stark färbbaren Körper (Benda). Mitosom (Niessing).	Proacrosome (PRA).
Idiosphaerotheca.	Archoplasmic vesicle (Moore). Bläschen (Meves). Vacuole (Benda). Helle Membran (Niessing).	Archoplasmic vacuole (V)

Papanicolaou and Stockard.	*Older Authors.*	*Suggested English Equivalent.*
Idiocalyptosome.	Periphere Zone des Spitzenknopfs (Meves). Äusserer Teil des Mitosomes (Niessing).	Outer zone of acrosome (OZA).
Idiocryptosome.	Innenkorn (Meves). Dunkler Teil des Mitosomes (Niessing).	Inner zone of acrosome (IZA).
Spermiocalyptra.	Spitzenknopf or Spitzenkörper of German authors. Acrosom of v. Lenhossék.	Acrosome (A).
Spermiocalyptrotheca.	Kopfkappe of German authors.	Covering membrane of acrosome (CA).
Idiophthartosome (Idioectosome)	Archiplasmarest (Benda). Idiozomrest (Meves).	Golgi elements with archoplasmic remains (GA).

4. Technique.

The guinea-pigs used for this work were nearly all supplied to us by Mr. H. M. Carleton and Mr. J. S. Haldane of New College, Oxford, to whom our thanks are given.

We used especially the Golgi apparatus techniques of Cajal and Mann-Kopsch, as well as many other methods. One of us (J. H. W.) carried out a large number of tests with the Cajal method in order to ascertain the best time to leave the testes in the formalin fixative. It was found that twenty-four hours in the fixative and twenty-four hours in the silver bath gave the best results, though it was always very difficult to get really satisfactory preparations with any of the formalin-silver nitrate methods.

We used the methods of Stockard and Papanicolaou with fairly satisfactory results, but never got preparations quite so clear as drawn in their figures. At a later stage in this work we tried Da Fano's new cobalt formalin method, which gave useful results. We also made some excellent Mann-Kopsch preparations (three hours Mann's fluid, two weeks 2 per cent. OsO_4), but Flemming without acetic acid and Champy gave poor results.

5. General Description of the Behaviour of the Inclusions of the Cytoplasm in Cavia Spermatogenesis.

We have compiled the following descriptions, and also Pl. 12, after a personal study of many preparations of guinea-pig testes, and also after a careful examination of the literature of the subject. The works of Niessing (**24**), Meves (**20**), Brown (**2**), Benda (**1**), v. Lenhossék (**18**), Moore (**22**), and Stockard and Papanicolaou (**26**), have been considered especially with reference to the formation of the acrosome. Regaud (**27**) and Duesberg (**6**) have also been consulted and their various statements examined. A good many of our results are quite new, especially with reference to the Golgi apparatus.

6. Period I. Growing Spermatocyte.

The mitochondria and Golgi apparatus are to be found in the so-called germinal epithelial cells: during the growth of the spermatogonium, the mitochondria, which hitherto tended to surround the region of the archoplasm, become spread throughout the cytoplasm, while the Golgi apparatus and archoplasm increase in size. Some time before the spermatocyte has become full-grown the archoplasm becomes distinguishable into two regions—an outer clearer part, and an inner chromophile part formed by the proacrosomic material.

In Pl. 12, fig. 5, is drawn the spermatocyte just about to begin the first maturation division. The chromosomes are appearing within the nucleus and are connected to one another here and there by chromatic or linin filaments. Throughout the cytoplasm the mitochondria (M) are scattered haphazardly. At CHB is the enigmatic chromatoid body, which later may be found in each spermatid, and which apparently therefore may divide during cell-division. The Golgi apparatus and the archoplasm are at GE. By this stage the inner region of the archoplasm containing the proacrosomic material has resolved itself into a large number of discrete granules which have been figured by Moore, Meves, Niessing, and Stockard and Papanicolaou, and which we propose to call the proacrosomic granules

(APG), as it is they which ultimately form the acrosome, or head-cap of the sperm.

In Pl. 12, fig. 5, the Golgi apparatus is seen to consist of a large number of semilunar platelets, rodlets, or dictyosomes (GE), which lie upon the outer surface of the archoplasm. By Mann-Kopsch technique the Golgi apparatus is not a reticulum, but is as drawn in Pl. 11, fig. 1 (AR), and Pl. 12, fig. 5. Examined after Cajal's method, or by Da Fano's modification of Cajal's formalin-silver nitrate method, the Golgi apparatus is seen to be in the form of a reticulum, or of flat plates joined here and there, as shown in figs. 2 and 3 of Pl. 11.

7. Period II. Maturation Divisions.

The periods of division of the spermatocyte are difficult properly to study. In very little of our material were mitoses to be found, and this part of our work is the section about which we feel the most diffident to write. Meves, Niessing, and Moore all failed to follow the proacrosomic granules through the phases of the maturation divisions, and we have been unable to establish Papanicolaou and Stockard's claim that these granules retain their individuality and become sorted out to the daughter cells during cell-division. Meves, Niessing, and Moore all agree that the proacrosomic granules soon become visible after the archoplasm is re-formed subsequent to division—that is in the late telophase. We have adopted Papanicolaou and Stockard's description for two reasons: firstly, it is extremely unlikely that the proacrosomic granules would gradually accumulate and grow, especially before the first maturation division—only to become disintegrated at the mitotic prophase; and secondly, we are aware that the Golgi elements or dictyosomes hitherto had not been followed through division, but we now know that in mammals as well as invertebrates the Golgi elements may become sorted out during division and do not lose their individuality.

In Pl. 12, fig. 6, we give a diagram illustrating the interpretations we at present consider to be the most likely to be correct: the mitochondria are spread haphazardly throughout

the cytoplasm, and they offer no remarkable behaviour for study. Around each mitotic aster are grouped approximately one-half of the Golgi elements or dictyosomes; for confirmation of this phenomenon in cells other than those of the guinea-pig testis see Deinecka (**3**), Golgi (**14**), Murray (**23**), Perroncito (**25**), Fauré-Fremiet (**9**), and Gatenby (**11, 13**). This behaviour of the Golgi element or dictyosome does not entail any sort of division of the element itself, but only a haphazard, though subequal, sorting out of the whole elements between the daughter cells.[1]

At APG in fig. 6 of Pl. 12 are the proacrosomic granules, which become scattered in the cytoplasm during division. As with the Golgi elements, the individual granules in the spermatocyte archoplasm are not themselves divided, but sorted out whole between the daughter cells.

At CHB is the chromatoid body whose fate in the maturation divisions has not been followed out; one fact, however, may be mentioned, it is that by far the majority of spermatids contain a chromatoid body (Pl. 12, fig. 7, CHB). In many animals the spermatocyte and spermatid contain a chromatoid body of some kind, and in the case of Smerinthus strong evidence has been accumulated which indicates that this body has the power of binary fission (**10**).

8. Period III. The Newly-formed Spermatid.

In Pl. 12, fig. 7, is a drawing of the newly-formed spermatid; it contains the same categories of cytoplasmic elements as the spermatocyte, only they are approximately one-quarter in amount. With reference to the fact that the spermatid cell is generally much more than one-quarter the size of the spermatocyte, it may be pointed out that between the stages drawn in Pl. 12, figs. 5 and 7, there must be a period during which the cells are rapidly growing. While it is certain that the spermatid Golgi apparatus and archoplasm is usually

[1] Dictyokinesis in the maturation of the germ-cells of Mus, Cavia, Stenobothrus, Limnaea and Helix is the subject of a forthcoming paper by Ludford and Gatenby. The process is even more haphazard than depicted in fig. 6.

more than one-half the size of the same structures in the spermatocyte, it is difficult to obtain satisfactory evidence of any increase in size of the individual mitochondria.

With reference to the sorting out of the Golgi elements or dictyosomes during the maturation divisions, attention is drawn to recent work on Limax agrestis, where it has been demonstrated that the number of dictyosomes in the spermatocyte is eight, and in the spermatid two (**13**). In all probability, though no count is possible in Cavia, the number of platelets or dictyosomes in the spermatid is approximately one-quarter the number in the spermatocyte.

Within the archoplasm of the spermatid the proacrosomic granules have collected (or according to Meves, Niessing, or Moore, now become visible again) (Pl. 12, fig. 7, APG); but very soon around each proacrosomic granule a clear ring appears, so that the granule reposes in a vacuole—the archoplasmic vacuole: the proacrosomic granules together with their vacuoles in which they lie, now tend to run together, so that one obtains the appearance of a number of granules, some larger than others (Pl. 12, fig. 7, APG).

At this stage the centrosome is dividing in the cytoplasm, near, but outside, the archoplasm (Pl. 12, fig. 7, c).

In the next stage the proacrosomic granules have run together so as to form two or three large grains, each surrounded by the clear vacuolar ring—the archoplasmic vacuole (Pl. 12, fig. 8, APG). The whole Golgi apparatus and archoplasm gradually passes to the anterior pole of the cell, i. e. that part of the cell which gives rise to the head end of the sperm, and which most commonly is directed towards the germinal epithelium. In Pl. 12, fig. 8, the Golgi apparatus and archoplasm are shifting in an upward direction (according to the way this cell has been drawn on the Plate). From the posterior end of the cell, the axial filament grows out from the centrosomes (c^1 and c^2).

The next stage in the formation of the acrosome is depicted in Pl. 12, fig. 9. A part of the nucleus is shown at N, and the Golgi apparatus plus the archoplasm lie nearly in front but to one side of the nucleus. The whole apparatus lies in contact

with the nucleus at one spot. A change has come over the proacrosomic structures: these have finally fused to form a single large bead, the proacrosome, within its vacuole (v), and around the entire periphery of the inner granule an outer rind has been secreted (.za). These two regions are known as the outer and inner region of the proacrosome (hitherto proacrosomic granules). The proacrosomic apparatus moves through the archoplasm and finally becomes stuck upon the surface of the nuclear membrane, towards the front end of the nucleus, and hereafter may be called the acrosome (Pl. 12, fig. 10). On the side of the acrosome which touches the nuclear membrane the outer region of the acrosome is completely pushed away, so that the inner region of the acrosome alone touches the nuclear membrane in the mid-region of the acrosome; at the edges, however, as shown in Pl. 12, fig. 11, the outer region of the acrosome lies in contact with the nuclear membrane.

The Golgi apparatus (i.e. all the dictyosomes), and the archoplasm upon which it lies, keeps its position, partly embracing both the acrosome and one side of the nucleus (as shown in Pl. 12, figs. 10 and 11) some considerable time, during which the two parts of the acrosome grow rapidly. Eventually, however, the apparatus and the archoplasm break away as shown in Pl. 12, fig. 12, and begin to drift back towards the tail end of the spermatid (Pl. 12, fig. 13).

The inner region of the acrosome gradually becomes flattened out on the front of the spermatid nucleus, and the whole structure undergoes the changes shown in Pl. 12, figs. 12–15.

9. On the Subsequent Behaviour of the Golgi Apparatus and Archoplasm.

By the stage drawn in Pl. 12, fig. 12, the Golgi elements and archoplasm have begun to drift down the elongating sperm cell, and in Pl. 12, fig. 13, this apparatus has completely flowed away from the nucleus. Between the stages depicted in Pl. 12, figs. 13 and 14, the definitive middle-piece Golgi apparatus appears as described by us on p. 269.

Between the stages in Pl. 12, figs. 15 and 16, the apparatus

and the archoplasm flow into the bead, which sloughs off, and take no part in the subsequent development of the spermatozoon. In Pl. 12, fig. 16, the apparatus and archoplasm have undergone degeneratory changes.

10. The Case of the Rat Spermatozoon.

Retzius (**29**), as we have mentioned above, does not figure a protoplasmic (Golgi) bead on the ripe spermatozoon of the rat or mouse, and apparently it would have seemed to be one of the exceptions to the rule that the ripe mammalian sperm carries a Golgi apparatus. Our friend Dr. Da Fano of King's College, London, who has made preparations of the rat testis by his new cobalt methods, examined at our request his preparations of rat epididymis, with the result that he found that each ripe sperm does carry a small bead which impregnates with silver nitrate. Retzius, therefore, overlooked this bead in the rat sperm, and may have done likewise in the other forms in which he does not draw the characteristic bead.

11. Discussion.

(*a*) On the Origin of the Acrosome in Animal Spermatogenesis.

The evidence that the Golgi apparatus is in some way intimately associated with the formation of the acrosome or perforatorium has accumulated considerably within the last few years.

In Paludina (**12**) and in Columbella (**30**), two molluscs, it has been shown that the Golgi apparatus adheres to the head end of the nucleus of the spermatid, and before breaking away deposits or secretes a small granule from which the acrosome finally develops. In Smerinthus populi, a moth (**10**), it has been shown that the acrosome is developed by changes which take place in crescentic 'acroblasts', which we now know as the dictyosomes or individual units of the Golgi apparatus. In the testis of Stenobothrus viridulus we have endeavoured to follow out the formation of the acrosome: in this cricket it seems likely that the Golgi apparatus

is intimately associated with the formation of the acrosome, but the form chosen did not provide the very clear evidence wanted. In the spermatogenesis of the louse, Doncaster and Cannon (**5**) observed that the acrosome was formed from a body which they took to represent the Golgi apparatus.

According to the account given for Smerinthus (**10**) by Gatenby, and for Pediculus by Doncaster and Cannon, all the Golgi apparatus is taken up in the formation of the acrosome. Our recent observations on Stenobothrus, and 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: this matter is far from being cleared up, but of one thing we may feel certain—that the Golgi apparatus of insects is related to the formation of the acrosome.

Turning now to our observations on the acrosome of the cavy, we note that the account we give agrees in general with that previously described for Paludina (**12**). In both animals we find a Golgi apparatus (plus archoplasm) which moves up to the front end of the nucleus of the spermatid, deposits a granule there, remains for a time, and finally passes away from the head end of the sperm into the lengthening tail.

Papanicolaou and Stockard describe the proacrosomic material as appearing inside the archoplasm as a differentiated area of the latter, which stains specifically in acid fuchsin. Here we have the crux of the whole matter: is the proacrosomic material, which later forms the acrosome, to be regarded as a product of the archoplasm, or of the dictyosomes or Golgi elements? We believe that this matter may be settled after the events in the formation of the acrosome of insect spermatids have been more fully examined: this remark refers especially to the Smerinthidae.

Another point to which we would like to draw attention is the fact that in the guinea-pig the Golgi apparatus (the 'Nebenkern' of some older authors) embraces the forming acrosome from the stage when the proacrosomic granule first touches the nuclear membrane, up to the stage when the

acrosome has reached almost its greatest size; the natural inference being that the Golgi apparatus and not the nucleus is concerned with the growth and perfection of the rudimentary acrosome. In this connexion it will be remembered that one of us has shown in Smerinthus (**10**) that the acrosome may form completely, while the nucleus lags behind in development, as occurs in degenerating spermatids.

We conclude at present that the animal acrosome is formed directly in association with the Golgi apparatus, and that the nucleus has little if any influence in the process.

(*b*) The Middle-piece of the Spermatozoon after Entry into the Egg.

That the middle-piece of the mammalian spermatozoon is carried into the egg is well known, and it is now established by the work of van der Stricht (**31**), Lams (**16**), and Levi (**19**), that excepting the centrosome the entire middle-piece of Vespertilio and Cavia, after having become carried bodily into the egg, remains inert and complete, and is passively borne into one or other of the two blastomeres (or one of three in Levi's case), and is ultimately lost sight of, probably degenerating at a later stage in the cleavage of the egg.

Lams' (**16**) work is particularly worthy of mention. Alone, and also in conjunction with Doorme, he showed that in the white mouse and the cavy the middle-piece (excepting the centrosome) remains unchanged after entry into the ovum. Many of the figures of Lams show the mitochondria lying upon the middle-piece, but in no case did he find any activation of these bodies. In both the cavy and the rat we are aware that the middle-piece bears a Golgi bead, but since Lams used no methods for the Golgi apparatus, it is hardly justifiable to use his work as evidence with regard to the behaviour of the Golgi bead after introduction into the ovum.

Henneguy, at the discussion following Lams' communication to the Brussels congress of 1910, suggested that the blastomere

containing the tail of the sperm became transformed into the embryonic part of the germ, the other blastomere into the trophoblast.

Meves (**21**) likewise suggests something similar for the case of Echinus. That part of the pluteus containing the sperm middle-piece is supposed to bud off the Echinus rudiment, a very unlikely suggestion indeed.

Levi (**19**) in remarking on these facts and suggestions says:

'Le ipotesi di Henneguy e di Meves non furono finora suffragate da alcun fatto, ed il solo argomento nuovo che io adduco, la possibilità della persistenza del pezzo intermedio dello spermatozoo in uno dei blastomeri provenienti dalla 2ª segmentazione, non contribuisce ancora ad illustrare il significato del condrioma maschile nello sviluppo ulteriore.'

As one of us pointed out before, the explanation of Henneguy for the case of the mammal does not accord with the generally accepted interpretation as to the origin of identical twins, for if the presence of a middle-piece was a factor of any sort of differentiation, the two separating blastomeres would not produce the identical twins.

We see no reason to suppose that the middle-piece Golgi apparatus is stripped off the sperm and left outside; there seems every justification for the supposition that the apparatus is carried into the egg with the mitochondria. What fate lies in store for this middle-piece Golgi apparatus is unknown to us, nor do the works of van der Stricht, Lams, or Levi bring forward any sort of evidence with regard to this point.

In all probability, the apparatus, like the mitochondria, first remains complete and inert and ultimately degenerates, after having fulfilled its function, whatever that may be.

The meaning of the stages in spermatogenesis during which most of the mitochondria and part of the Golgi apparatus become applied to the middle-piece of the spermatozoon, is difficult to understand. If the mitochondria and Golgi apparatus of the spermatozoon remain inert, unlike those of Ascaris which persist in the egg and live (**15**), we are forced to conclude that the function fulfilled by these bodies is carried

out between the time the sperm leaves the spermatic tubule, and enters the egg.

Two suggestions are obvious and may be set forth : (*a*) Both mitochondria and Golgi apparatus are concerned with the production of the energy used up by the movements of the sperm tail. (*b*) Either the mitochondria or the Golgi apparatus (or both) carry some active substance which is set free just as the sperm enters the egg, or after it has penetrated the egg, and whose function is related in some obscure way to the phenomenon of heredity.

It seems to be established that every mammalian sperm is partly formed of mitochondria, and we may find that every such sperm has a Golgi apparatus. The experimental evidence which is necessary for the elucidation of the function of these two categories of cell inclusions within the structure of the spermatozoon would be very difficult to procure, and it appears to be very doubtful whether mere observation of the behaviour of these inclusions during fertilization will provide any conclusive facts.

It has been said that the animal spermatozoon is merely a much modified cell, and it has been shown in this paper that the remark is true to the smallest detail, for a sperm such as that of Cavia is a complete cell with nucleus, mitochondria, Golgi apparatus, and centrosome. In one fact, however, the two gametes differ widely : While the nuclear matter (chromosomes) of both gametes is similar in quantity, the mitochondria and Golgi apparatus of the spermatozoon are infinitely less in quantity than those of the ripe ovum. Are we to look upon the presence of the mitochondria and Golgi apparatus in the animal spermatozoon as being merely of phylogenetic importance, and indicative of a period when the two gametes were equal in size and metabolic potentialities, or should we entertain the view that the mitochondria and Golgi apparatus are specially concerned with a ' cytoplasmic heredity ', as apposed to a ' nuclear ' one ?

It has never been shown satisfactorily that either the mitochondria or the Golgi apparatus can originate from the nucleus,

though some indications of this have been noted (see **11**, p. 581), and until such is established we are not justified in dismissing the hypothesis of a special ' cytoplasmic heredity '.

More than this we cannot at present write ; the very function of the mitochondria and the Golgi apparatus is not understood, and those paths which will lead to this understanding are only now being entered.

University College, London,
April 12, 1920.

12. Summary.

(*a*) The Middle-piece Golgi Apparatus.

1. The middle-piece of the mammalian spermatozoon is formed from part of the mitochondria of the spermatid which become grouped around a central rod or skeleton. Not all the mitochondria of the spermatid pass into the middle-piece, a certain proportion always sloughs off.

2. On the middle-piece of many mammalian spermatozoa there is a protoplasmic bead which can be seen in the fresh, and which, on fixation, stains in plasma dyes.

3. With formalin and silver nitrate techniques the protoplasmic bead is found to contain a number of argentophil platelets or rods, which impregnate exactly like the Golgi apparatus of younger sperm cells.

4. The spermatid of Cavia contains a Golgi apparatus consisting of an inner core of archoplasm, and a cortical region formed of curved plates and rods—the dictyosomes. With formalin-silver nitrate techniques, the Golgi apparatus either appears as a reticulum, or the whole cortex of the apparatus reduces the silver, and then appears homogeneous : with Mann-Kopsch techniques the individual dictyosomes are often very clearly marked.

5. At a stage when the spermatid is elongating the Golgi apparatus buds off a small part of itself. This part becomes

separated from the main Golgi apparatus, and ultimately comes to lie in the middle-piece bead referred to in paragraph 2.

6. The rest of the Golgi apparatus of the ripening spermatozoon sloughs off.

7. While all the chromatinic substance of the young spermatid eventually goes to form the nucleus of the spermatozoon, only the majority of the spermatid mitochondria, and a very small part of the spermatid Golgi apparatus, form the representatives of these cell organs in the ripe spermatozoon.

8. Attention is drawn to the works of Lams and Doorme, van der Stricht, and Levi, where it has been shown that the whole middle-piece of the mammalian sperm (Cavia or Vespertilio) enters the egg at fertilization, but, so far as these authors could observe, thereafter remains inert, and is carried whole and haphazardly into one of the blastomeres.

(*b*) The Formation of the Acrosome.

9. The acrosome of the spermatozoon of Cavia is formed from the proacrosomic granules which are differentiated within the archoplasm during the later growth stages of the spermatocyte.

10. The archoplasm in the spermatocyte of Cavia is covered by the Golgi elements or dictyosomes, which in all probability are associated with the differentiation within the archoplasm of the proacrosomic granules.

11. Each of the spermatids derived from the spermatocyte contain an equal share of Golgi elements, archoplasm, and proacrosomic granules. According to Papanicolaou and Stockard the latter granules do not disintegrate during mitosis, but, keeping their individuality, become scattered in the cytoplasm, are subequally sorted out among the daughter cells, and eventually come to lie within the re-formed spermatid archoplasm.

12. Each proacrosomic granule has a liquid-filled space formed around it, so that it comes to lie in an archoplasmic vacuole.

13. The several proacrosomic granules within their archo-

plasmic vacuoles approach and fuse into fewer larger granules, which eventually all come together to form a single large granule lying in a single archoplasmic vacuole. This structure is known as the proacrosome.

14. The Golgi apparatus complex now consists of numbers of dictyosomes lying on the surface of the archoplasm : the latter contains near its centre the proacrosome. The latter soon becomes distinguishable into an inner darkly-staining bead surrounded by a paler cortical zone, the whole lying in the archoplasmic vacuole.

15. The Golgi apparatus complex has moved up towards the anterior end of the spermatid nucleus, and it now becomes applied to the nuclear membrane. Where the complex touches the membrane the Golgi elements or dictyosomes are pushed aside, so that the archoplasm comes into direct contact with the spermatid nuclear membrane.

16. From its more or less central position the proacrosome passes through the archoplasm and becomes applied to the nuclear membrane, upon which it becomes flattened so as to form a hemisphere. The proacrosome is now spoken of as the acrosome : it has an inner zone, an outer zone, and it is still covered on its outer side by the archoplasmic vacuole. Where the latter comes into contact with the archoplasm there is differentiated the covering membrane of the acrosome, which is rarely very clear.

17. The acrosome grows rapidly, and at a stage when it has differentiated to form a conspicuous cap at the anterior end of the spermatid nucleus, the Golgi elements with archoplasmic remains, which hitherto covered and embraced the developing acrosome, gradually drift away and pass towards the posterior end of the spermatid.

18. The acrosome now develops by itself. The lower part of the archoplasmic vacuole spreads down past the equator of the spermatid nucleus, and the lower edges of the outer zone of the acrosome cover the equatorial region of the nucleus. The archoplasmic vacuole becomes less evident.

19. The outer zone of the acrosome grows very rapidly,

becomes cone-shaped, and later flattened and crescentic in shape when the broad side of the sperm is examined. In the fully formed acrosome the outer zone of the acrosome is much greater in extent than the inner zone of the acrosome.

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14. DESCRIPTION OF PLATES 11 AND 12.

Illustrating Dr. J. Brontë Gatenby and Mr. J. H. Woodger's paper on the ' Cavy Sperm '.

Explanation of Lettering.

A =acrosome. APG =proacrosomic granules. AR =archoplasm (centrosphere). C, C^1, C^2 =centrosome (first and second). CA =covering membrane of acrosome. CH =chromosome. CHB =chromatoid body. GA =Golgi apparatus plus archoplasm. GAX =middle-piece Golgi apparatus elements. GAXC =cytoplasmic bead containing the Golgi elements of the middle-piece. GE =Golgi element or dictyosome. IZA =inner region of acrosome. LPV =lower part of archoplasmic vacuole embracing nucleus. M =mitochondrium. MC =manchette. MD =middle-piece. MX =degenerate mitochondria coalescing to form von Ebner's granules. N =nucleus. OZA = outer zone of acrosome. T =tail of sperm. V =archoplasmic vacuole. V.V. =archoplasmic vacuoles.

In each plate the scale is on the left-hand side.

Plate 11.

Fig. 1.—Spermatid of Cavia, at the time when the Golgi apparatus (GE and AR) is still in contact with the forming acrosome: the latter is distinguishable into two regions, an inner zone applied to the nucleus (IZA) and an outer zone (OZA). The mitochondria are scattered throughout the ground cytoplasm. This cell is drawn from a Mann-Kopsch preparation; note the discrete dictosomes, GE.

Fig. 2.—Later spermatid, showing the double appearance of the Golgi apparatus (GA). At GAX is the bead which later becomes attached to the middle-piece, as in fig. 3 at GAX. Preparation by Cajal formalin-silver nitrate method.

Fig. 3.—Ripe sperm just before the residue cytoplasmic bead strips off. The middle-piece Golgi apparatus bead is at GAX, the definitive Golgi apparatus, which is cast off, at GA. Preparation by Cajal's method.

Fig. 4.—Sperm at same stage showing mitochondria heavily impregnated by reduced OsO_4. Preparation by Mann-Kopsch method. The Golgi apparatus did not impregnate in the region of the testis from which this cell was drawn. The bead protoplasm is seen at GAXC.

Plate 12.

This plate is drawn from three separate sets of preparations by (*a*) Mann-Kopsch, (*b*) Cajal's Golgi apparatus technique, (*c*) a mitochondrial method.

So far as possible we have utilized the work of previous observers. All the figures are drawn to scale excepting fig. 16, in which the tail of the sperm is much shorter than it should be.

Fig. 5.—Ripe spermatocyte, I, in the prophases of the first maturation division, chromosomes appearing in the nucleus. The Golgi complex contains in the middle the divided centrosome (C). Around the latter are the proacrosomic granules (APG) which constitute the inner zone of the archoplasm. Between the Golgi elements or dictyosomes (GE) which lie on the surface of the archoplasm, and the inner region of the archoplasm, is a space free of proacrosomic granules. The space constitutes the outer zone of the archoplasm. The whole Golgi complex is drawn in optical section. In the ground cytoplasm lie the mitochondria (M) and the chromatoid body (CHB).

Fig. 6.—Second spermatocyte division metaphase viewed from side. The mitochondria lie haphazardly around the spindle. Following Papanicolaou and Stockard we have drawn the proacrosomic granules (their idiogranulomes) as preserving their individuality and becoming distributed here and there in the cytoplasm around the spindle (APG).

Fig. 7.—Newly-formed spermatid showing the same elements as the spermatocyte in fig. 5, only the proacrosomic granules are now surrounded by the archoplasmic vacuoles (V.V.). The centrosome is dividing. The mitochondria tend to pass to the periphery of the cell.

Fig. 8.—Later stage: the Golgi complex begins to move towards the anterior pole of the cell. The proacrosomic granules have fused one with another till only three are left, the large main one in the middle (APG) being surrounded by its archoplasmic vacuole (V.V.). The mitochondria tend to lie on the periphery of the cell. The centrosome has divided into two, and from one part the flagellum is growing out.

Fig. 9.—Golgi complex and part of nucleus of later spermatid. The proacrosomic granules have all run together to form the proacrosome (PRA), lying in the archoplasmic vacuole (V); the proacrosome is differentiated into an outer (OZA) and an inner zone (IZA). The proacrosome has left its position in the middle of the archoplasm and has approached the nuclear membrane (N).

Fig. 10.—Later spermatid, after the proacrosome has become partly flattened against the nuclear membrane. The outer and especially the inner zones (OZA, IZA) of the acrosome have become much larger. The Golgi apparatus and archoplasm surround the entire acrosome. The archoplasmic vacuole has begun to grow down on each side of the nucleus (LPV). The Golgi complex is placed to one side of the nucleus, but in later stages the acrosome comes to lie at the head end of the nucleus, possibly by a partial rotation of the latter.

Fig. 11.—Spermatid at a later stage just before the Golgi apparatus flows away from the acrosome. The front and back parts of the nucleus

show thickenings. The mitochondria have left the periphery and are collecting towards the middle of the posterior end of the cell.

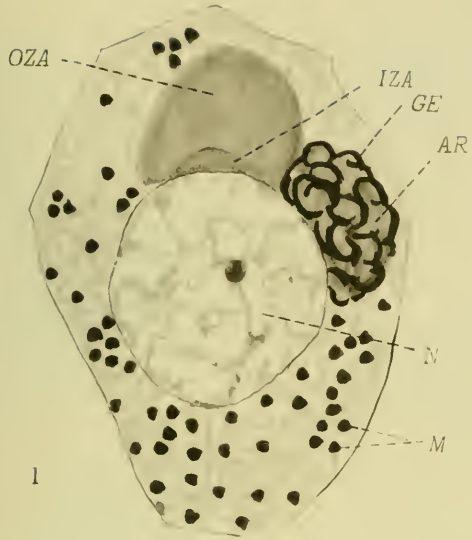
Fig. 12.—The Golgi apparatus has left the head end of the cell, and is beginning to regain its spherical shape. The centrosome apparatus and flagellum have moved up towards the posterior end of the nucleus. From this region of the latter manchette fibres (MC) begin to grow back. The acrosome become plastered over the entire front of the nucleus. Nearly all the mitochondria have left the anterior pole of the cell.

Fig. 13.—Later stage showing great development of the outer zone of the acrosome (OZA). The manchette has become tubular (MC). From the spermatid Golgi apparatus (GA) has begun to grow out a small bead (GAX) which later forms the middle-piece Golgi apparatus. The mitochondria are collecting in the region of the manchette. The centrosome ring is beginning to pass from the posterior part of the nucleus.

Fig. 14.—The acrosome become more oval in contour. The centrosome ring is passing near the Golgi apparatus (c^2). From the latter the middle-piece Golgi apparatus bead is just separating (GAX). The manchette is less evident, and around the axial filament or flagellum a distinct thickening is visible. It was not settled whether the parts in figs. 13 and 14, MC and MD, were inter-related.

Fig. 15.—The acrosome is now fully formed. The nucleus has gained its characteristic shape. The middle-piece Golgi bead (GAX) has become fixed to the middle-piece (MD) just behind the nucleus. The mitochondria begin to become attached to the middle-piece skeleton (MD) from before backwards. The Golgi apparatus is drifting down and undergoes staining changes.

Fig. 16.—Spermatozoon viewed edgewise, just before skinning off of residue bead. The middle-piece bead is at GAX, but not all the mitochondria (M) have become applied to the middle-piece skeleton; in the residue protoplasm many of the mitochondria run together and undergo changes, forming von Ebner's granules (MX). The Golgi apparatus is degenerating.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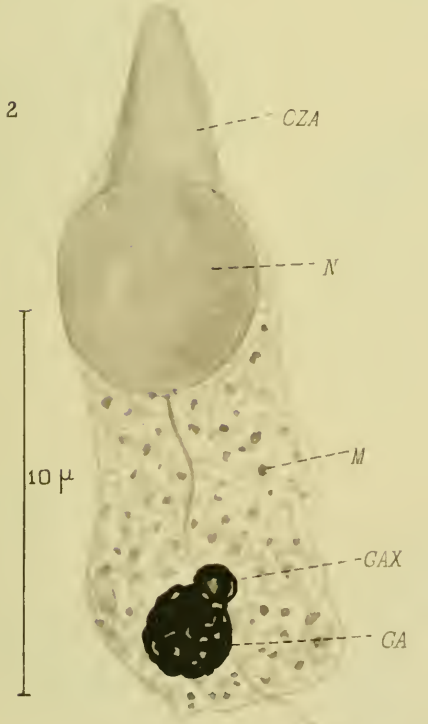
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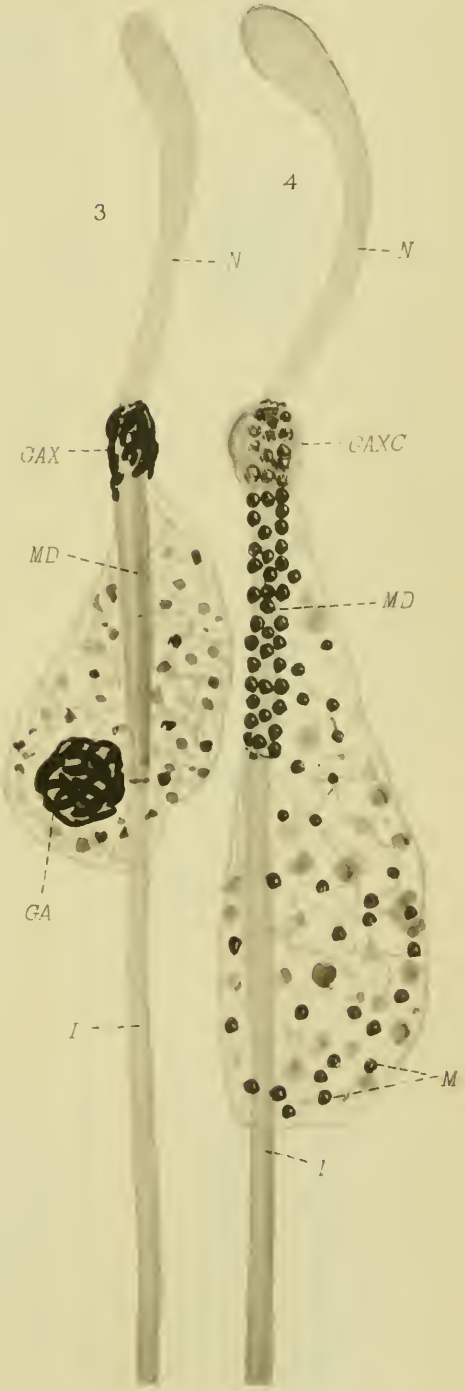
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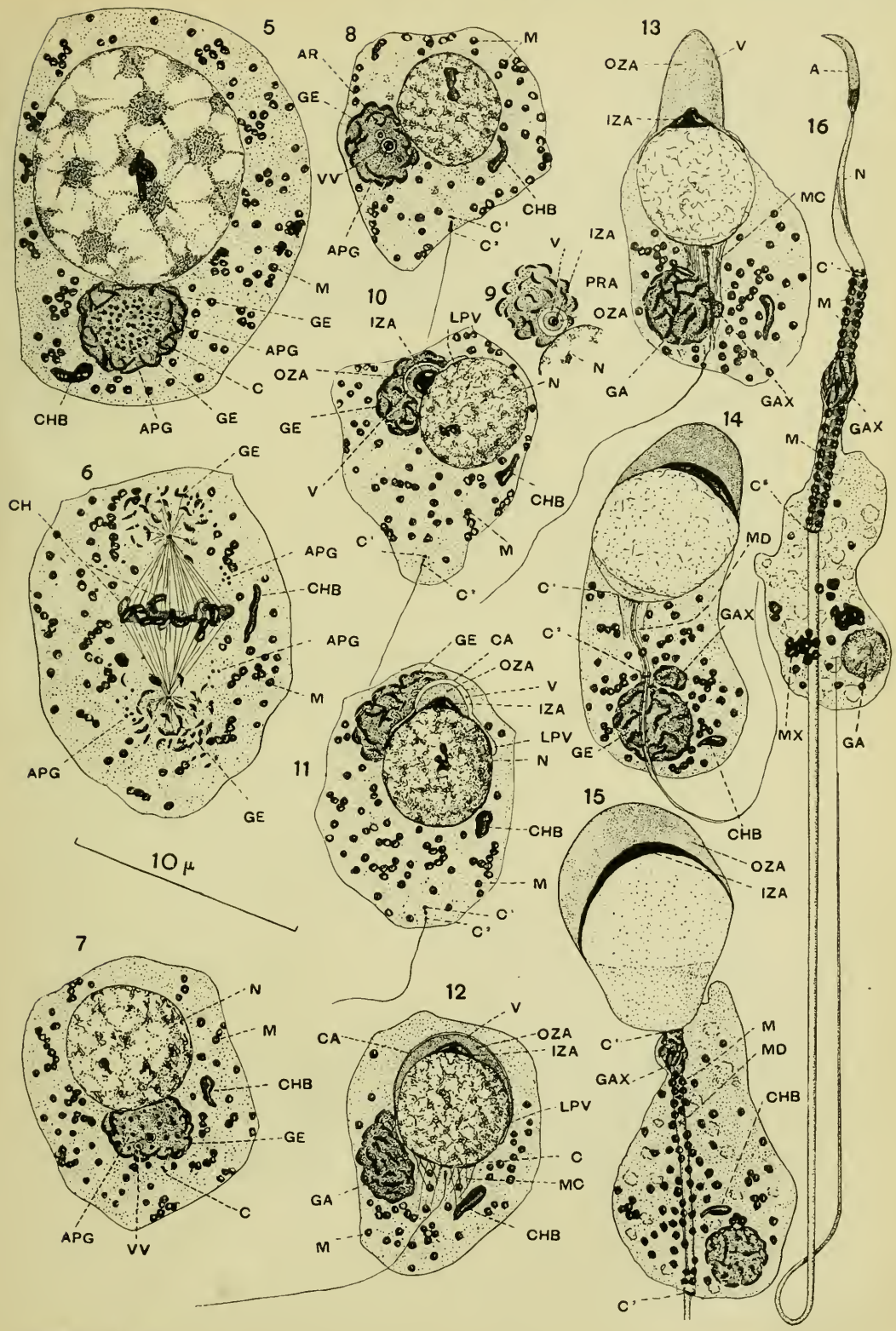

Gatenby and Woodger del.

Further Studies on Restitution-bodies and free Tissue-culture in Sycon.

By

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With Plates 13 and 14.

Contents.

1. Introduction.

In a previous paper (Huxley, **7**) I showed that the remarkable phenomena of regeneration from dissociated cells, first observed by H. V. Wilson (**15**) in monaxonid sponges, later extended by him (**16**) and by de Morgan and Drew (**4**) to coelenterates, could be studied in a simpler and more satisfactory form in

heterocoelous calcareous sponges than in any other types investigated. I further showed that, by certain methods, restitution-bodies composed entirely or almost entirely of collar-cells could be produced, and that these assumed a form quite unlike anything found in normal sponges, but with a resemblance to a Choanoflagellate colony. Simple excess of collar-cells, or, apparently, larger masses composed almost entirely of collar-cells, led to the formation of what I called choanocyte blow-outs—a part of the solid mass becoming blown out to form the segment of a collar-cell sphere.

Since then I have continued making observations on the subject as opportunity offered. Although these cannot pretend to completeness, they have brought certain new facts to light, which I publish in the hope that other workers may extend them by observation on the same favourable material. Some of the work was done at Wood's Hole, Massachusetts, and some at the M.B.A. Laboratory, Plymouth. I have to thank the authorities at both institutions for their help in getting material and in other ways. I have also to acknowledge much efficient help at Wood's Hole from Mr. I. J. Davies, laboratory assistant in the Rice Institute, Houston, Texas.

2. Material and Methods.

A species of Sycon was used at both places. That used at Plymouth was S. coronatum, obtained from piles in the Millbay docks. Orton (**12**) has recently drawn attention to the fact that this sponge grows actively during the winter without reproducing; but during the summer it reproduces so long as the temperature is above a certain level, and scarcely grows at all. The same is to be presumed true of other species of the same genus. If so, it follows that the best time for conducting similar experiments will be during the cooler half of the year.

Experiments were tried on homocoelous sponges such as Clathrina and Leucosolenia, but without much success. Restitution masses are formed, but are small and do not live well. The collars and flagella are withdrawn on very slight provoca-

tion, and the organisms and their parts appear to be more delicate.

The method originally adopted was that discovered by H. V. Wilson—the squeezing of the chopped-up sponge through fine-meshed silk bolting-cloth.

In order to procure ' pure cultures ' of collar-cells, the sponge or a transverse segment of it is held with one needle and briskly teased with another. By this means large sheets of collar-cells are obtained. If the pieces are shaken together in a solid watch-glass, they will cohere and larger masses result.

A method which will give an excess of collar-cells but not an almost pure culture is simply to perform the teasing process as above, and then remove the portions of original sponge. The collar-cells, being more easily detached than the others, will form the bulk of the tissue present.

Finally, simple squeezing of the whole sponge with the fingers into water will give a thick suspension of single cells and very small cell-aggregates, which is very similar to the culture produced by squeezing through gauze. By different dilutions of this suspension, different results can be achieved.

These methods will be called squeezing through gauze, choanocyte isolation, teasing, and squeezing without gauze respectively.

The experiments at Wood's Hole were done in late July and August ; those at Plymouth in July and early August.

3. Subdivision of Restitution-bodies.

(Work done at Plymouth.)

A teased culture was made on August 3, 1920. Many of the restitution masses were of rather large size. They began to blow out in normal fashion, and after six days a number of very fine choanocyte blow-outs were present. On the seventh day they were even better. On the eighth day a certain quantity of bodies consisting of a number of small spherules, rather closely packed together, were observed in the dish (fig. 1). They were attached to the glass, and some force was

necessary to squirt them free. On examining them I at first thought that they might be derived from the Sycon restitution-bodies, but dismissed the idea as improbable. Later in the same day I took some of them to Miss Lebour, Naturalist at the Laboratory, to see if she could identify them. On examining them under pressure with a high power, it was found that they contained fragments of spicules. Thus the suspicion that they were of sponge origin was strengthened.

Two days later a restitution-body which had for four days been isolated for other purposes on a slide in a moist chamber was examined and was found to have subdivided into six spherules (fig. 2, *a*). Thus their sponge origin was conclusively proved. Meanwhile the original dish was picked over, some of its contents preserved, and the remainder separated into divided and normal undivided masses. The normal masses were examined two days later (the tenth day of the whole experiment) and found to be still undivided, many with active flagella and protruded collars still visible externally. On the thirteenth day, eight out of fourteen masses were still single, but the remaining six had subdivided. They were similar in every way to those observed on the eighth day, except that they were not so closely packed, and that I could see no traces of a gelatinous membrane round the spherules. It would, however, of course be expected that those which subdivided earlier would be of slightly different composition from these later-divided ones.

A detailed observation of one of the earlier divided masses on the ninth day (fig. 1, *a*) showed that the spherules were tightly packed and mutually compressed. The whole body was surrounded by a faint gelatinous membrane, which apparently caused the whole to adhere to the glass. Under a higher power (fig. 1, *b*) the single spherules were seen to consist of a one-layered epithelium surrounding a central mass. The epithelium was composed of extremely clear cells, with a few minute granules; the central mass did not touch the epithelium at all points, and was dense and of a yellowish colour; cell outlines were not visible in it. The single spherules did not

appear to possess separate membranes. My attention, however, had not yet been drawn to this point, and I cannot be sure of it. Broken spicules were present in some. Another mass examined on the same day contained many more fragments of spicules. It was very similar to the first, but the epithelia were not so sharply marked off from the central masses, which in their turn were not quite so dense. When gelatinous membranes were present, numerous bacteria were usually seen along their outer edges.

The spherules were of various sizes, as is shown in fig. 4, which illustrates an isolated specimen on the tenth day. This same specimen was examined again on the thirteenth day. The same individual spherules were identified, but their appearance had changed, their outlines being less regular and the general effect more transparent. On examination with a high power this was seen to be due to the fact that in the majority most of the individual cells had separated from each other (fig. 5). Each spherule was surrounded by a definite layer of jelly. Within this, isolated clear cells, all sub-spherical, were scattered. At one point, either central or at the side, a denser yellowish mass was seen. This appeared to consist of larger cells, still adherent, containing many granules (of two types, large and small). A few of the small clear cells could still be seen embedded in some of the yellow masses. A minority of the spherules showed a different appearance (fig. 6). In them the spherule had simply subdivided into a small number of pieces, of somewhat irregular shape, each apparently consisting of clear cells round the periphery, yellow cells within. Finally, one or two spherules intermediate in type were seen, i. e. with a few large masses and also some isolated clear cells.

The independent gelatinous coverings of the separate spherules were also seen in other specimens, e. g. in that shown in fig. 2.

A variant of the types already discussed is shown in fig. 3, which illustrates a small mass found in the culture-dish, consisting of an epithelium of dermal cells surrounding a central mass, presumably mainly of collar-cells, which had subdivided

into spherules. No gelatinous layer was seen round this mass. This was paralleled in the development of some other masses; e. g. that shown in fig. 2, *a*, had, three days later, assumed the appearance shown in fig. 2, *b*. The smallest spherule was unchanged. The remaining five, however, were all surrounded by a well-marked epithelium of dermal cells very different from the epithelium shown in fig. 1, which I take to be choanocytic. The masses had swollen up by the secretion of fluid. The central portion had in three of the spherules begun to fragment. The gelatinous layers were of the same thickness as before. Although no cell-outlines had been visible in the spherules when examined three days previously, it had been noticeable that their outer boundary was very sharp. Other observations give colour to the idea that this sharp boundary heralds the formation of a dermal epithelium.

In this case the dermal epithelium is formed after the spherules have been produced. In the mass shown in fig. 3, the whole mass forms a dermal epithelium, and the spherules are then produced internally.

The further history of the spherules was as follows. The majority showed disintegration of the types shown in figs. 5 and 6. A few degenerated directly. No recovery was observed in spite of change of water. This, however, may only mean that laboratory conditions were unfavourable. It is to be remarked that the general appearance of the tissues in stages like that of fig. 5 was perfectly healthy.

Another culture was later found, where the same processes were observed. It was unfortunately not possible to carry out experiments to determine if subdivision could be initiated at will.

The subdivision appears to be primarily a reaction to unfavourable conditions (witness the accumulation of bacteria round the edges of the subdivided masses). In all the dozens of dissociation cultures I have made at Naples, Wood's Hole, and Plymouth, these two were the only ones where subdivision was observed. Both these cultures were from teased, not squeezed, material.

The secretion of gelatinous membranes is of interest. Other noteworthy points are as follows:—(1) The size of the spherules produced varies within considerable limits. Those produced by a single mass might be approximately equal, or of very different sizes. (2) I at first thought that the phenomenon was determined by the proportion of dermal cells present, subdivision continuing until enough surface was formed to accommodate all the dermal cells in the state of simple epithelium. Appearances like that of fig. 1, *b*, however, seem to negative this, for there the epithelium surrounding the spherules is cuboidal, and quite unlike any dermal cells seen by me. This epithelium seems to consist of the healthiest choanocytes present. The difference of colour between them and the cells of the inner masses, however, is to be noted, and it is possible that they represent a dedifferentiated condition of the dermal cells. If so, they would resemble the cuboidal form of the ectoderm cells seen in dedifferentiated stolons, &c., of the Ascidians, Perophora and Clavellina-cells which are normally as flattened as the normal dermal cells of Sycon. At all events the phenomenon must be determined by some surface-volume relation, the cells not being able to cohere in large masses when in certain conditions.

In any case, the spontaneous segmentation of the masses into regularly-arranged portions of smaller size is of interest. This phenomenon never occurs, as far as is known, in the normal life-history of Sycon; yet the process is regular, and at first sight would be taken for a normal occurrence. It is an example of the determination of physiological processes by the direct action of external circumstances, without any modification by way of heredity. A somewhat similar phenomenon was found by Müller (**10**) in restitution-bodies of Spongilla, but it was not so regular, nor, since it only occurred in large masses, does it seem to have been due to identical causes.

(3) The separation of the clearer cells from each other, apparently when the circumstances have become slightly more unfavourable, is also of interest. In Perophora and in Hydroids, a slight concentration of toxic substances starts dedifferentiation

in the zooids (results in course of publication). The further progress of events in these organisms, however, is determined by the emergence of the cells from the tissue into the blood, leading to the resorption of the zooid. Here, in these spherules, the cells emerge from the tissues, but must remain in position, since there is no means by which they can migrate elsewhere. Slight mercury poisoning also causes emergence of some of the endoderm cells from the gut of late Echinoid plutei. It is probable that total or partial resolution of the tissues into separate cells is a general occurrence in dedifferentiation, but that it is masked in many cases, e. g. in Clavellina. A study of these phenomena, together with that of dissociation of cells as observed in particular chemical solutions, as, e. g., observed by Gray (**5**), will throw light on the problem of cell-coherence in general.

(4) The production of a definite dermal epithelium late in the history of many subdivided spheres is to be considered in relation to the observed fact that restitution-bodies with dermal epithelium are more viable than those composed of choanocytes alone (Huxley, **8**).

(5) The transition from a state in which no cell-outlines are visible (figs. 2, *a* ; 6) to one where the cells are distinct (fig. 2, *b*) or separated (fig. 5) is to be compared with the formation of syncytia in Coelenterate restitution masses, as noted by Wilson and by de Morgan and Drew, and their subsequent resolution into cells. Here again a very important general phenomenon is made accessible to study.

4. Dermal Blow-outs.

In my previous paper three types of restitution were described, leading to : (1) normal regenerates, consisting of dermal and gastral cells in normal proportion. These formed spicules, and those that lived long enough produced normal miniature sponges. (2) Collar-cell spheres : small hollow spheres, consisting of a single layer of choanocytes, with no, or very few, other cells. (3) Collar-cell blow-outs : masses consisting mainly of collar-cells, blown out in one or more regions

to form segments of spheres. The external epithelium of the solid remainder might be formed: (*a*) by collar-cells only, (*b*) by dermal-cells only, (*c*) by patches of both.

Other types have now been observed. The most interesting are the dermal blow-outs. These appear to be formed whenever the mass contains a preponderance of dermal cells. A mass of collar-cells generally fills most of the interior; it is covered closely with a single layer of dermal epithelium, which at one point is swollen out to form a segment of a sphere which thus consists entirely or almost entirely of dermal cells. Often cells are to be seen adhering to its inner surface; these were sometimes rounded and of a fair size, presumably typical amoebocytes, oftener of the minute elongated type which I have called finger-cells (see p. 304). A few detached cells might sometimes occur in the cavity. These were occasionally seen to be forming spicules. A typical mass of this kind is shown in fig. 12.

Shaking caused contraction and disappearance of the blown-out regions, as with the collar-cell blow-outs.

One very peculiar mass was seen (at Wood's Hole). This was isolated together with a number of others shortly after concrescence, when they were solid and irregular in shape. Four days later this was found to have a large hemispherical collar-cell blow-out, which in its turn showed a small dermal blow-out on one side. Under the surface finger-cells were visible. It would thus appear that local as well as general excess of dermal cells can occur, leading to the formation of mixed blow-outs.

Previous experience (Huxley, 8) has led me to conclude that when a small proportion of dermal cells is present in a culture, they exercise an attraction for each other. This leads to the production of a few normal regenerates in a culture consisting mainly of collar-cell blow-outs. In a similar way this congregation of dermal cells can lead to the formation of dermal blow-outs. This was so in the mass shown in fig. 12.

This and other facts would indicate that the formation of dermal blow-outs is mainly a matter of the number of dermal

cells present in a particular mass. That this is not all, however, is shown by the following experiment (Wood's Hole).

July 12. Several sponges squeezed through bolting-silk into a finger-bowl. Three dilutions of the resulting cell-suspension were made: (1) dense, (2) medium, (3) dilute.

Results:—After one day: (1) large, sometimes irregular, masses; (2) medium-sized spheroids, many with good collars and flagella, many with larvae embedded in them; (3) as (2), but smaller, and fewer with larvae.

After two days: (1) no blow-outs; most seem normal restitution masses; (2) most with collar-cell blow-outs; (3) some with collar-cell blow-outs.

After three days: (1) as before; (2) fewer collar-cells than the previous day; (3) none seen blown out.

After five days: (1) the smaller masses forming small dermal blow-outs; (2) many with large dermal blow-outs; (3) solid.

After nine days: (1) mostly dead; (2) many attached to glass; (3) as before, none attached.

A repetition of the experiment gave similar results, except that dermal as well as choanocyte blow-outs were formed early in the middle dilution.

It will thus be seen that dermal blow-outs did not begin to appear until the fifth (or fourth) day, and that they appeared most notably in the same culture which had previously produced the best choanocyte blow-outs. Their failure to appear in the large masses of (1) may be due to the fact that these in this experiment were not very healthy. It would appear, since the only difference between (2) and (3) lay in the size of the masses formed, that the eventual production of dermal blow-outs is determined partly by the total, and not only by the relative number of dermal cells present. It appears that first of all the collar-cells on the surface protrude collars and flagella towards the water; these are, however, very susceptible to noxious influences, and as culture conditions became less good they retracted into a spheroidal form. The dermal cells then migrated to the surface and covered the collar-cells with an epithelium, which they were apparently unable to do when

the external collar-cells were functional. Since, however, the total number of dermal cells in a mass is proportional to its volume, while the number required to form a single external layer of epithelium is proportional to its surface, there will be in large masses an excess of dermal cells above those needed to form the epithelium. This excess apparently forms the dermal blow-outs. The replacement of choanocytes by dermal covering is of interest in view of the greater viability or protective capacity of the dermal cells shown by other considerations (Huxley, **8**).

Fig. 13 shows another type. A number of very large masses had formed in a culture produced by squeezing without gauze. The larger masses had first been very irregular in shape, and had demonstrably been formed by the coherence of original smaller spheroids. (The culture was made in a finger-bowl. The flat bottom was covered with small spheroids, while a ring of the large irregular masses was found at the foot of the sides. This was due to the opportunity given here for many masses to come in contact by rolling down the steep sides.)

These irregular large masses later rounded up, and shortly after this produced blow-outs. Some were similar to that seen in fig. 12. Others, however, consisted of a much-distended sphere surrounded by an epithelium of dermal cells, the contained gastral cells not forming a well-marked mass, but spread in layers of varying thickness over part of the inner surface of the sphere (fig. 13). The majority of the larger masses in the culture were of this type, while the majority of the smallest were not blown out at all, but were normal regenerates. This bears out the conclusion drawn above as to the rôle of size of mass.

Wilson, in his experiments with Coelenterates, also found that size of mass was very important, the larger masses failing to metamorphose. A study of restitution-bodies from this point of view would probably throw light upon the reasons for the sizes of the larvae in many low types.

An interesting feature of most dermal blow-outs examined

with the high power was the association of the small types of amoebocytes I propose to call finger-cells with the dermal cells in the blown-out region. This was observed both at Wood's Hole and at Plymouth. In surface view the dermal epithelium is seen to consist of a number of granular areas (fig. 12)—cell-bodies—separated by transparent areas, where the protoplasm is extremely thin. Cell-junctions cannot be seen in vivo. Below each granular area is seen an irregular stellate figure. On careful examination this is seen to consist of a number of finger-cells radiating from below the centre of the cell-body. Optical section of the periphery gives a profile view, when the body of the dermal cell is seen to be sharply marked off from the underlying finger-cells. Similar finger-cells are seen to protrude, but singly, from the inner mass of choanocytes. The meaning of this arrangement of finger-cells remains obscure.

The cultures containing the large dermal blow-outs above referred to were examined again later. Almost all had produced some spicules, and a considerable proportion had metamorphosed into functional young sponges of the ' Olynthus ' stage.

In my previous work (Huxley, 7, p. 169) I never obtained fixed Olynthus stages from restitution masses. Here, however, some 25 per cent. of them were firmly attached. A few of these were of great regularity of form (fig. 7, *a*), again surpassing any obtained previously. Others, however, showed marked irregularities, more pronounced than any seen in 1910, often appearing as if preparing to form a second osculum. In no case, however, was a second osculum seen, or even a rudimentary second oscular crown of spicules. The most remarkable thing about these forms was the large size shown by many of them, far exceeding that of a newly-metamorphosed larva. Thus, although large size is associated with less viability of restitution masses, yet even very large masses, provided they remain healthy, can metamorphose into normal-type Olynthi if the various sorts of cells are present in correct proportions.

Some idea of the normal size of Olynthi from larvae can be got by comparing the figures of larvae (figs. 9, *a*; 10, *a*.

drawn to a larger scale than figs. 7, *a* and *b*). It is possible that the abnormal-shaped Olynthi were produced by dermal blow-outs of the types of fig. 12, or by coalesced masses of irregular shape.

Numerous other masses, however, were seen of the type shown in fig. 8. Here spicule formation had progressed well, but no osculum was present. The most noticeable point was the restriction of the gastral layer to part of the sphere (as already seen, e. g. in fig. 13). The gastral layer was usually one cell-layer in thickness, but in some masses was several layers thick at certain spots only (fig. 8).

The disproportionate number of dermal cells had certainly delayed development. As I had to leave Plymouth the day after discovering this type of regenerate, their fate could not be ascertained.

5. Dark-centred Masses.

These were both seen at Wood's Hole and at Plymouth. Typically (fig. 11), they consisted of a dermal epithelium, surrounding several layers of pale cells, apparently choanocytes, which in their turn surrounded a central mass of dark yellowish-brown material, whose cellular nature could not be seen in vivo. The central mass is separated from the pale cells by a space. In one or two cases the central body was seen to be revolving. If this was so, then the collar-cells must have developed flagella on their inner side.

The meaning of these masses is obscure. There is a strong resemblance between the inner mass and the yellow-brown masses seen in the subdivided spherules (fig. 5), and some resemblance also between the intermediate pale layer and the isolated cells of fig. 5.

A few specimens were observed where a dark central mass was present, together with active choanocyte epithelium on the outside.

6. Adherence of Larvae to Masses.

Both at Wood's Hole and at Plymouth it was noticed that when cultures were made from sponges containing nearly

mature larvae, these might adhere to and be actually embedded in the restitution-bodies (figs. 9, *a* ; 10, *a*). In this situation their flagella would continue to beat. Transferring masses with embedded larvae by means of a pipette often resulted in detaching the larvae (fig. 10, *b*). Larvae that remained attached appeared to become resorbed into the masses, finally disappearing (fig. 9, *a–c*). Histological investigation of this has not yet been undertaken.

7. Adhesion and Unification of Restitution-bodies.

In section 4 an account has already been given of the fusion of a number of spheroidal bodies to form irregular masses, which later became spheroidal in their turn. These were all masses with excess of dermal cells. Some observations on masses with excess of choanocytes may also be given. Some four-day restitution-bodies were isolated in a hanging drop. The chief are shown in fig. 9, *a*. Most are covered with dermal cells, but two have dermal epithelium in part. Some have attached larvae. After two days these were seen to have fused (fig. 9, *b*). Three larvae and two other small restitution-bodies, not shown in fig. 9, *a*, had not shared in the fusion.

On the next day the larvae were still visible, but the general form was not so irregular. The day after (fig. 9, *c*) the larvae were no longer visible, the blown-out region had increased, and the traces of the separate original masses have been almost lost, the mass looking quite unitary, though with slight irregularities. Two days later (fig. 9, *d*) slight regressive changes had set in. The form was more unified, but the blow-out was smaller, and the collars had been entirely, the flagella partially, retracted. Gaps in the blow-out appeared, bridged by dermal cells. Three days later the blow-out, together with all traces of flagella, had disappeared, and four days later the mass had still further shrunk, and was apparently covered entirely with dermal cells, though I could not be quite sure of this.

The most interesting feature of this is the gradual assumption of unitary form by artificial aggregation of cell-masses, which

in their turn are produced in a totally abnormal, artificial way. The form produced, however, as also in the case of the choanocyte spheres, though typical, is not in the least like anything occurring in the normal life of the species. Here, typical form and form-changes of an organic type are seen in artificial aggregates. Once more we see a series of organic forms very clearly as an equilibrium between external environment and inner constitution. Here, however, the inner constitution is simple, the changes are not running in grooves of heredity. It is probable that many adult forms of simple organisms, as well as simple developmental forms, are in this way determined almost entirely by a direct relation of not highly-differentiated tissues with environment. The blastula, for instance, may or may not represent an ancestral adult form. It certainly is a primitive ancestral developmental form ; but it is this not for any adaptive or eventually ' organismal ' reason, but because it is the simplest way in which a number of undifferentiated cells can arrange themselves in a fluid medium. See also Child (**2**) for an account of the way in which adult form may be largely determined thus in flat-worms.

8. Restitution-bodies and Tissue-culture.

It is obvious that the spheres produced by isolation of sheets of collar-cells are ' tissue-cultures ' in that they consist of one sort of cells only. Their history in ordinary sea-water is a history of gradual starvation, followed by involution, since the fluid does not contain sufficient nutriment. Numerous experiments were tried with a view to finding a suitable nutrient medium, but so far without success.

(1) Pure culture of the Diatom Nitzchia, so successfully employed by Allen and others for feeding Echinoid plutei, were obtained and mixed with water containing preponderatingly choanocyte restitution-bodies. In a few cases, diatoms were seen in the collars of collar-cells, or partly embedded in the cell-body ; but they were apparently too long for convenient ingestion.

(2) Suspensions of common sea-water Bacteria killed by heating were added daily.

(3) Masses were put in vessels, together with fresh Ulva, to see whether they would ingest the swarm-spores.

(4) Sterile solutions of Peptone in sea-water, of various strength, were prepared. The restitution-bodies were transferred to this through four changes of sterilized sea-water, the pipette being sterilized between each operation. Although somewhat over 50 per cent. of the cultures thus prepared became contaminated, yet a number remained free of bacteria. In all these, however, the choanocytes underwent regressive changes, actually sooner than in normal sea-water, and the masses died within a few days.

(5) 'Sponge Broth'. 3 c.c. of chopped sponge was extracted in 20 c.c. of sea-water and sterilized, and restitution-bodies transferred to it as under (4), but again with no success.

(6) Ammonium lactate of 0·1 per cent. concentration was prepared and a trace of phosphate added (cf. Peters, **14**), and then sterilized. Again some restitution-bodies were transferred to the medium without infection, but all contracted and died speedily.

(7) Under gauze, in the circulation (at Wood's Hole). Unfortunately these experiments had to be discontinued. The restitution-bodies remained healthy for some time, but growth could not be detected. The fact that normal regenerates throve better and actually grew in these conditions, warrants the belief that some modification of this method might be successful.

Although all methods so far tested have proved unsuccessful, yet I feel sure that choanocyte masses could be supplied with food. It is possible that experiments in circulation would succeed best at Naples, where Sycon establishes itself and grows in the tanks. Further, experiments of this nature would most profitably be undertaken in the cooler months (see § 1 of this paper). At Wood's Hole I found that covered cultures kept cool in the circulation throve better than those exposed to air-temperature.

The cultivation of the collar-cell spheres, if successful, would open out many points of interest. What, e.g., would happen if considerable cell-multiplication took place? The resemblance of the collar-cell spheres to colonial protozoa, and the fact that the collar-cells are the nutritive organs of the sponge, make the research still more interesting. Finally, the ease with which sheets of pure collar-cells can be obtained, and the fact that they will remain healthy, with expanded collars and active flagella, for one to two weeks without being fed, renders them very suitable as material. Detached tissues which assume characteristic form in this way and live for a considerable period in the normal medium may be termed free tissue-cultures.

One or two interesting points concerning ingestion by the collar-cells may be mentioned here.

(1) Addition of powdered carmine to a culture of choanocyte masses was followed within an hour or so by ingestion of some of the particles. Very many particles adhere to the flagella, so that the masses appear reddish. Such particles as find their way within the collar are ingested by a pseudopodial extension of the intrachoanal protoplasm. No extrachoanal ingestion was observed.

(2) When Nitzchia was added, very few were ingested, and these only partially. They were usually caught, like the carmine particles, by the ends of the flagella, and lashed to and fro. This adhesive condition of the flagella is of interest. (In fresh dissociation cultures, finger-cells may often be seen adherent to the flagella and being waved from side to side with their beat.) Some were also seen adherent to the inner side of the collars.

9. Mechanical Shock. Toxic Agents.

Mechanical shock, such as repeated pipetting, or even transfer to a hanging drop, will cause marked changes in the cells, both dermal and choanocytic. A collar-cell blow-out treated in this way shows marked reduction of the size of the blow-out region,

together with a thickening of its walls. The collars are usually retracted. (See figs. 10, *a*, and 10, *b*.)

This sensitiveness to mechanical stimuli is shown by many other tissues in culture (cf. Holmes, **6**).

Exposure to very dilute solution of mercuric chloride in sea-water ($\frac{n}{500,000}$ to $\frac{n}{2,000,000}$) causes retraction, first of the collar and then, gradually, of the flagellum, together with slowing of the flagellar beat. The effect is proportional to the strength of the solution. This retraction of the flagellum is a remarkable phenomenon, and the short stumps of the flagella still beating provide a curious spectacle.

A record of an experiment is appended.

Record of Experiment.

Five or six collar-cell blow-outs in each solution (100 c.c. each).

A. Control. Collars and flagella remained normal for twenty-four hours.

B. $\frac{n}{1,500,000}$ $HgCl_2$.

1 hr. 25 m. Two masses with short collars. Three masses with very short or no collars, and sharp smooth outline of epithelium, all with fair to good flagellar movement.

2 hrs. 10 m. None with more than very short collars. Some with short flagella. These beating faster than unretracted flagella of other masses. Smooth outline still visible.

19 hrs. No collars. Only two with flagella (moderate length), two with slight cell-disintegration.

C. $\frac{n}{750,000}$ $HgCl_2$.

1 hr. 10 m. One mass with short collars in most cells, one mass with vestigial collars, remainder without collars. Flagellar action moderate, one with shortened flagella. Blow-outs shrunken in all but one. Some cell-disintegration.

2 hrs. No collars. Flagellar action slow and spasmodic.

18 hrs. No flagella. All masses with much disintegration into separate cells.

D. $\frac{n}{200,000}$ $HgCl_2$.

1 hr. No collars. Flagellar action very slow or nil. Flagella absent or normal length. Two blow-outs still present.

1 hr. 55 m. No flagella. Disintegration starting in all.

18 hrs. 45 m. Masses present, but more disintegrated than C.

E. $\frac{n}{100,000}$ $HgCl_2$.

50 m. Collar-cell blow-outs no longer visible. One dermal blow-out unaffected. Two masses with a few flagella moving (slow or spasmodic). Flagella somewhat shortened. Masses with irregular outlines.

1 hr. 50 m. All cells rounded off, total disintegration of masses starting.

18 hrs. 30 m. Completely disintegrated into groups of one to twenty dead cells.

F. $\frac{n}{50,000}$ $HgCl_2$.

40 m. No collars or flagella. Blow-outs burst, contracted, or disappeared. Masses with irregular outlines.

1 hr. 50 m. and 18 hrs. 30 m. As E.

10. Discussion.

(*a*) Dedifferentiation. Position and Fate.

Wilson, in his work on dissociation and subsequent regeneration in Monaxonid sponges, left the question entirely open as to whether regeneration was due wholly to the 'totipotent' amoebocytes, or whether the differentiated tissue elements underwent a process of 'despecialization' (dedifferentiation) into an 'indifferent or totipotent state', after which they took their shares in restitution. He does not seem to have envisaged the possibility of the differentiated cells sharing in the restitution-process without undergoing total dedifferentiation. In his later paper, on dissociation and restitution in Hydroids (**16**), he returns to the subject, and decides that in these forms, where undifferentiated cells form but a fraction of the normal body, the differentiated tissue-elements definitively become despecialized 'to form masses of totipotent regenerative tissue'. The cells in these masses later differentiate in accordance with their position, the outer cells forming ectoderm, the central core endoderm. This is, of course, in accordance with Driesch's well-known dictum that the fate

of a cell is a function of its position. He finally concludes that, since the restitution-masses of sponges are so like those of Hydroids, the processes occurring in them are of the same nature.

He further stated that the cells in the early stages of the restitution-mass formed a syncytium, few or no cell-boundaries being distinguishable.

De Morgan and Drew, in their later work on restitution-masses in other Hydroids, while confirming Wilson in some points, differ from him in others. In the first place, although restitution-bodies with perisarc, ectoderm, and typical endodermic coenosarcal tubules were produced and lived for as long as sixty days, no hydranths were formed. As Orton (**12**) suggests, this may be due to the fact that de Morgan and Drew's experiments were performed from December to March, when the growth of Hydroids appears to be at a standstill, while Wilson worked in the summer.

In the second place, although they describe a syncytial phase, their figures do not show any such complete cell-fusion as Wilson's, and they mention that a small portion of endoderm cells are always to be recognized as such. They do not pronounce definitively one way or the other as to whether dedifferentiation of all cells to a ' totipotent ' condition occurs.

Müller (**10**) also believes that collar-cells do not take part in the redifferentiation of restitution-masses in Spongilla, but that the amoebocytes and thesocytes form the new gastral cells. In view of the great rôle played by the amoebocytes in monaxonid sponges, and the specialization, small size, and relatively small number of the choanocytes, this is not surprising. In gemmule development, for instance, the flagellated chambers arise from archaeocytes. The same author (**11**), in describing dedifferentiation in Spongillidae, notes that the choanocytes early dedifferentiate and disappear, apparently ingested by amoebocytes. It would appear that they cannot maintain themselves as such in unfavourable conditions. In this connexion, mention may be made of the work of Maas (**9**), who found that slow deprivation of calcium led to similar

dedifferentiation in various calcareous sponges, including a heterocoelous form (Sycandra). He also describes degeneration and phagocytosis of the collar-cells in late stages of the process.

In view of my work (see discussion below), it would appear that in both Calcarea and Monaxonida the choanocytes are more susceptible than the amoebocytes, and will degenerate in certain conditions. In Calcarea, however, this difference in susceptibility is less marked, and the choanocytes will remain capable of maintaining existence in dissociation-masses, while this is not possible for those of Spongilla.

My own work (7) on Sycon indicated that the conclusions of Wilson as to the fate of the cells in restitution do not apply in the case of Sycon. On dissociation the tissue elements all become dedifferentiated morphologically, e. g. the choanocytes lose both collar and flagellum and become rounded, the dermal cells lose their extended flat shape for a spheroidal one ; but this dedifferentiation is not complete in the sense that the various kinds of cells become physiologically similar, or acquire the same potentialities of development. After this dedifferentiation caused by shock the cells redifferentiate in appropriate directions, the dermal cells producing an external epithelium round a central choanocyte mass, which in its turn becomes hollow with epithelial walls. The normal form of the post-larval sponge is thus produced by a process exactly the reverse of that envisaged by Driesch and Wilson. The fate of the cells is not a function of their position, but their eventual position is a function of their constitutional differences. The development of a restitution-body is primarily a process of sorting-out of different kinds of cells, followed by a redifferentiation of the individual types of cells. We have thus to distinguish sharply between two types of cellular dedifferentiation : (1) that which leads to complete loss of the character of the tissue to which the cell belongs, and a return to a totipotent, or at least, if I may coin a new word, to a pluripotent condition. This may be called ultra-typical (or pluripotent) dedifferentiation; (2) that which leads to a temporary

suppression of the characters of the cell, also with the assumption of a simple spheroidal form. Redifferentiation, however, is only possible in the direction of the original form, and the cell has not acquired pluripotency by dedifferentiating. This may be called intra-typical (or unipotent) dedifferentiation.[1]

The existence of pluripotent dedifferentiation is rendered probable by various observations which cannot be entered into here. It has frequently been assumed, however, on insufficient evidence; and in view of its theoretical importance, and the difficulty of proof, very thorough investigation is required to establish its existence in any particular case.

Further evidence against its occurring in Sycon was afforded by the artificial production of masses composed entirely, or almost entirely, of collar-cells. These, though they lived healthily for a number of weeks, never produced a dermal epithelium or spicules. This is paralleled by the failure of endoderm or ectoderm alone to regenerate in Hydra, as has been shown by various observers.

In a later paper (Huxley, **8**) attention was drawn to the fact that masses composed only of collar-cells were less viable than those containing dermal cells also, although both were kept under identical conditions, and although the collar-cells are the organs of nutrition.

In the present paper, the converse of the collar-cell blow-outs is shown to occur in the form of masses with an excess of dermal cells. These form blown-out vesicles exactly as do the choanocytes when they are in excess.

It is thus clear that, in Sycon at least, the form and composition of the restitution-mass depends (apart from questions of size) upon the proportions of the different types of cells which entered into its composition.

It is clear from the observations of Wilson that some process of dedifferentiation does occur in restitution-masses of Hydroids.

[1] Since writing the above, I find that a very similar classification of types of dedifferentiation from the point of view of tumour-growth has been adopted by Adami and McCrae (**1**, p. 324. See also pp. 318–22).

E. g. in Pennaria the endoderm cells enter in large numbers into the composition of the restitution-masses, and are distinguishable immediately after dissociation by large granules. Within twenty minutes, however, a syncytial mass has been formed, in which very few of these granular elements can be distinguished. Presumably the granules have been resorbed in the new conditions. On the other hand, neither his observations, nor those of de Morgan and Drew, in the least exclude the idea of migration of ectoderm or endoderm cells to their proper stations after intra-typical dedifferentiation.

In this connexion, the facts concerning the possible attraction of the various types of cells for each other may be mentioned (Huxley, **8**). In cultures consisting almost entirely of collar-cells, a small proportion of normal regenerates usually occurred. In other cultures made at Plymouth, where the great majority of the masses were choanocyte blow-outs, with partial dermal covering, a small proportion were dermal blow-outs. These facts may be due either to accidental distribution of dermal cells, or else to an attraction of dermal cells for each other. This point could only be settled by appropriate experiments. The probable attraction of spermatozoa by choanocytes was mentioned in the same paper.

(*b*) Formation of Blow-outs.

The secretion of fluid by epithelia, whether dermal or choanocyte, and consequent formation of spheres or segments of spheres (' blow-outs '), is an interesting phenomenon.

In this connexion, Mr. J. Gray, of King's College, Cambridge, has kindly allowed me to refer to some unpublished observations of his own, which he is at present investigating, on the formation of similar blown-out spheroidal masses by fragments of the gills of Mytilus. The phenomenon would thus seem to have more than isolated significance. It perhaps involves changes of the same nature as those taking place in the formation of a blastocoele.

(*c*) Size Relations; Viability.

Wilson (loc. cit.) found that the size of the restitution-masses produced by Hydroids was of great importance. Large masses almost invariably died early, while too small masses, though living for a long time, failed to produce Hydranths or even coenosarcal outgrowths.

In Sycon also, very small masses, though reaching a two-layered stage and occasionally forming spicules, fail to metamorphose. Similar failure to produce normal structure from pieces below a certain definite size is well known in studies on regeneration, both in unicellular and multicellular organisms. It may be partly due to mere lack of material, but undoubtedly also, in some way not as yet properly understood, to the relatively greater surface and the consequences thereon attendant—differences of gaseous exchange and difference of stimulation by the environment being prominent.

Similarly, in too large a mass, it does not appear that proper oxygenation for the central cells can be provided, and so disintegration sets in. Wilson found the interesting fact that successfully-metamorphosing masses were of the same order of size as normal planulae. The same is roughly true for Sycon, although here the upper limit of size for successful masses is much further above the larval size than in Hydroids.

De Morgan and Drew comment on the fact that their restitution-masses, although not metamorphosing, were much more resistant to laboratory conditions than the normal colonies, and regard it as surprising. There should be no ground for surprise in this—the cells of the restitution-masses are definitively, as we have seen, in a dedifferentiated condition. Experiments on Perophora and Obelia show that the undifferentiated stolons and hydrocaulus remain perfectly healthy in conditions causing dedifferentiation and resorption of the zooids. Clavellina and other Ascidians hibernate in the form of 'winter-buds', which are of somewhat similar nature to restitution-masses; and the normal gemmules of sponges have also something in common with them. In the laboratory the hydriform

larva of the medusa Gonionema becomes transformed into a syncytial, undifferentiated mass, as was shown by Perkins (**13**).

The obverse of this condition is shown by the failure of highly differentiated parts of the organism to maintain themselves in the restitution-masses. Wilson and de Morgan and Drew found that portions of tentacles fail to become incorporated in the masses. This is paralleled by the failure of Hydra tentacles to regenerate. Apparently, on the one hand they are too highly specialized to dedifferentiate ; and on the other cannot exist as such in the conditions afforded by the restitution-bodies. The nematocysts also are gradually resorbed in the restitution-bodies.

If we seek to embrace the phenomena in one general view, we may say that Hydroid tissues in unfavourable or abnormal conditions lose much of their differentiation, come to have a low metabolic rate (in the general sense in which the term is used by Child (**3**)), and are more resistant. In these conditions specialized organs cannot exist. The same tissues in optimum conditions possess a higher metabolic rate, and are capable of maintaining specialized organs such as the tentacles in existence.

(*d*) 'Normal' and 'Abnormal' Phenomena.

Attention has already been drawn to the fact that many of the processes occurring in restitution-bodies and free tissue-cultures run parallel with various phenomena of development. The normal phenomena constitute an interlocking series, each stage of which is determined by the preceding and helps to determine that which comes after. By studying processes which occur in 'abnormal' conditions, e.g. by dissociation methods, we remove the tissues of the organism from this developmental chain, where it is often impossible to say what occurrences are palingenetic, what adaptive, what the direct consequence of changes in the environment, and what conditioned by previous processes in the series ; by varying the conditions, we may then throw light upon the normal processes.

So far my work has been mainly devoted to elucidating

roughly the course of events in restitution-bodies in Sponges. It is clear, however, that in Sycon we have an admirable material for qualitative experiment, as to the rôle of size of masses, the proportion of the tissues in the mass, the coherence of cells, their mutual attraction, &c.

The elucidation of these problems will need many workers, and it is hoped that others may be induced by the facts here set forth to take up the work.

Meanwhile two tendencies should be noticed. The first is a tendency to discuss the results from a morphological standpoint. This is shown, e. g., in Wilson's discussion of results. He compares the development of the restitution-masses in detail with that of normal development, and goes so far as to apply the term ' yolk ' to the central syncytial portion which remains in the middle of the masses while the two layers are differentiating. This, and indeed his whole discussion, though of great interest, seems to me to be putting the cart before the horse. We should rather expect to find some of the causes determining the presence and form of the normal yolk by examining the mode in which the abnormal conditions of a restitution-mass influence the internal cells, rather than vice versa.

A word is also in order as to the use of the terms ' normal ' and ' abnormal '. Abnormal is often used as if it were synonymous with pathological. This is not the case in any of the forms of restitution-mass here described (until we reach degenerative change at the close of their history, this being due to lack of nutriment and to laboratory conditions). Dedifferentiation, aggregation, sorting-out, &c., are all perfectly healthy phenomena.

11. Summary of Results.

(Including those recorded in previous papers.)

1. Various methods can be used to dissociate the tissues of Calcarea Heterocoela.

2. Mixture of the various types of cells in normal proportions may lead to the formation of normal regenerates, resembling post-larval Sycon, with spicules, osculum, and pores.

3. The development of these masses consists primarily in the sorting-out of the dermal and gastral cells. The former produce a single-layered epithelium, below which spicules are subsequently formed, the latter a central mass which later becomes a hollow one-layered sac, into whose cavity the cells put forth collar and flagella. Thus their fate is not a function of their position in the whole, but their position a function of their nature.

4. The two types of spicules are formed in the same order as in normal development.

5. Free tissue-cultures consisting only of collar-cells can be obtained by appropriate methods. These form spheres resembling choanoflagellate colonies with the collars directed outwards. These live for a considerable time, but do not regenerate other forms of tissue or produce spicules.

6. All grades from these to masses containing an excess of dermal cells may be formed. They may be classified as follows :

(*a*) Collar-cell spheres.

(*b*) Collar-cell blow-outs. These consist of a solid mass with one or more portions blown out to form a segment of a collar-cell sphere.

(*b* 1) With active collar-cell epithelium over the whole surface.

(*b* 2) With mixed collar-cell and dermal epithelium over the solid portion.

(*b* 3) With dermal epithelium over the solid portion.

(*c*) Normal regenerates.

(*d*) Dermal blow-outs, resembling (*b* 1), but with dermal epithelium over the whole surface.

7. In almost pure collar-cell cultures, a few normal regenerates may be found. In cultures consisting almost entirely of collar-cell blow-outs, a few dermal blow-outs may be found. This is probably due to mutual attraction of dermal cells.

8. Normal regenerates are more viable than collar-cell spheres or collar-cell blow-outs of type (*b* 1).

9. Dermal blow-outs may be formed from collar-cell blow-outs. They are in such cases produced more readily from large masses.

10. Numerous methods have been tried for feeding the collar-cell spheres and blow-outs, but so far without success.

11. The flagella of collar-cells are adhesive.

12. Larvae may become embedded in the restitution-masses ; they are gradually resorbed.

13. Restitution-masses, if brought into contact, will cohere. The irregular masses thus produced gradually round up and become unified.

14. Mechanical shock causes a contraction of both dermal and choanocyte blow-outs, and a retraction of the collars and partial retraction of the flagella in the latter.

15. A peculiar small finger-shaped amoebocyte (' finger-cell ') is numerous in normal sponges and restitution-masses. These cells are arranged in a remarkable manner below the dermal epithelium of dermal blow-outs.

16. Spontaneous segmentation of restitution-masses into small spherules may take place, apparently in unfavourable circumstances. The spherules usually secrete a gelatinous covering. They may differentiate a normal dermal epithelium. The bulk of the component tissue (presumably choanocyte) usually separates into its constituent cells after a time.

17. A type of restitution-body with dark central mass is described.

18. Dedifferentiation of all cells takes place after dissociation, but does not lead to a totipotent condition.

New College, Oxford.
October 1920.

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EXPLANATION OF PLATES 13 AND 14.

The figures are all drawn from life with the Abbé camera lucida. The magnifications are given as follows: 3+4 oc., denotes drawn at table level with a no. 3 ($\frac{1}{3}''$) objective and no. 4 Huyghenian ocular. The objectives and oculars were Reichert unless otherwise stated.

Plate 13.

Fig. 1.—A subdivided restitution-mass. (Eight days.) *a*. The whole mass. The spherules are mutually compressed and show a definite cubical epithelium. (3+4 oc.) *b*. A single spherule under higher power. The central mass is distinct from the epithelium. (6+2 oc.)

Fig. 2.—Different stages of another subdivided mass. (3+4 oc.) *a*. A nine-day mass. The spherules have separate gelatinous layers, and no sharp epithelia. Dark areas are seen within them. *b*. Three days later. All but one possess well-marked dermal epithelia and have somewhat expanded. The central masses are irregular, and several have fragmented.

Fig. 3.—A small eleven-day mass with dermal epithelium; the contents are subdivided into small spherules. No jelly-layer. (3+4 oc.)

Fig. 4.—Ten-day subdivided masses. The individual jelly-layers of the spherules are not shown. (3+4 oc.)

Fig. 5.—A single spherule of the mass of fig. 4, three days later, under higher magnification. The layer of jelly, the separation of the clear cells, and

the dense mass of yellow-brown cells are seen. (Zeiss $\frac{1}{16}''$ water-immersion + 4 compens. oc.)

Fig. 6.—Another spherule from the same specimen, same date. The layer of jelly is thinner. The spherule has subdivided into irregular masses with clear outer layer and yellow inner centre. From one, cells are beginning to separate. (Same magnification as 5.)

Fig. 7.—Olynthus stages from restitution-masses. Osculum and oscular crown are well developed. (3+2 oc.) *a*. Large, fixed, of normal shape (spicules figured at the edge only). *b*. Smaller, of abnormal shape (spicules omitted). A small patch (undotted in the figure) lacks the gastral layer.

Plate 14.

Fig. 8.—A further stage in the development of the type shown in fig. 13. The gastral layer is markedly incomplete (spicules only figured at the edge). (3+4 oc.)

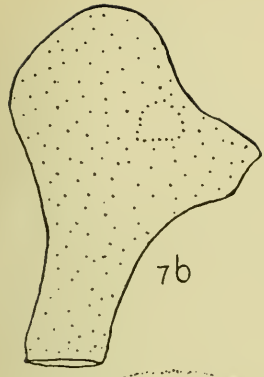
Fig. 9.—Successive stage in one hanging-drop culture. (3+4 oc.) *a*. The chief masses present in the drop, two hours after isolation (four days from beginning of experiment). *b*. After two days. The masses shown in (*a*) have fused together (in addition, in (*a*) there were three embryos and two small masses which had not fused). Note three larvae and one sphere partially attached. *c*. After four days. Larvae no longer visible. Blow-out larger; more unification of the separate masses. *d*. After six days. No collars. Flagella shorter and fewer. Still more unification. Gaps in the blown-out region bridged by dermal membranes with amœbocytes on the inner surface. *e*. After nine days. Disappearance of blow-out. No collars or flagella. *f*. After thirteen days. Still further contraction. A few cells had separated from the mass (not shown).

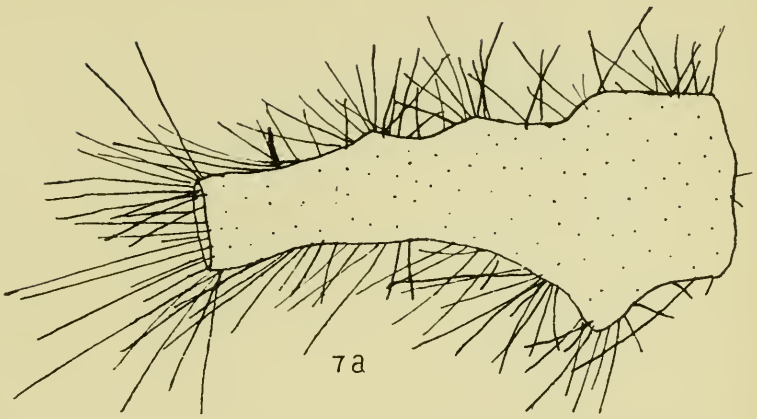
Fig. 10.—To show the effects of mechanical shock. (3+4 oc.) *a*. A mass with good choanocyte blow-out and attached larva. *b*. The same mass after repeated pipetting. The larva is detached, the epithelium of the blow-out has contracted and thickened, the collars have been retracted.

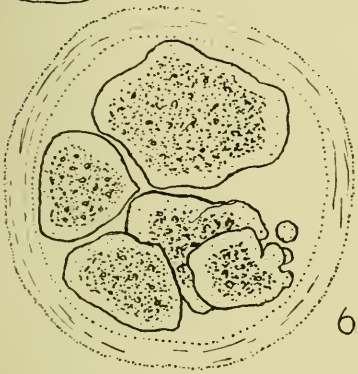
Fig. 11.—Restitution-mass with dermal epithelium and central dark yellow-brown sphere, separated from intermediate layers of collar-cells. (6+2 oc.)

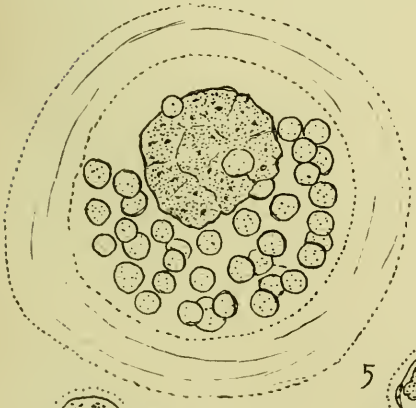
Fig. 12.—Small dermal blow-out under high power. (6+2 oc.) The dermal cells are granular. Adhering to the inner side of each are a number of finger-cells. A few dermal cells are figured in surface view. From others, the subjacent finger-cells have been omitted. Over the rest of the surface, dermal cells are not figured. The bulk of the interior mass is composed of choanocytes. From the edge of this, finger-cells protrude into the blow-out cavity.

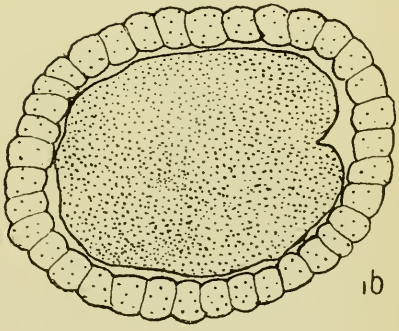
Fig. 13.—Very large dermal blow-out, spherical type. (3+4 oc.) Here there is no sharp internal mass, but the collar-cells form irregular areas of varying thickness adherent to the dermal epithelium. Those on the upper side are represented darker than those below. (The cells of the dermal epithelium have been represented too large.)

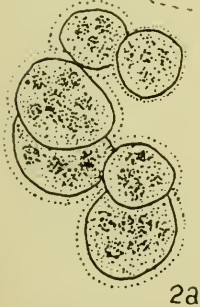


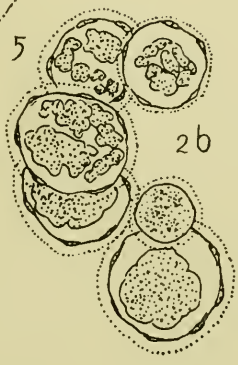


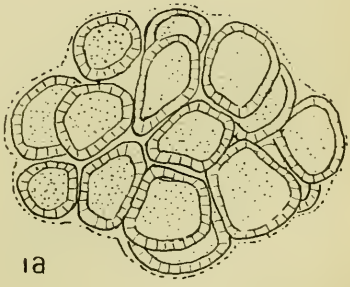












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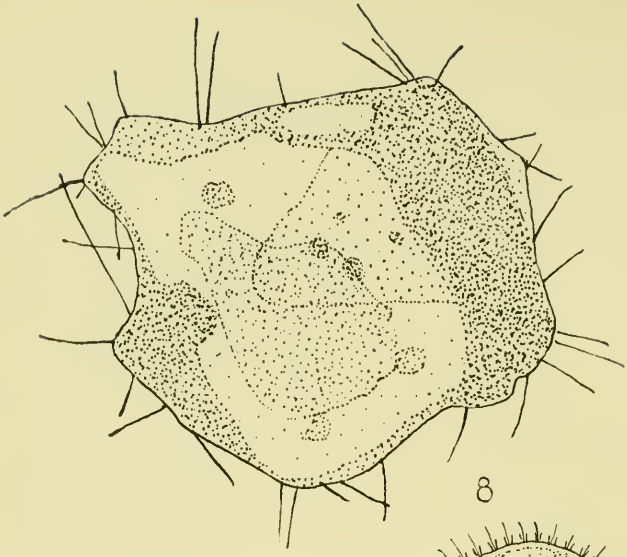

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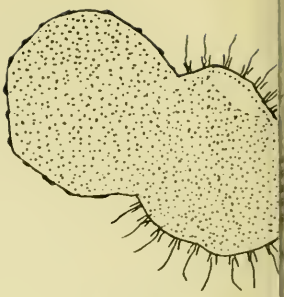

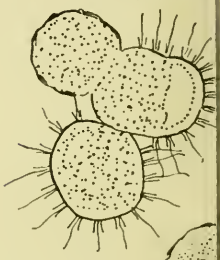

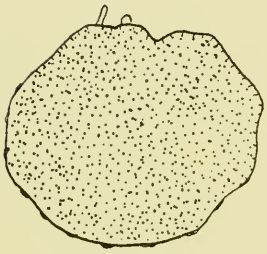

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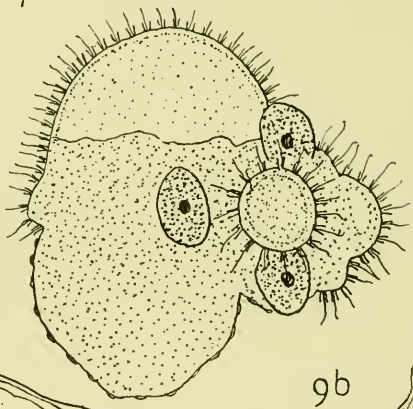

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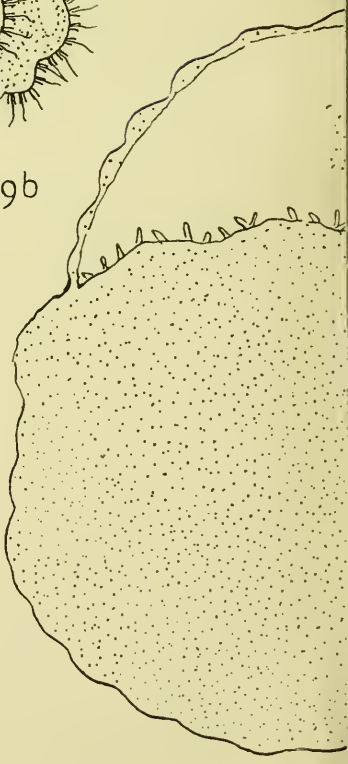

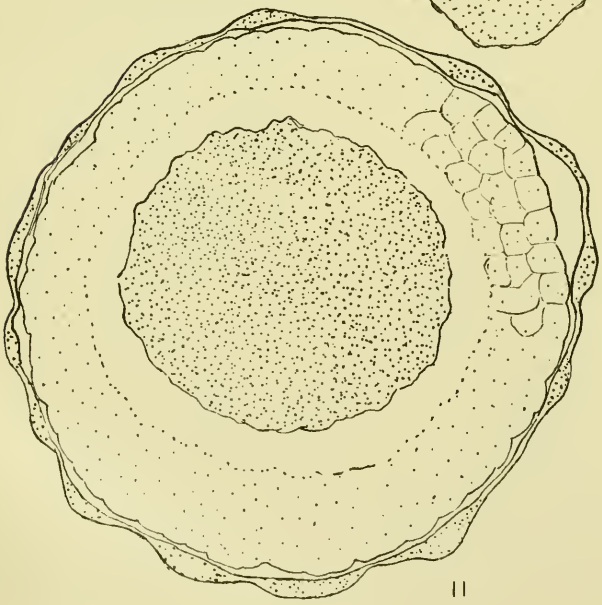

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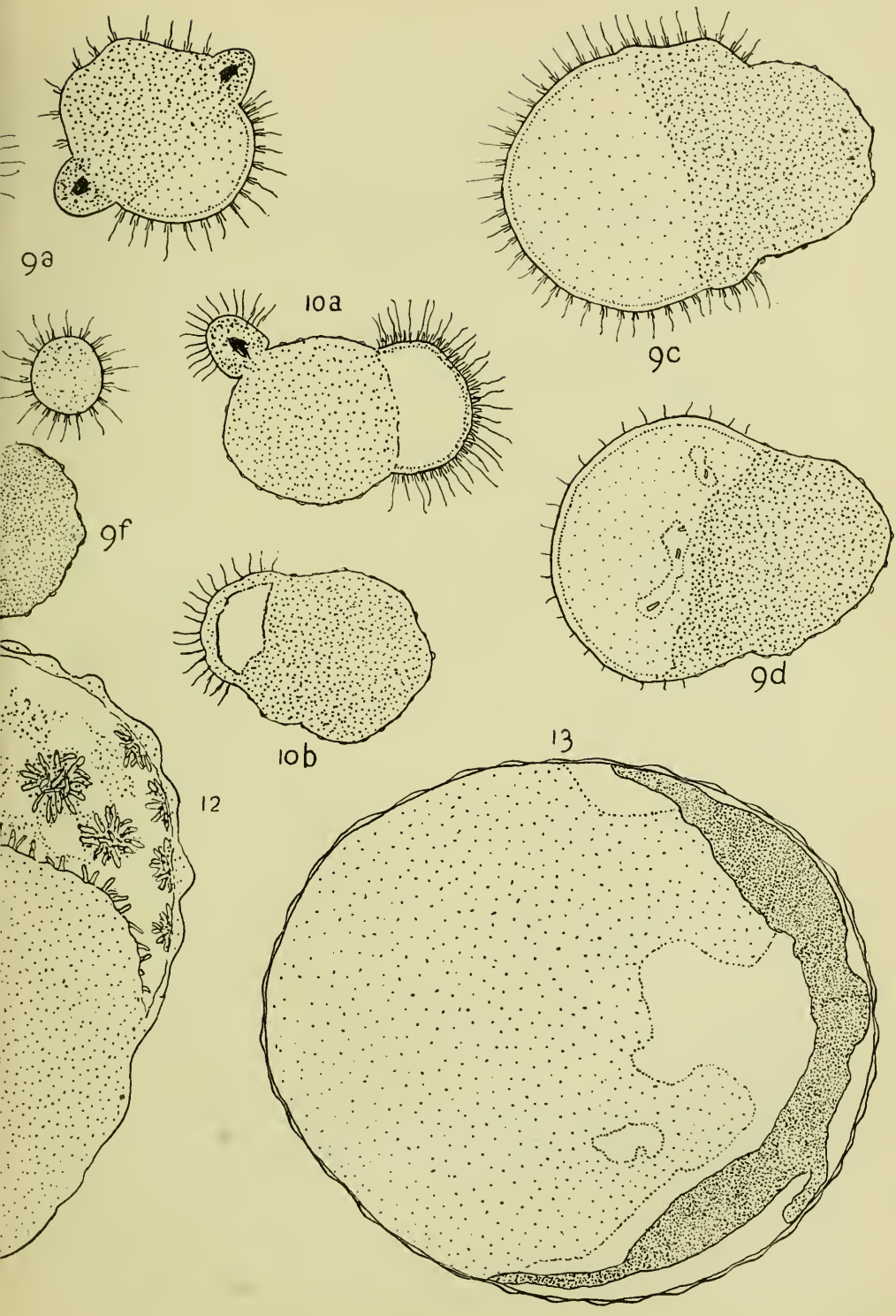
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The Proboscis of the Syllidea.
Part I. Structure.

By

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With Plate 15.

I. Divisions of the Proboscis Region.

The proboscis of the Syllidea (here taken as comprising all that part of the digestive tube lying in front of the intestine) is made up of five parts (fig. 6), which are all (with the exceptions presently to be noted) sharply marked off from one another. These will be referred to here as (1) the buccal chamber, (2) the pharynx, (3) the proventriculus, (4) the ventriculus, and (5) the post-ventriculus with a pair of caeca appended to it.

Ehlers (1864) recognized in the region (1) 'Rüsselröhre' (buccal cavity), (2) 'Schlundröhre' (pharynx), (3) 'Drüsenmagen' (proventriculus), and (4) 'Uebergangstheil' (ventriculus plus post-ventriculus).

De Quatrefages (1865) (**10,** tome ii, p. 3) recognized buccal cavity, and pharyngeal, dentary, and oesophageal regions of the proboscis.

Claparède (1868) distinguished: 'gaine de la trompe' (buccal cavity), 'trompe' (pharynx), 'proventricule', 'ventricule' with its glands (caeca).

Eisig (1881) describes the 'Rüsselösophagus' as made up of three sharply-separated regions—the first (pharynx), the second ('Drüsenmagen'), and the third, which he does not name, but which is the ventriculus: this is followed by

the ' Vormagen ' (post-ventriculus) from which the caeca are given off.

Malaquin (1893) designates the divisions ' gaine pharyngienne ' (buccal chamber), ' trompe pharyngienne ' (pharynx), ' proventricule ', and ' ventricule ' (ventriculus plus post-ventriculus).

McIntosh (1908) describes the region as consisting of (1) pharyngeal cavity, (2) protrusible proboscis, (3) proventriculus, followed by (4) a short portion which ends in a dilated region often with two lateral caeca (see Pl. 15, fig. 6).

II. The Buccal Chamber.

This is the only part which becomes actually evoluted when the proboscis is protruded. It is a short chamber with a cuticle thinner than that of the outer surface : its wall in the ordinary retracted condition is thrown into a number of folds.

III. The Pharynx.

The pharynx is a cylindrical tube, usually of considerable length, straight in the majority, sinuous or coiled in the Autolytidae and in Amblyosyllis. It has a greatly thickened cuticle, the thickened lining terminating abruptly in front in an entire, lobed, or denticulate edge. In front of this is a circlet of papillae on the surface of which open the numerous fine ducts of the pharyngeal glands. In most cases the pharynx contains a single triangular tooth (or rather stylet) with the base embedded in its dorsal wall. This is nearly always situated at the anterior end, and is so placed that, when the proboscis is protruded, its apex projects freely in front. In some cases the single tooth is replaced by a paired crescentic group of several teeth (Odontosyllis), or by a circlet (Trypanosyllis, Autolytus).

The cellular layer of the pharynx in the anterior part of its extent is a simple epithelium complicated only by being perforated by the system of splanchnic nerves. Posteriorly it becomes greatly modified by the development of numerous gland-cells, so that it virtually assumes the character of

a gland. In the Exogoneae this gland, which I have termed the anterior proventricular, is more conspicuous than in the other groups of the Syllidea owing to its being more distinctly marked off; but in the latter it is quite as important so far as relative development is concerned (fig. 7). The cuticle in this region is as thick as it is throughout, and appears quite imperforate, so that the secretion of the gland must find its way out elsewhere. As in Exogoneae, in fact, the ducts of the gland-cells run back through the epithelium to the anterior region of the proventriculus, where the cuticle is very thin and, apparently from its staining reactions, not strongly chitinized. Here most of the ducts terminate, though some appear traceable for some distance in the region behind the chitinous plates.

Eisig describes the structure of the pharynx correctly as regards the greater part of its length. The change which takes place at the posterior end he describes rightly as regards the epithelium, but he falls into an error in stating that in this region the structure corresponds closely with that of the ventriculus, not only in the modification of the epithelium, but in the development of radial muscular fibres.

Malaquin gives a more exact account of the structure as far as the Syllidae and Eusyllidae are concerned. He recognizes the glandular modification of the epithelium at the posterior end, but assumes that this has to do with the growth of the pharynx and the formation of additional chitin. In Amblyosyllis and Autolytus, with elongated coiled pharynx, he places the glandular region towards the middle instead of at the posterior end, i.e. instead of at the opening into the proventriculus, where it occurs exactly as in the Syllidae and Exogonae.

In connexion with the pharynx and its papillae mention has been made of the pharyngeal glands, the secretion of which is discharged on the surface of the latter. As I have pointed out (**7**, p. 229), these glands were referred to by Claparède (**2**) and De Saint-Joseph (**11**) and were fully described by Malaquin (**9**). They consist, in most cases, of about

ten narrow cylindrical bodies of varying length surrounding the pharynx, with which they run parallel, ending blindly behind, and in front terminating in the pharyngeal papillae.

They are solid bodies each of the nature essentially of a group of greatly elongated cells, the anterior end of each of which is produced into a narrow duct terminating in a very minute aperture on the surface of the corresponding papilla.

In Odontosyllis the arrangement of these glands is, as pointed out by Malaquin, somewhat modified by their restriction to the ventral side. In Amblyosyllis and in certain species of Autolytus, as also observed by Malaquin, they are fused together into a pair of irregular masses of considerable size. These divide up in front into narrow lobes running forwards to the papillae.

IV. The Proventriculus: General Structure.

The proventriculus is an exceedingly conspicuous and very characteristic structure to which reference is made by all writers who have dealt with this group of the Polychaeta. But it was not till, in 1881, Eisig published his paper entitled 'Ueber das Vorkommen eines schwimmblasenähnlichen Organs bei Anneliden' that an approximately correct interpretation was given of the structure of this complex organ.

In Eisig's account, though it marks a distinct advance in our knowledge, there are certain omissions and certain misstatements. Of the former one of the most important is the failure to recognize that the muscular tissue of the radial columns, which make up the bulk of the substance of the wall of the organ, is of the striated type. The true nature of this tissue was pointed out by the present writer in a short paper published in this journal in 1886; and the subject, as regards the histology of the muscular tissue, was further developed in 1889 (**6**).

In 1893 was published Malaquin's 'Recherches sur les Syllidiens'. In this comprehensive work the author gives a very full account of the proventriculus, summarizing previously published results and adding numerous observations

of his own. He gives many details, more especially regarding the radial columns of striated muscle and the variations which they undergo in different families and genera. Since the publication of Malaquin's valuable work there has not, so far as I am aware, been any further contribution to the subject with the exception of the brief reference to it contained in a paper on the Exogoneae contributed by me to the Linnean Society (7).

On approaching this subject anew, with a wider command of material, I have found that Malaquin's account, excellent though it is, with many new observations, is yet not altogether correct in some respects, and leaves untouched several structural features that seem to be of some importance in connexion with the study of the proboscis as a mechanical system.

The proventriculus is of cylindrical or sub-cylindrical form, usually with a small degree of lateral compression, and varies greatly in length in different members of the group. The surface is marked by a series of rings, an appearance which examination with a low power of the microscope shows to be due to the presence of annular fine lines and rows of dots. The fine lines correspond to annular bands of non-striated muscular fibres: the dots, which are frequently coloured in the living animal, are the outer ends of the cores of the radial columns of striated muscle. Along the dorsal side of the organ runs a longitudinal light or coloured line, the dorsal raphe, and a similar ventral raphe runs along the ventral side.

A comparison of the pattern on the surface of the proventriculus in representatives of different groups of the Syllidea reveals the occurrence of three main types. In one of these the annular lines alternate with the rows of dots. In a second the lines run through the dots. In the third type, which is the prevalent one in the Syllidae and in the Exogoneae, while the lines perforate the dots in all the lateral regions, they leave that position in the neighbourhood of the raphes, and pass to the latter in the intervals between the rows of dots.

These three types of pattern arrangement mean respectively:

(1) that the annular bands run throughout in the intervals between the radial muscle-columns; (2) that the annular bands perforate the muscle-columns throughout; and (3) that the same arrangement as in (2) holds good except in the neighbourhood of the raphes, where the annular rings pass to the position they occupy throughout in (1).[1]

The lumen of the proventriculus may be described as a vertical slit the upper and lower ends of which lie near the dorsal and ventral raphes respectively. This is the form assumed in the contracted state; in complete contraction the sides of the slit are in close contact: when dilated the slit expands till in transverse section its outline becomes ellipsoidal.

The thick wall of the proventriculus (Pl. 15, fig. 1) consists of the following layers: (1) splanchnic layer of coelomic epithelium; (2) outer fibrous membrane; (3) layer of radial muscle-columns and annular muscle-bands: (4) inner fibrous membrane; (5) enteric epithelium; (6) cuticle.

The coelomic layer is a very thin one, recognizable by its infrequent flattened nuclei. The outer fibrous membrane is the layer described by Malaquin, and earlier by myself, as a layer of non-striated muscle. Of its contractile character I am by no means certain. It is a thin layer, only about 0·003 mm. in thickness in the largest forms, and is made up of two strata in the outer of which the fibres run transversely and in the inner longitudinally: the fibres are exceedingly fine and there are no nuclei. The chief function of this layer seems to be to serve for the insertion of the radial fibres and the fibres of the annular bands. The inner fibrous membrane is a similar layer, also composed of outer transverse and inner longitudinal fibres: it has the inner ends of the radial fibres inserted into it. At the raphes paired trabeculae pass at regular intervals from the inner fibrous membrane to the outer and bind the two layers firmly together.

The enteric epithelium and the cuticle need not be specially

[1] Towards the anterior end of the proventriculus the regularity of the rings on the surface is broken owing to a modification in the arrangement of the radial muscles associated with the presence of the chitinous plates.

described here. They both become specially modified towards the anterior end of the organ in connexion with the valvular apparatus to be described later.

V. The Proventriculus: Muscular Elements.

The greater part of the substance of the thick wall of the proventriculus (figs. 1–5) is made up of the radial muscle-columns and the annular bands. The former are hollow fibres, squarish or polygonal in cross-section, arranged in annular rows, and extending radially from the outer fibrous membrane to the inner.

The hollow of each column is occupied by a protoplasmic core. In the columns which are perforated by the annular bundles the protoplasm is divided into anterior and posterior halves, and this division may extend to the inner end, but not to the short portion of the core outside the annular bands, the two halves being here continuous. In the Exogoneae and in certain members of the other groups each core contains only a single nucleus. But in the rest the structure is more complicated and the number of nuclei increased. The maximum of complexity is reached in the case of Syllis coruscans. In this species (fig. 4), in which the arrangement of the muscles is of type 2, the core is permeated by a system of exceedingly fine fibrils—forming an irregular meshwork with a prevailing longitudinal arrangement: this is more condensed towards the outer end. Communications occur between adjoining cores of the same row along the lines of the annular bands, and there are also communications, irregularly arranged, between the columns of neighbouring rows by means of processes which perforate the cortex. Fibrils from the meshwork of each core radiate outwards and penetrate through fissures into the substance of the cortex. Such communications are most numerous opposite the Z membranes (Krause's membranes) of the cortex, if they are not entirely restricted to such an arrangement.

Nuclei are present in large numbers in each core. These are of two main varieties—larger, clearer nuclei of about 0·0075 mm. in diameter, and smaller, denser, of a diameter of

about 0·005 mm. The former are less numerous, mainly situated towards the outer end, but occurring throughout the core to its inner extremity. The smaller nuclei are extremely numerous, distributed fairly uniformly throughout the length of the core. In addition there are a comparatively small number of nuclei belonging to what appear to be distinct cell-elements with fine-grained cytoplasm embedded in the core. Surrounding the cortex is a layer continuous with the core at the longitudinal fissure, composed apparently of similar material, and containing an occasional nucleus: the investments of contiguous columns coalesce completely.

Slightly less complex than Syllis coruscans are the cores in Trypanosyllis zebra. In this species the arrangement of the muscles conforms to type (3). The cores here consist of two kinds of material—an axial part, split into two in the perforated fibres, and a peripheral part. The former is loaded with rounded granules which are strongly coloured by haematoxylin; the latter appears as a meshwork of delicate threads, prolongations of which pass into the substance of the cortex. Strands of granules similar to those in the central part of the core run longitudinally between the fibrils of the cortex, and the latter is enclosed in an investing layer which encloses similar granules. The central part of the core contains numerous nuclei.

In Syllis variegata (figs. 2 and 3), in which also the arrangement of the muscles conform to the third type, the core is greatly simplified. In the perforated columns it is split longitudinally into anterior and posterior halves which unite together only at the extreme outer ends outside the annular bands. The substance of the core and the layer investing the cortex is a finely granular homogeneous material which does not become very readily stained. In this are embedded some five or six nuclei, one (or two) of which are larger than the others (about 0·008 to 0·01 mm. in long diameter), and are situated usually about the middle of the length of the fibre, while the rest are mostly towards the outer end. The core has a thin investment of what looks like fibrillated material.

As regards the cortex of the column. This consists of a bundle of fibrils among which penetrate branching processes from the protoplasmic core. Each column or fibre is characterized, except in the Exogoneae, by the presence of one (Typosyllis variegata, T. closterobranchia, T. truncata), or more 'striations'. In all essentials these fibres resemble the striated fibres of Arthropods and Vertebrates. The fibrils of each are bound together by one or more transverse membranes (Krause's membranes, telophragms) which pass through the fibrils, and, through the interfibrillar substance, bind all the fibrils intimately together. The fibre itself is composed of alternating zones of singly and doubly refracting material, the telophragms passing through the latter. Moreover, gold-chloride methods reveal systems of J-granules (sarcosomes) and transverse networks in the neighbourhood of the telophragms, exactly as is the case in the striated muscles of Arthropods and Vertebrates.[1]

At their outer and inner ends the fibrils of the striated muscular fibres are firmly fixed into the outer and inner fibrous membranes.

Occupying much less bulk than the radial fibres are the annular bundles of non-striated fibres. The extent of this system, its relations and the part which it plays in the movements of the proventriculus, have not hitherto received adequate attention. Malaquin, a little misled by his idea of a system of transverse septa separating the annular rows of muscle-columns from one another, pays little heed to these bundles. He says in his account of the proventriculus of the Autolytea (p. 217), 'Comme nous aurons l'occasion de le voir plus loin pour d'autres types, il est des points du diaphragme où les fibrilles, s'arrangeant en faisceaux, ont tout à fait l'apparence de fibres musculaires, et on peut croire alors que ce tissu conjonctif fibrillaire passe au tissu musculaire proprement dit. Nous reviendrons sur ce point à propos d'un autre type'.

[1] Mesophragms and Q-granules I have not hitherto succeeded in detecting, except somewhat doubtfully in the case of Syllis (Typosyllis) variegata.

The only further mention is under Syllis hyalina (p. 227) : ' Les diaphragmes transversaux ont la même disposition et la même structure, à part ce fait que le tissu fibrillaire qui les compose présente vers la périphérie un arrangement en faisceau très marqué.'

But these annular muscles, as they may be termed, are of much greater importance than such casual mention as that given above would imply.

Each annular muscle is a bundle of non-striated fibres, compressed in the antero-posterior direction, running (in the prevailing third type) transversely between two adjoining rows of radial striated fibres in the immediate neighbourhood of the raphes, and, farther on, passing through the outer ends of the radial fibres. At the raphe the annular muscle is continued straight across the middle line to the opposite side. From the raphe the muscle runs in an annular way in the position indicated above, and is inserted at intervals into the outer fibrous membrane. These insertions occur between the radial fibres of the row, around the corresponding accessory fibres (non-striated radial fibres) described below.

It will thus be seen that the annular muscles are so arranged as to form a system of constrictors by means of which the lumen of the proventriculus, dilated by the action of the radial fibres, is contracted.

The striated fibres, though the most important, are not the only radial fibres in the wall of the organ. Another set of radial elements, hitherto entirely overlooked, play a part which must be of some consequence, since their occurrence seems to be universal, and their arrangement varies little. These elements, which for the sake of distinction may be called the accessory or non-striated radial fibres, like the striated, run from the outer fibrous membrane to the inner. They are single fibres (usually bifurcated close to the outer end in S. variegata, usually branched in S. coruscans), placed at regular intervals between the striated fibres, as shown in figs. 1 to 4. As mentioned above, the main relations of these fibres are with the annular strands of non-striated

muscle, and their chief function would seem to be to provide a series of 'points d'appui' for the latter. It may be mentioned here that it is largely to the presence of these fibres in transverse sections in certain planes that the illusion of regular partitions between the rows of striated fibres is due.

VI. The Proventriculus: Chitinous Plates.

In the interior of the proventriculus towards its anterior end is an elaborate structure which has hitherto failed to receive the notice which its importance in the mechanism of the proboscis seems to demand. It occurs in essentially the same form in all the members of the group which I have examined for it—not only in the Syllidae and Eusyllidae, but in the Exogoneae and Autolytidae.

De Saint-Joseph seems to have been the first to direct attention to the appearance presented by this structure, though he misunderstood its significance. In his description of *Typosyllis alternosetosa*, he says, 'le proventricule, avec 30 rangées de points gris, qui a à sa partie supérieure un anneau chitineux', with a foot-note, 'Cet anneau, qui se remarque souvent chez les Syllidiens, me paraît être la continuation de la trompe qui pénètre dans le proventricule'. On the other hand, he refers to the same structure in *Pterosyllis spectabilis* as 'deux valves cornées' (pp. 65 or 189). Malaquin (p. 213) gives a much more consistent and complete description: 'Dans la région antérieure de l'organe, l'épithélium prend un autre aspect, il devient en quelque sorte fibrillaire; les cellules en sont très allongées avec noyau médian (Pl. v, fig. 7, *Ep. pr.*). Cette structure correspond à une disposition particulière, à un épaississement de la cuticule formant en avant du proventricule un anneau chitineux. Cet anneau chitineux, visible sur le vivant (Pl. iv, A. *ch.*, figs. 1, 2, 3. 4, 5), peut surtout s'étudier dans une coupe horizontale du proventricule (Pl. v, fig. 6). Dans la région antérieure de l'organe l'épithélium est beaucoup plus épais et les parois se touchent à l'état de repos de manière à fermer totalement la lumière.

En arrière de cet épaississement existe l'anneau chitineux auquel correspond une disposition particulière des colonnes musculaires : celles-ci, au lieu d'être régulièrement radiaires, sont obliquement disposées, au moins dans le plan horizontal médian du proventricule, de façon à agir dans deux sens perpendiculaires. Cette disposition est destinée probablement à faire glisser et au besoin à comprimer fortement les aliments avalés par l'animal.'

A short distance behind the abrupt posterior edge of the thickened cuticle of the pharynx (fig. 5) is a deep transverse (circular) groove in the thick epithelium, and a little farther back a second similar groove, the two separated from one another by a prominent band of thickened epithelium. Just behind the posterior groove the cuticle is developed on either side into a dense chitinous plate. These plates are of no great length in the direction of the long axis of the body, but considerably elongated vertically, extending downwards so as to bound almost the whole of the slit-like lumen (Pl. 15, fig. 1). At the dorsal and ventral edges of each run grooves in the epithelium. Dorsally and ventrally these plates pass into the unmodified cuticle which bounds the lumen of all the rest of the organ : anteriorly the same holds good, but posteriorly each plate projects a little beyond the general level of the surface, as the free edge of a finger-nail, elsewhere lying close on its bed, projects beyond the general surface of the digit. The radial muscle-columns of the wall of the proventriculus in the belt through which these chitinous plates extend, depart from their arrangement in regular annular zones (fig. 5), and, as observed by Malaquin, run obliquely inwards and forwards or inwards and backwards. The object of this oblique direction would seem to be to enable the two plates to be tilted up so that their edges may be brought into contact.

VII. The Ventriculus.

The ventriculus is a small chamber, reduced or absent in some. It has fairly thick walls with a correspondingly reduced lumen. Numerous thin bundles of muscular fibres run radially

through the substance of the wall ; but the chief space is taken up by the epithelium modified into a mass of gland-cells similar to those composing the anterior proventricular glands. They are apparently syncytial, and in most specimens present the appearance, in the aggregate, of a mass of sinuous and anastomosing tubules and vacuoles with thin walls and without distinct contents : more rarely the spaces are filled with a secretion capable of taking a strong stain with haematoxylin. In the Exogoneae the ' ducts ' from this mass of unicellular glands do not seem to open—in great number at least—into the cavity of the ventriculus itself, but run forwards to open into the recess at the extreme posterior end of the proventriculus. It is in very few preparations that this destination is traceable : the specimen must happen to have been fixed when the secretion was actually being discharged, and the strands of secretion by which alone the course of the ' ducts ' is traceable, must have become differentially stained. In the other sections of the Syllidea I have not been able to trace this connexion, and I am led to conclude it is not universal.

VIII. The Post-ventriculus.

Sharply marked off both from the ventriculus in front and the intestine behind is the chamber from which are given off laterally the two caeca present in most of the Syllidea with the exception of the Autolytea.

This, as already noticed, is recognized as a separate chamber by Eisig, and he gives prominence to it as the second main division of the alimentary canal—the first being the whole proboscis-oesophagus (Rüsselösophagus) and the third the intestine. De Saint-Joseph, on the other hand, and Malaquin do not recognize the distinctness of this chamber from the proventriculus. Its walls have only a thin layer of muscle [1] without radial fibres. Its epithelium is ciliated and is loaded

[1] It may be pointed out here that Malaquin was in error in stating that the intestine is devoid of a muscular layer. There is a thin layer of flattened fibres, not placed in close contact with one another, composed of outer longitudinal and inner circular elements.

with unicellular glands. The caeca are of essentially the same structure, with numerous unicellular glands which discharge their secretion into the lumina. A name is needed to designate the small but distinct part of the digestive canal from which the caeca are given off. The term oesophagus is in general use for a corresponding part in the Nereids; but, whatever its claims, it seems inappropriate to a compact glandular chamber with a ciliated epithelium. I propose instead the term post-ventriculus as not involving any doubtful homologies and indicating simply position.

Though the post-ventriculus, with the caeca, resembles the intestine in its ciliated epithelium, it differs from the latter in the presence of the very numerous and characteristic unicellular glands. It is also sharply constricted off from it, and the narrow aperture of communication between the two is guarded by a valve composed of folds of the intestinal epithelium which must prevent the passage of liquid forwards from the intestine.

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EXPLANATION OF PLATE 15.

Lettering common to all Figures.

an., annular bands of non-striated muscle; *a.pr.g.*, anterior proventricular glands; *a.r.*, accessory radial fibres; *c.p.*, chitinous plates of proventriculus; *cu.*, cuticle; *e.m.*, external membrane; *ep.*, epithelium; *i.*, intestine; *i.m.*, internal membrane; *p.*, pharynx; *pa.*, pharyngeal papillae; *p.g.*, pharyngeal gland; *pr.v.*, proventriculus; *pt.*, post-ventriculus; *r.*, radial muscle-columns.

Fig. 1.—Diagram of a transverse section of the proventriculus of Syllis: about one quadrant shown. The section is represented as passing through the chitinous plates; but the typical arrangement of the muscles is illustrated—not the modified arrangement in the chitinous plate region (see fig. 5). The coelomic epithelial layer is not represented in this or the other figures.

Fig. 2.—Part of a transverse section of the proventriculus of Syllis variegata (Grube). ×330. The dark transverse lines passing across the radial muscle-columns indicate the telophragms. The accessory radial (*a.r.*) fibres are drawn in black, as in the other figures, for the sake of contrast.

Fig. 3.—Portion of a tangential section of the proventriculus of S. variegata internal to the annular bands. ×330. The pattern of the transverse sections of the cortex ('Cohnheim's areas') is not represented in this or in the following figure.

Fig. 4.—Portion of a tangential section of the proventriculus of S. coruscans (Haswell), internal to the annular bands. ×330.

Fig. 5.—Part of a horizontal section of the proventriculus of Syllis closterobranchia (Schmarda), passing through the chitinous plates. ×330.

Fig. 6.—Diagrammatic general view of the proboscis of a Syllis from above: part of the dorsal wall of the pharynx and proventriculus removed to show the region of the anterior proventricular glands and the chitinous plates. Only one of the pharyngeal glands is represented. *a.pr.g.*, anterior proventricular glands; *c.*, caeca; *c.p.*, chitinous plates; *cu.*, thickened cuticle of the pharynx; *i.*, intestine; *p.*, pharynx; *pa.*, pharyngeal papillae; *p.g.*, pharyngeal gland; *pr.v.*, proventriculus; *pt.*, post-ventriculus.

Fig. 7.—Semi-diagrammatic view of a horizontal section through the junction of the pharynx and proventriculus of Grubea to show the position and relations of the anterior proventricular glands. ×780.

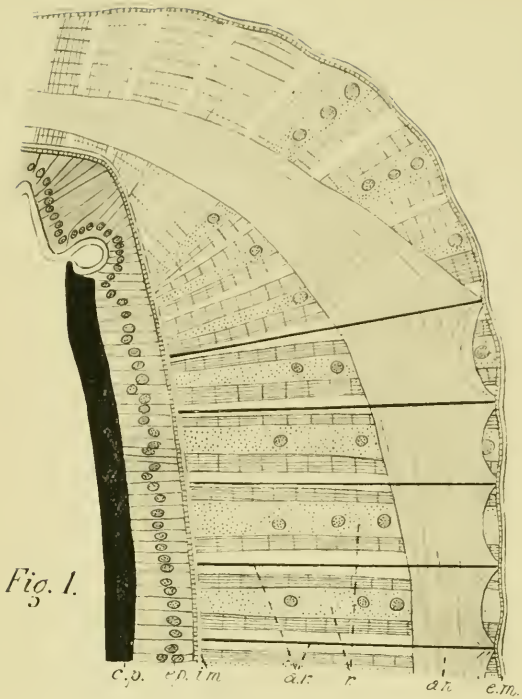
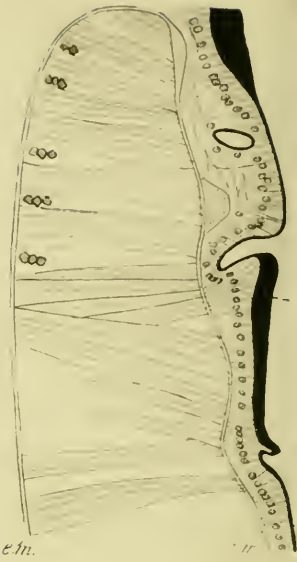
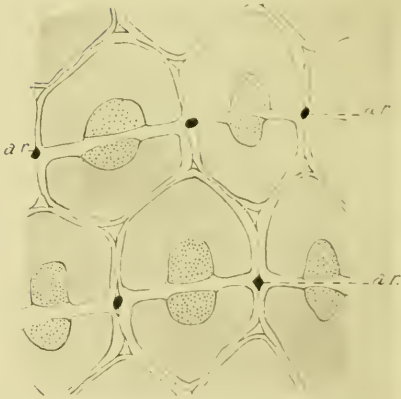


Fig. 1.





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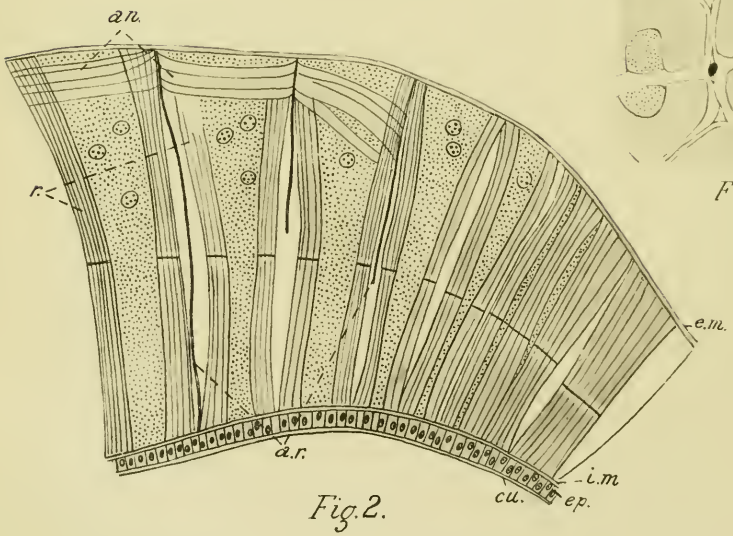

Fig. 2.

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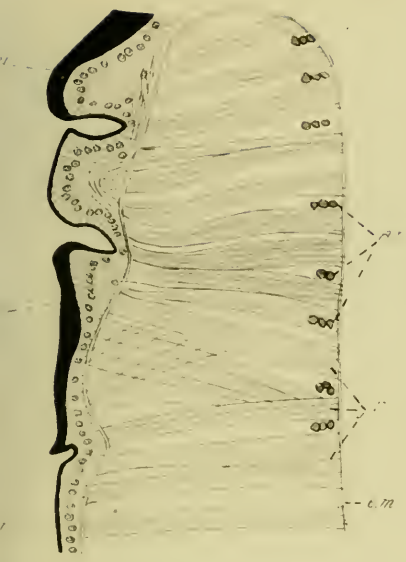

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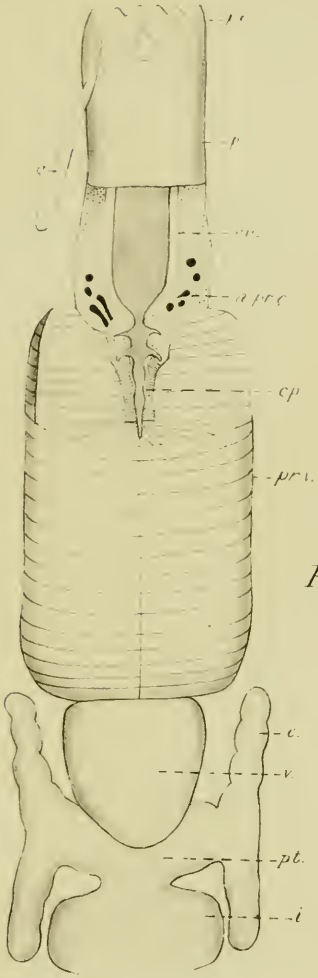

Fig. 6

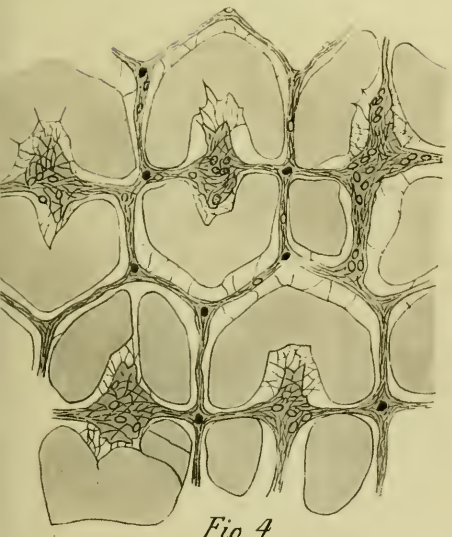

Fig. 4.

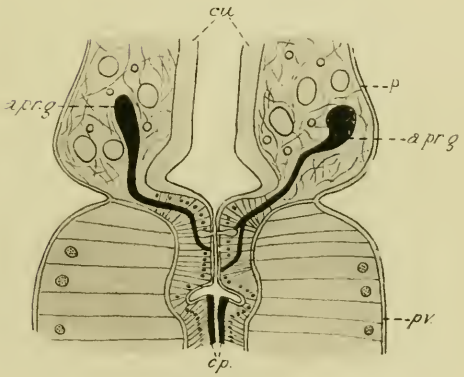

Fig. 7

The Life-history of Melicertidium octocostatum (Sars), a Leptomedusan with a theca-less Hydroid Stage.

By

Prof. James F. Gemmill, University College, Dundee.

With Plate 16.

Contents.

This well-known medusa (fig. 19) is classified among the Thaumantiadae, and is characterized by the presence of eight 'radial' canals on which the gonads are developed. The marginal tentacles are numerous (up to 140) and of unequal size, larger and smaller ones alternating more or less regularly. There are no lithocysts, cordyli or ectodermal ocelli. The manubrium is short, the mouth four-angled and without oral tentacles. The medusa has a fairly wide distribution in the North-east Atlantic ranging from Bergen to Falmouth. (See E. T. Browne, **4**, for details and a discussion of the nomenclature.) The hydroid, as I have ascertained by rearing the eggs, proves to be a hitherto undescribed species identical with one which has been noted for several years (with a year's interval of absence) growing abundantly and spontaneously in the tanks at the Millport Biological Station.

An allied form Melicertum campanula (Agassiz) occurs in West Atlantic Canadian and U.S. waters. In 1863

A. Agassiz (1) described the young hydroid reared from the eggs of Melicertum, but this hydroid has not up to the present been recorded in nature from the American coasts.

Development of Eggs of Melicertidium.

Ripe examples appeared in the tow-nettings at Millport towards the end of June 1918. By keeping specimens in aquaria in the Research Fellowship Laboratory at Glasgow University I obtained numbers of fertilized eggs. These are small (0·08 mm. in diameter), homogeneous-looking, faintly yellowish in tinge, and with delicate closely-adherent membrane. They are ripe before extrusion and pass outwards through the mouth, as also do the spermatozoa in the males. No membrane of fertilization is formed. Segmentation is total and equal (figs. 1–5), the two-celled stage beginning with a notch or groove on one side of the egg. A blastocoele cavity is recognizable even at the eight- or sixteen-celled stage. Early blastulae are irregular in outline, the blastula wall being a single layer, but exhibiting folds and inpocketings which soon straighten out and do not seem to have any subsequent formative importance (figs. 6 and 7). The larva now becomes pear-shaped, and, having acquired cilia, progresses with the blunt end in front and rotates in the solar direction as viewed from the blunt end (figs. 8 and 9). At this stage the endoderm arises by inward budding from the blastula wall (figs. 8, 9, 10). The budding occurs first near the pointed end, and then all round, gradually filling up the blastocoele cavity, the last part of this cavity to be filled being at the blunt end (fig. 11). The endoderm cells are rounded, slightly granular, and less transparent than the ectoderm. The planula now elongates, becoming almost worm-like, and swims vigorously through the water at any depth. Later it seeks the bottom and becomes attached. The mode of attachment presents certain peculiarities which I hope to elucidate later. The free end becomes swollen and rudiments of the first tentacles appear (fig. 12). Figs. 12–14 illustrate four-tentacled and eight-tentacled stages. Both show a delicate perisarc covering hydrorhiza and hydrocaulus,

and ceasing at the base of the hydranth without forming even a rudimentary hydrotheca. At no stage are the bases of the tentacles united by a web or membrane. The sixteen-tentacled stage is entirely similar to young polyps (fig. 15) of the tank hydroid described later in this paper, though the latter are relatively rather larger, no doubt because they could draw during growth on a nutritional reserve greater than was at the disposal of the parent of the colony. This year (1919) I have repeated the rearing experiments and obtained the same results.

Description of the Tank Hydroid.

In the early spring of 1916, 1917, and 1919 colonies of an apparently new theca-less hydroid appeared on stones and on glass in several of the tanks at the Millport Biological Station. Dr. James Ritchie, Royal Scottish Museum, Edinburgh, to whom I sent a specimen in February 1917, made the conjecture, which has proved right, that it might turn out to be the hydroid of some Leptomedusan. A little later in the same year young medusae budded off from a colony were obtained. They had four radial canals, eight tentacles, no lithocysts, and no ectodermal ocelli or oral tentacles. I tried to rear them, but without success. The matter remained there till July 1918, when the results (given above) of rearing Melicertidium eggs unexpectedly connected the tank hydroid with this medusa, and made me undertake more careful experiments (see below) on rearing the young medusae, when these were budded off from the tank colonies in the spring of the present year (1919). The characters of the hydroid are as follows :

Hydranth: entirely theca-less. Tentacles: long, slender, tapering, with solid core of endoderm cells in a single row, studded with nematocysts, not united at their bases by a membrane, arranged in a single circle but tending when fully extended to curve upwards and downwards alternately, commonly sixteen in number, but often more numerous especially in sterile colonies, in which individuals with as many as thirty-two may be noted. Hypostome: conical when closed, shaped like a shallow wide-mouthed urn when

fully opened, lined for a very short distance downwards from the margin by close-set columnar cells having the characters of ectoderm. Body of Hydranth: sometimes slender, elongated (1·7 mm. in length), sometimes short (0·9 mm.) or vase-shaped according to contraction, usually showing constriction below hypostome, furnished with stinging cells near middle, merging insensibly into hydrocaulus, except in contracted condition, when junction becomes evident. Hydrocaulus: short but varying in length (1 mm. to 1·7 mm.), often irregularly bent, evidently weak, unbranched except in giving off the stalk of a medusa bud. Hydrorhiza: creeping, branching but not anastomosing, 0·1 mm. across (including perisarc). The distinction between hydrocaulus and hydrorhiza is not always sharply apparent. In the thicker parts of a colony hydrorhizae may intertwine, and leaving the surface of attachment become equivalent to low irregular branching hydrocauli. When, however, the hydrorhizae are not too crowded they remain adherent and give off unbranched hydrocauli. Perisarc: thin, wrinkled irregularly but not ringed, enclosing hydrorhiza and hydrocaulus and separate from these except at occasional points of 'anchorage', thinning away at distal end of hydrocaulus and fusing with ectoderm at base of hydranth which is entirely theca-less. Medusae: Gonophore production takes place from the beginning of February till the end of March. Parts of the colony were isolated, kept in filtered sea-water, and in course of time a number of young medusae were collected. The buds appear at the end of short stems arising from the hydrocaulus well below the base of the hydranth, each hydrocaulus only producing a single medusa. The medusa buds, especially at full size, are more elongated than the free medusae, but the characteristic shape is acquired during the period immediately prior to detachment when vigorous pulsations may be noted. The young medusae have four rather wide radial canals, four tentacles opposite these, four small tentacles or tentacle buds in the interradii, and no lithocysts or ectodermal ocelli (figs. 16, 17, 18). The bell is dome-like and moderately deep: the stomach is quadrangular and the

manubrium short, showing four blunt, radially-placed, grooved angles. At first the bell shows a small pit in the middle of the aboral surface, to the bottom of which a cone-like projection of the stomach is anchored. Later this remnant of the connexion between bud and stalk becomes severed, and the summit of the dome shows an upward convexity (fig. 17). Over the rest of the bell, the mesogloea superficial to the plane of the stomach and radial canals forms a relatively thin layer. At their bases the tentacles are hollow and slightly swollen, the endoderm here containing yellowish intracellular pigment. The measurements of the young medusa at rest are: height 1·2 mm., breadth 1·3 mm., interradial diameter of stomach 0·45 mm.; breadth of radial canal 0·06 mm., depth of superficial mesogloea 0·075 mm. The surface of the bell shows numerous minute glancing-points which do not disappear on treatment with acid. The medusae were kept alive for a time, and increased in size; the four interradial tentacles grew almost as big as the radial ones, and new tentacle buds appeared in irregular sequence, one for each interspace between a radial and an interradial tentacle. Stages with ten, twelve, fourteen, and sixteen tentacles were thus obtained. Medusae four weeks old and with *c.* ten tentacles showed a single blunt outgrowth from the stomach in each interradius (fig. 18, *b*). A week later (*c.* twelve tentacles) these outgrowths had extended over the summit of the bell, becoming pointed at their ends. In another week or fortnight (*c.* fourteen to sixteen tentacles) the outgrowths had extended downwards along the sides of the bell and become continuous with slender corresponding upgrowths from the ring canal (fig. 19). I failed to rear the medusae further, but they had already reached the eight-rayed condition characteristic of Melicertidium.

I have not obtained the early four-rayed medusae in townettings off the Millport Station, but they were moderately abundant during April 1919 in plankton from the Gareloch,[1] an inlet farther up the Firth of Clyde.

[1] Since this paper was written, I have found the intermediate stages described above in May plankton from this locality, and the adults at the end of June.

General.

As far back as 1865 A. Agassiz (**1**, p. 130) inferred from the results of tow-nettings that the eight-rayed condition in Melicertum campanula was reached by the formation of four new interradial outgrowths from the stomach in an originally four-rayed young medusa.

Mayer (**7**, p. 208) thinks that Melicertum campanula (Agassiz) and Melicertidium octocostatum (Sars) are probably identical species, and that Melicertum should have priority as the generic name. However, there are sufficient reasons (especially under (1) and (2) in the following comparison) for keeping Melicertum and Melicertidium as distinct genera, at least in the meantime.

Melicertum (hydroid)	Melicertidium (hydroid)
(1) Tentacles united at their bases by a membrane.	(1) Tentacles not united at their bases by a membrane.
(2) A small theca at base of hydranth.	(2) No theca.
(3) Tentacles up to ten in number.	(3) Tentacles sixteen or more (up to thirty-two) in number.
Melicertum (medusa).	**Melicertidium (medusa).**
(4) Earliest free stage with only two marginal tentacles.	(4) Earliest free stage with four marginal tentacles and four intervening tentacle buds.
(5) No 'radiating lines' on sub-umbrellar surface.	(5) Numerous 'radiating lines' on sub-umbrellar surface.
(6) Marginal tentacles, in adult equal or sub-equal in size.	(6) Marginal tentacles in the largest specimen examined consist of about sixty small tentacles and about eighty much larger ones.

I agree with Romanes' opinion (**9**, p. 527) that the ' radiating lines ' referred to under Melicertidium (medusa) above are bands of muscle fibres, and not of nematocysts as is thought by Browne (**4**, p. 764) and others.

Additional instances in which theca-less hydroids have been reared from Leptomedusae are recorded by Claus (**5**), Metchnikoff (**8**), and Brooks (**2**). The medusae concerned belong to the genus Eutima (McCrady), the species being *Eutima campanulata* (Claus), *Octorchis gegenbauri* (Haeckel), in the first two cases, and *Eutima mira* (McCrady) in the third. Eutima differs from Melicertum and Melicertidium, among other things, in having marginal lithocysts, and in having the stomach mounted on a long peduncle. In the hydroid of *E. campanulata*, described by Claus and named by him Campanopsis, the tentacles are up to twenty-four in number and are united at their bases by a membrane. A theca is entirely absent, and the young medusae are formed near the middle of the hydranth body. Brooks (**2**) describes the hydroid of *E. mira* as small, Perigonimus-like, with eight tentacles united at their bases by a membrane.

E. Stechow (**10**) has described a theca-less hydroid, with short hydrocaulus having definitely ringed perisarc, with hydrorhizae forming a network, and with fourteen to eighteen tentacles which were not, so far as could be made out in the preserved material, united at their bases by a membrane. The specimens were in a tube left by a former assistant at Munich and were labelled ' Polyp of Octorchis '. Stechow names it *Campanopsis dubia* and considers the medusa to have been an Octorchis Eutima.

On the whole, the life-history of Melicertidium supports the generally-accepted view that Leptomedusan hydroids are derived from Anthomedusans. The hydroid is theca-less, the medusa is deep and has no lithocysts or ectodermal ocelli, and though the gonads are on the eight radial canals in the adult, the mode of development of the second four radial canals by outgrowths from the stomach makes it clearly possible that ontogenetically or phylogenetically the gonad tissue of the

other four originates in the region of the stomach or manubrium. Indeed, in the earliest stage of the Melicertidium medusa identified by Browne (**4**, p. 763) the gonads extended outwards from the stomach only along the proximal halves of the radial canals.

The Leptomedusan Family Thaumantiadae, to which Melicertum and Melicertidium belong, contains other twelve typical genera. The hydroid stages of only three of these, viz. Thaumantias (Wright, **11**), Laodicea (Metchnikoff, **8**), and Dipleurosoma (Browne, **3**), are known, and, curiously enough, they all possess complete thecae. In having a rudimentary theca Melicertum recalls the Anthomedusan Perigonimus, while Melicertidium having no theca is in line with Eutima (Campanopsis) and Tima, which are members of the Leptomedusan Family Eucopidae. Dr. James Ritchie compares the general facies of the Melicertidium hydranth to that of Halecium. The just liberated medusa of Melicertidium resembles that of Podocoryne carnea except in having a slightly shorter manubrium and no oral tentacles. It is evident that on the borderland between the Antho- and the Leptomedusae there are numerous forms which, whether in their hydroid or their medusoid stages, exhibit features characteristic of better-defined members of either group.

I have to thank the Trustees of the Carnegie bequest for a grant in aid of the expenses of this investigation, and the staff of the Millport Station for help in obtaining material and rearing the young medusae.

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EXPLANATION OF PLATE 16.

Figs. 1–15.—Development of Melicertidium.

Figs. 1–7.—Stages in segmentation and blastula formation of the egg of Melicertidium.

Figs. 8–11.—Change to the planula, formation of endoderm (end), &c. The arrow and circle between 9 and 10 indicate respectively the direction of progression of the larva, and its rotation as viewed from the narrow end.

Figs. 12–18.—Fixation of the larva: formation of first tentacles.

Fig. 14.—Stage with eight tentacles.

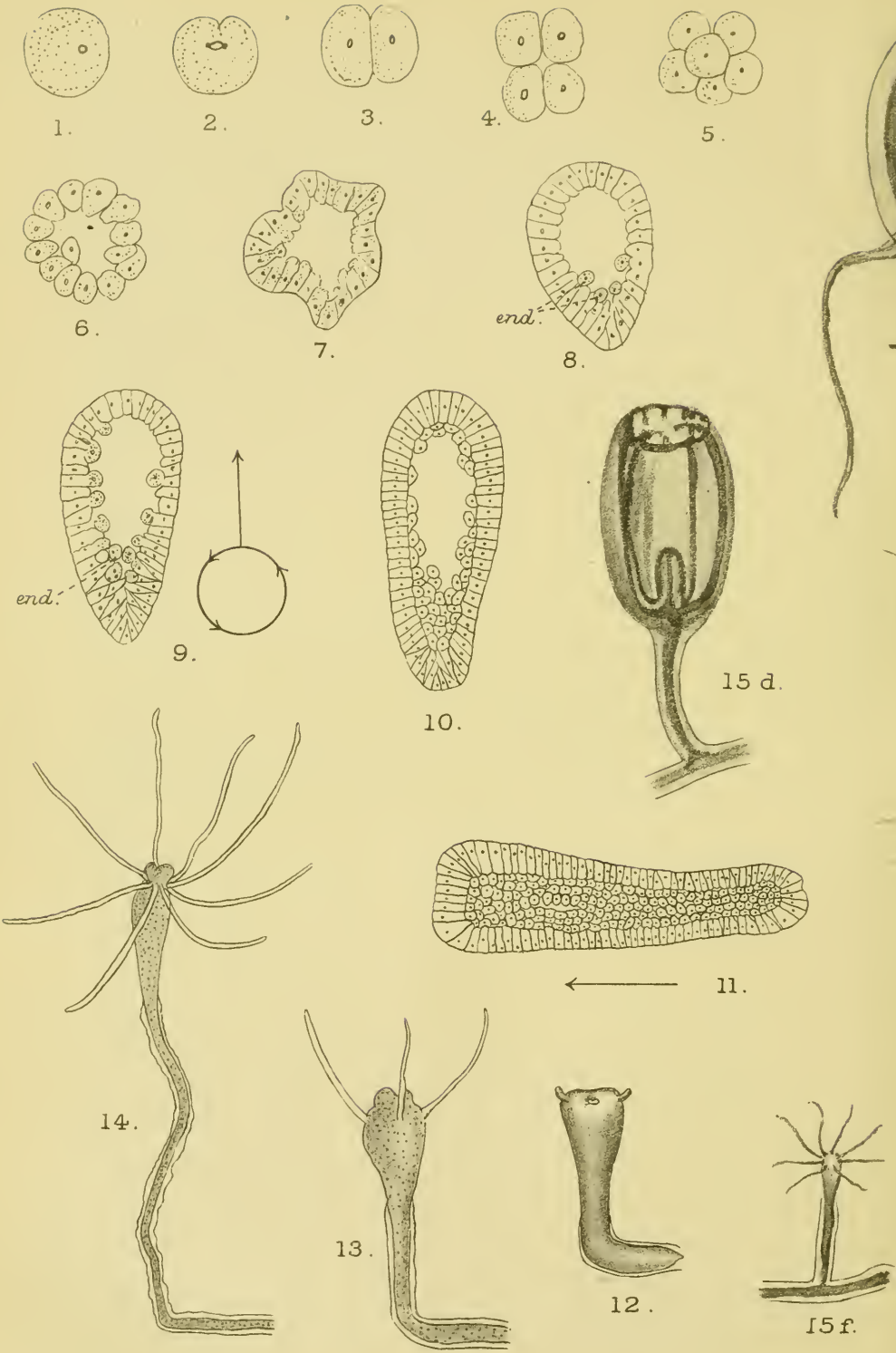
Fig. 15.—Portion of a colony, (*a*) hydranth; (*b*) medusa ready for liberation; (*c*) young hydranth and young medusa bud; (*d*) medusa bud almost fully grown; (*e*) hydranth fully stretched out; (*f*) young polypite arising from a hydrorhiza.

Fig. 16.—Just liberated medusa.

Fig. 17.—Aboral part of medusa, two weeks old, showing mesoglocal projection on summit of bell.

Fig. 18, (*a*), (*b*), (*c*).—Stomach and radial canals viewed from above in two days, three weeks, and six weeks' old medusae respectively, showing the formation, by interradial outgrowths from the stomach, of four new 'radial' canals.

Fig. 19.—Medusa, seven weeks old, showing interradial outgrowths from the stomach which have met corresponding upgrowths from the ring canal. R, one of the four original radial canals; I.R., one of the four new interradial canals formed in the manner described above.



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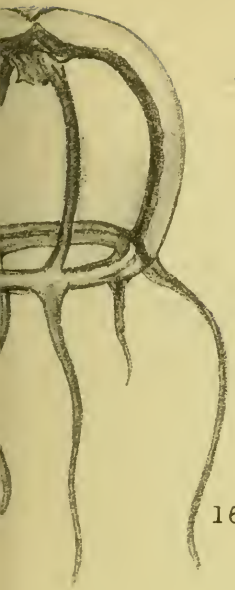

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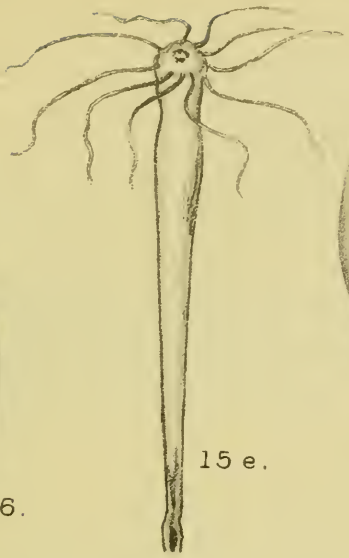

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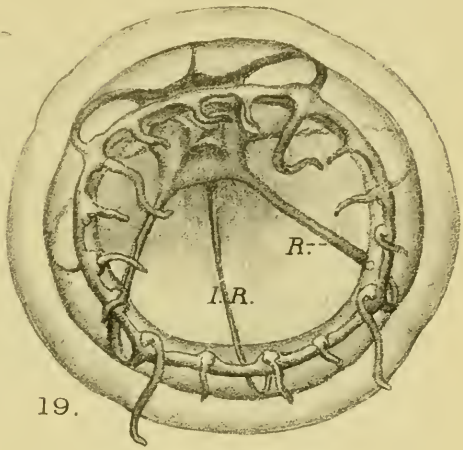

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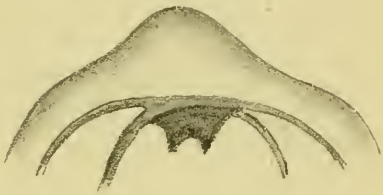

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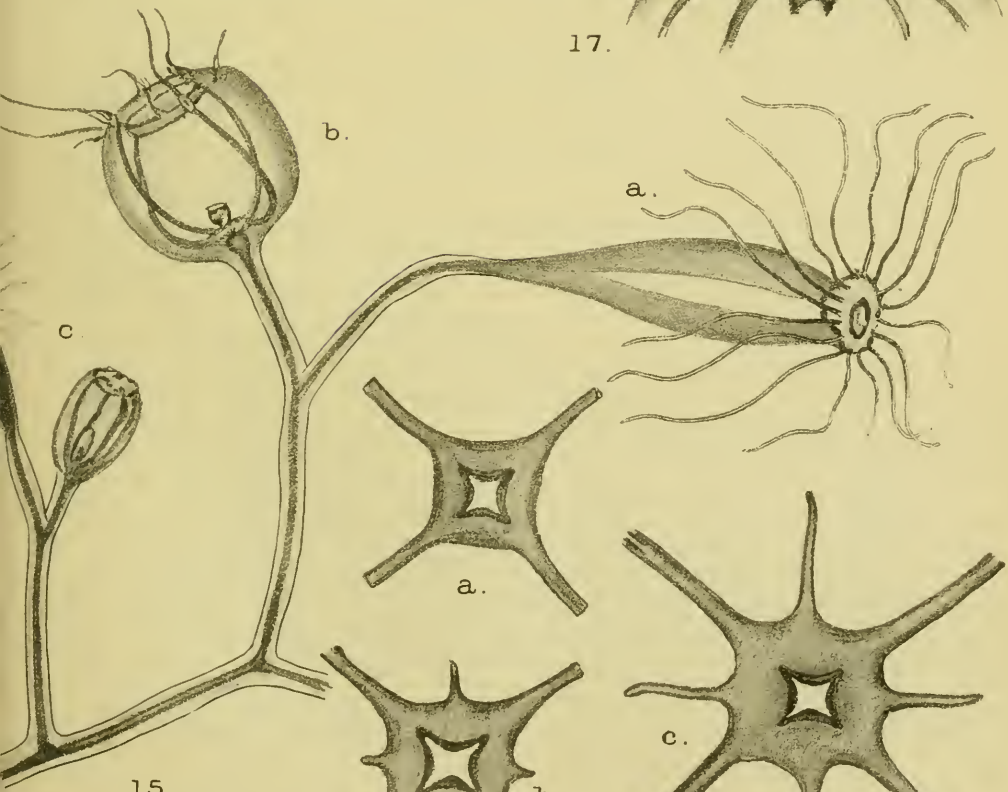

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Huth, London.

On the Blood-Vascular System of the Earthworm Pheretima, and the Course of the Circulation in Earthworms.

By

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(From the Department of Comparative Anatomy, Oxford.)

With 11 Text-figures.

Table of Contents.

1. Introductory.

The blood-vascular system of earthworms has engaged the attention of many distinguished observers. Lankester (**12**) described the blood-vessels of Lumbricus in one of his memoirs on the 'Anatomy of the Earthworm', which forms about the earliest contribution to this subject. Jaquet (**9**) gives a comparative account of the vascular system in Annelids, describing the system in typical genera of the various classes of the group. Of the Oligochaeta, he selects Lumbricus as a type. Perrier (**13**) and Benham (**5**), also working on Lumbricus, describe the course of flow in all the blood-vessels from a study of the disposition of the valves; to Benham we also owe our knowledge of the blood-supply of the nephridium in Lumbricus (**6**). Harrington (**8**) gives a detailed account of the anatomy of the blood-system in Lumbricus with elaborate diagrams, and was the first to describe the arrangement of blood-vessels in the integument. Recently, Johnstone and his student, Miss Johnson (**10** and **11**), have published two papers on the course of blood-flow in Lumbricus demonstrating the course in various vessels by a series of interesting experiments and observations. The blood-system has thus been thoroughly studied in Lumbricus since that is the form studied as a type in Europe and America. Amongst the Oriental forms of Oligochaeta, Bourne (**1**) has described the blood-system in some detail in the Perichaete worm Megascolex and also in Moniligaster grandis (**2**, 1894), a huge worm about two feet long placed by Beddard in the group Microdrili. Besides Bourne's work on Megascolex, very little attention has been paid to the blood-system of the Perichaetidae, the largest family of earthworms.

The earthworm Pheretima (the genus Perichaeta sensu stricto) is now studied as a type of the Oligochaeta in Northern India and also at the Universities of Bombay and Calcutta, and it has become necessary, therefore, to have as complete a knowledge as possible of the anatomy of this form. An attempt has been made in this paper to present an account

of the blood-system of Pheretima and the course of blood-flow about which, even in Lumbricus, there has been a great divergence of opinion amongst the various observers. Some of the observations were made in India, but in this country, besides having an opportunity of examining the two English genera Lumbricus and Allolobophora, I was able to complete my work on Pheretima, having been lucky to obtain specimens of this Oriental form in the Lily-house of Kew Gardens.

The work was carried out in the Department of Comparative Anatomy at Oxford. I am indebted to Professor E. S. Goodrich for his keen interest in my work; he has made valuable suggestions, and has also found time to read through and correct the manuscript of the paper.

Although essentially the blood-systems of both Lumbricus and Pheretima can be reduced to a common type, there are important differences in the system in the two genera, which I have indicated in the text. Pheretima resembles Allolobophora rather than Lumbricus so far as the blood-system in the general body-region is concerned, while the system differs in important respects from that of Megascolex. As regards the course of the blood-flow studied by holding the vessels with fine forceps, by cutting the vessels and observing the direction of blood-flow, and by a study of the valves, I am led to confirm the observations and conclusions of Johnstone (**10** and **11**) and to reject part of Bourne's theory of the course of the circulation (**1**).

The typical arrangement of the blood-system in Pheretima is found behind the fourteenth segment, being metamerically repeated behind that segment. In the first fourteen segments, on the other hand, this typical arrangement is considerably modified, this modification, together with that shown in the digestive, reproductive, and nervous systems, being spoken of as cephalization. It will be convenient, therefore, to describe, as Harrington (**8**) does in the case of Lumbricus, first, the typical arrangement as it occurs in the region of the body of the worm behind the fourteenth segment, and then the

blood-vessels in the first fourteen cephalized segments, and finally to discuss the course of the circulation in the system.

2. The Typical Arrangement of the Blood-system in the Intestinal Region of the Body behind the Fourteenth Segment.

The blood-system in this system in this region of the body consists of (*a*) three longitudinal trunks running parallel to one another, namely, the dorsal, the ventral, and the subneural vessels; (*b*) the intestinal blood-plexus, situated in the wall of the gut, is directly connected with the dorsal and ventral vessels, and indirectly with the subneural; and (*c*) the commissural, integumentary, and nephridial vessels.

(*a*) The Longitudinal Trunks.

1. The dorsal vessel.—The dorsal vessel is the most prominent of all the blood-vessels in the worm and is rhythmically contractile. It runs along the mid-dorsal line immediately beneath the body-wall, between the latter and the intestine, and is at once seen lying on the gut, when the worm is opened by a mid-dorsal incision. In Lumbricus the dorsal vessel is heavily covered over with 'yellow cells', which must be removed before the vessel is seen: but in Pheretima the 'yellow cells' do not cover the dorsal vessel, so that the latter is at once prominent on dissection. Although lying close upon the gut, the dorsal vessel is not actually attached to the wall of the former in any portion of its course. It is single throughout its length and has thick muscular walls which are responsible for its contractility. The average diameter of this vessel is about 220 μ; it is narrowest at places where it pierces the intersegmental septa. On opening a narcotized worm, we can easily see the wave of contraction in this vessel travelling from behind forwards and consequently driving the blood in that direction. During its course through the body, the dorsal vessel, on piercing each septum, has a pair of forwardly-directed valves (figs. 7 and 10) in its lumen. These valves,

as I shall show later, prevent the flow of blood backwards when the vessel contracts. There are also valves (vide infra) at the orifices of the dorso-intestinal and commissural vessels.

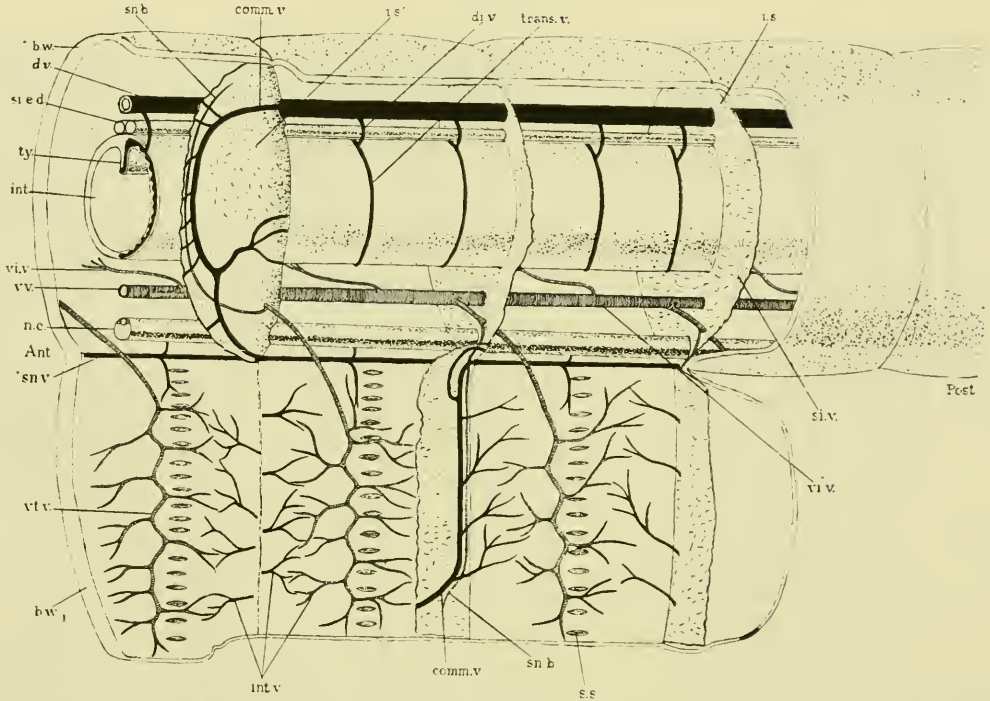
It will be seen from fig. 1 that the dorsal vessel is connected with the intestine by two pairs of dorso-intestinal vessels (*di.v.*) in each segment; these vessels serve to establish a communication between the internal intestinal plexus and the dorsal blood-vessel (fig. 2). The anterior pair of dorso-intestinals come off from the dorsal in the anterior third of the segment, while the posterior pair lie in the posterior third, nearing the hinder septum of the segment, in close association with the so-called 'lymph-glands' which lie on each side of the dorsal vessel in every segment here. These dorso-intestinals are very short vessels, being only about 450 μ in length, on an average. They soon enter the intestinal wall, in which they are continued as 'transverse vessels' (vide infra).

Again, just before piercing each septum from behind, the dorsal vessel receives a commissural vessel (the dorso-lateral or the parietal vessel), which is connected ventrally with the subneural (*comm.v.*, figs. 1 and 2). This commissural vessel runs along the posterior face of each septum very near and parallel to its outer edge, i. e. the edge joining the body-wall; and is connected with capillaries of the nephridia and the body-wall.

As I shall show later on, both the dorso-intestinal and the commissural vessels bring blood into the dorsal vessel and replenish its supply. No blood leaves the dorsal vessel in this region of the body.

2. The ventral vessel.—The ventral vessel, like the dorsal, is single throughout its length and extends from the anterior to the posterior end of the body. In the region of the intestine it has an average diameter of 115 μ and gives off a pair of ventro-tegumentary branches in each segment. Each of these branches leaves the ventral vessel just anterior to the septal wall in each segment and, after running alongside the anterior face of each septum for a little

TEXT-FIG. 1.



A diagrammatic representation of the blood-system in the region of the body behind the fourteenth segment, in the typhlosolar region of Pheretima posthuma. Five segments are shown, and the greater part of the skin of the left side of the four anterior segments has been cut and reflected out, in order to expose the blood-vessels in position. *b.w.* = body-wall; $b.w._1$ = body-wall cut and reflected out; *comm.v.* = commissural vessel; *d.v.* = dorsal vessel; *di.v.* = dorso-intestinal vessel; *int.* = intestine; *i.s.* = intersegmental septum; $i.s.^1$ = intersegmental septum turned forwards; *int.v.* = integumentary vessels; *n.c.* = nerve-cord; *si.e.d.* = supra-intestinal excretory ducts; *si.v.* = septo-intestinal vessel; *sn.v.* = subneural vessel; *sn.b.* = septo-nephridial branch; *s.s.* = setal sac; *ty.* = typhlosole; *trans.v.* = transverse vessel; *v.v.* = ventral vessel; *vi.v.* = ventro-intestinal vessel; *vt.v.* = ventro-tegumentary vessel.

distance, it pierces the septum and gets into the succeeding segment (*vt.v.*, fig. 1). Here it lies on the inner surface of the body-wall near the middle line of the segment just in front of the row of setal sacs, going right up near the mid-dorsal line (figs. 1 and 2). As it ascends along the body-wall transversely, the ventro-tegumentary vessel (*vt.v.*) gives off backwards and forwards capillaries that supply blood to the body-wall (epidermis and the muscles) and the integumentary nephridia. Besides, the septal nephridia and the prostates also receive their blood-supply from the ventro-tegumentaries. The septal nephridia are supplied by a septo-nephridial branch (*sn.b.*, fig. 1) of the ventro-tegumentary given off in each segment at the place where it pierces the septum ; while the prostate glands in the segments sixteen to twenty-one receive small branches from the ventro-tegumentary in each of these segments.

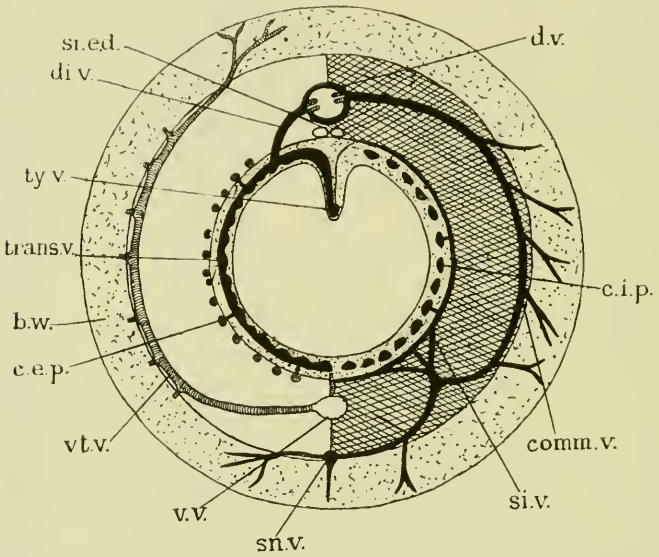
Besides the paired ventro-tegumentary branches the ventral vessel gives off dorsally a single unpaired ventro-intestinal vessel in each segment (*vi.v.*, fig. 1). This vessel originates from the ventral a little behind the middle of each segment, and runs forward to enter the ventral wall of the intestine, by three or four branches, close to the anterior intersegmental septum. The ventro-intestinal, though generally overlooked in this worm, is, however, an important vessel, and measures as much as 1·5 mm. in length in some worms from its place of origin on the ventral vessel to its place of entrance into the intestinal wall. It puts the ventral vessel into communication with the intestinal plexus. There are no valves anywhere along the course of the ventral vessel.

The ventral vessel is the main and, in fact, the only distributing channel in the intestinal region of the body. All parts in this region get their supply of blood from the ventral vessel.

3. The subneural vessel.—The subneural vessel runs along the mid-ventral line of the body-wall, being intimately attached to it, and lies, as its name indicates, beneath the nerve-cord. It is a very slender vessel and extends from the

posterior end of the worm to the fourteenth segment anteriorly, being absent from the first fourteen segments. The commissural vessel, connecting the subneural with the dorsal in the septal regions, has already been referred to above. At about the middle of each segment just in front of the line

TEXT-FIG. 2.



A diagrammatic transverse section through the region of the intestine, the right half showing a section through the intersegmental region and the left half through a segment proper passing through one of the dorso-intestinals. *b.w.* = body-wall; *c.e.p.* = capillaries of the external plexus; *c.i.p.* = capillaries of the internal plexus; *comm.v.* = commissural vessel; *d.v.* = dorsal vessel; *di.v.* = dorso-intestinal vessel; *si.v.* = septo-intestinal vessel; *s.v.* = subneural vessel; *trans.v.* = transverse vessel; *ty.v.* = typhlosolar vessel; *v.v.* = ventral vessel; *vt.v.* = ventro-tegumentary vessel.

of setal sacs, the subneural receives a pair of very small branches from the ventral part of the body-wall. One also finds in sections the subneural receiving a branch on its ventral side from the body-wall in the mid-ventral line (fig. 2).

The subneural is connected with the intestinal plexus

through the septo-intestinal (*si.v.*, figs. 1 and 2), a vessel which I describe below along with the commissural vessel.

This vessel collects blood from the small ventral part of the body-wall and the nerve-cord; and as the area over which its branches ramify is very small and the quantity of blood received is also small, the vessel itself is very slender as compared with the other longitudinal trunks.

There are no supra-intestinal vessels in this region in this worm: a pair of longitudinal ducts attached to the mid-dorsal line of the gut and described as supra-intestinal blood-vessels by Stephenson (**14**) have already been shown by me to be excretory ducts (**7**).

There are also no lateral neural vessels as found in Lumbricus.

(*b*) The Intestinal Blood-plexus.

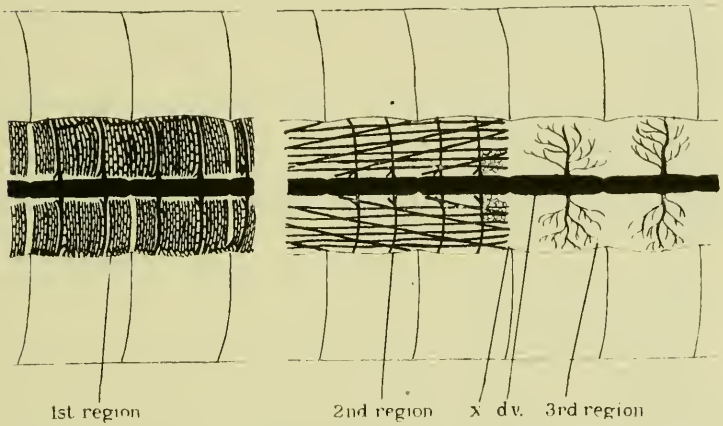
The intestinal blood-plexus (fig. 3) consists of a close network of capillaries and blood-vessels in the walls of the intestine. In Pheretima as in Megascolex (**1**) there are two capillary networks in the alimentary canal, i.e. (1) an internal deep-lying network, and (2) an external more superficial one. The internal network lies deep in the wall of the gut inside the layer of circular muscle-fibres, between it and the internal epithelial lining; while the capillaries belonging to the external network lie on the surface of the gut-wall amongst or even outside the yellow cells (chloragogen cells) which form the splanchnic layer of the peritoneal lining of the coelom. When a freshly-killed worm is opened in saline solution it is at once seen that the blood-plexus on the gut is marked out into three distinct regions—the first region is from the fourteenth to the twenty-sixth segment, where the intestinal capillaries are very thickly set and lie at right angles to the longitudinal axis of the body (transverse capillaries); the second is the longest portion and extends from the twenty-sixth segment to twenty-three to twenty-eight segments in front of the anus, the main

portion of the plexus in this region consisting of longitudinal capillaries lying parallel with one another along the intestine all round the circumference ; and the third region comprises the last twenty-three to twenty-eight segments of the animal, where the blood-plexus differs markedly from what we have in the first two regions. The difference in appearance of the blood-plexus in the three regions is illustrated in fig. 3, where at the point marked *x* there is a sudden change in the arrangement of capillaries from the second to the third region. While there is a regular, almost rectangular arrangement of the capillaries in the anterior two regions of the gut, the capillaries in the posterior region (last twenty-three to twenty-eight segments) branch off in a tree-like fashion from the dorso-intestinal vessels. That the three regions mentioned above are distinct from one another will be evident from the fact, ascertained by a study of sections passing through the three regions, that in the first region (fourteenth to twenty-sixth segment) the intestinal capillaries form only the internal plexus, the external plexus being absent, that in the second region (twenty-sixth segment onwards) there are both the internal and external plexuses well developed, while in the third region (last twenty-three to twenty-eight segments) we have no internal plexus at all, all the capillaries belonging to an external plexus.

Besides the difference in the arrangement and position of capillaries in the three regions there is another feature which also distinguishes these three regions from one another, and that is the presence and absence of a typhlosole and the typhlosolar vessel. Taking the last region first, we have to note the entire absence of a typhlosole in this region. Beddard (3) describes the absence of typhlosole in the last few segments of *Acanthodrilus*, and calls this last part of the gut without a typhlosole the 'rectum'. Similarly, the typhlosole is absent in the gut in the last thirty-six segments of *Lumbricus*, and we can apply the term 'rectum' to these last thirty-six segments of *Lumbricus* and the last twenty-three to twenty-eight segments of *Pheretima*.

It seems reasonable to suppose that by the time the earth reaches the last rectal portion of the gut there is hardly any nutriment left in it for absorption, and hence we have the absence of the typhlosole as well as of internal blood-plexus in this region, both of these structures being the likely media for absorption of nutriment from the earth. A well-developed external network of capillaries is, however, present in the

TEXT-FIG. 3.



Semi-diagrammatic representation of the intestinal blood-plexus in the three regions of the intestine. The *1st region* extends from the fourteenth to the twenty-sixth segment; the *2nd region* from the twenty-sixth to twenty-three to twenty-six segments in front of the anus and the region includes the last twenty-three to twenty-six segments (rectal region). *d.v.* = dorsal vessel; *x* = the place where there is a change from the regular geometrical plexus to the branching tree-like plexus of the rectum.

rectal region and serves to supply blood to the wall of the gut, and also, being distributed amongst the chloragogen cells, allows the latter to take up the excretory products from the blood capillaries.

In the second region, which is the most extensive (twenty-sixth segment to twenty-three to twenty-eight segments in front of the anus) of the three regions, we have a typhlosole as well as both the internal and the external plexus equally

well developed. The internal plexus is a dense network of capillaries appearing as a sort of blood-sinus interrupted at places by the foldings of the gut epithelium (fig. 2). The typhlosolar vessel, which should be regarded as part of the internal plexus, communicates with it at two places in each segment. The external blood-plexus, which is not continuous from segment to segment, has capillaries of varying diameters. The blood apparently passes from the external to the internal plexus, as, like the case in Megascolex (1), we can see the capillaries of the external network communicating with the capillaries of the internal network at numerous places in sections.

In the first region we have only a well-developed internal plexus but no external one. Neither is there a typhlosole, although, of the specially large mid-dorsal and mid-ventral capillaries, the mid-dorsal one simulates the typhlosolar vessel.

(1) Alimentary plexus in the first region (fourteenth to twenty-sixth segment).

In this region of the gut the internal blood-plexus is best developed. The network is very dense, almost a blood-sinus interrupted at certain places ; the interspaces in the dorsal half of the plexus are very small indeed, even less than one-fourth the size of the vessels which surround them. The capillaries run parallel to one another transversely to the length of the gut, and towards the ventral half break up into capillaries of smaller calibre, so that in the ventral half of the gut a continuous blood-sinus gives place to a coarse network of capillaries. In a freshly-opened worm this region of the gut presents a very bloody appearance.

Besides the richness in capillaries of this region we have a pair of well-marked vessels lying on the dorso-lateral aspect. These begin ventrally in the intestinal plexus about the fourteenth segment, and incline gradually dorsalwards up to the twenty-sixth segment, where they join the posterior pair of dorso-intestinal vessels of that segment at the inner angles of the roots of the intestinal coeca, and also communicate at that

place with the other blood-vessels on the walls of the coeca themselves.

An equally well-developed vessel runs along the mid-dorsal line of the gut, being only a specialized capillary of the internal plexus and being also continuous with the typhlosolar vessel behind.

The external blood-plexus is almost completely absent in this region. There are, however, a few capillaries present, which can be seen attached to the outside of the gut; for example, at places where the ventro-intestinal and septo-intestinal vessels join the wall of the intestine. But they soon enter the intestinal wall and pour their blood into the internal blood-plexus; so that a regular external plexus such as we find in the second and third regions (*vide infra*) is absent in this part of the gut, the internal plexus being very strongly developed.

(2) The alimentary plexus in the second region (twenty-sixth segment to twenty-three to twenty-eight segments in front of the anus).

In this region we have both the external and internal plexuses well developed. The external plexus consists of capillaries of various sizes which are continuous on the ventral wall of the gut but not on the dorsal. They are connected with the septo-intestinals and the ventro-intestinals which apparently form their source of blood-supply. They open into the capillaries of the internal plexus as shown in fig. 2.

The internal plexus in this region of the gut presents a very regular geometrical arrangement, as shown in fig. 3. This network consists of (*a*) Longitudinal capillaries, which are very closely set around the wall of the gut, extending all along its length. They are continuous from segment to segment and number about forty all round. These capillaries form the main portion of the plexus and in transverse sections are seen to lie in the folds of internal gut-epithelium.

(*b*) Transverse Channels.—We have already mentioned that in each segment the dorsal vessel is connected with the gut by means of two pairs of dorso-intestinal vessels. These dorso-intestinals on leaving the dorsal vessel enter the

intestinal wall about ½ mm. from their origin and go round the wall of the gut to its ventral side. I propose to apply the term dorso-intestinal to the vessel from its point of origin from the dorsal to the point of its entrance into the intestinal wall. The continuation of the dorso-intestinal on the wall of the gut I propose to call a transverse channel.[1] Corresponding to the two pairs of dorso-intestinals there are two pairs of transverse channels in each segment; each of these transverse channels is joined at its point of junction with the dorso-intestinal by a branch from the typhlosolar vessel (vide infra) (fig. 2, left half): so that these transverse channels serve to connect not only the longitudinal capillaries with each other but also the whole plexus with the typhlosolar vessel.

(*c*) Oblique Channels.—These begin at the mid-ventral line of the intestine at the intersegmental plane and run forwards and dorsalwards, passing through three segments before reaching the mid-dorsal line, where they join the typhlosolar just in front of the septa (fig. 3).

(*d*) Typhlosolar Vessel.—The typhlosolar vessel runs along the free edge of the typhlosole all down the second region of the gut (fig. 2). The typhlosole itself cannot be compared to the structure of the same name in Lumbricus, for in Pheretima it is really a bigger fold of the gut-epithelium containing not yellow cells, like those which fill up the typhlosole of Lumbricus, but only connective tissue which has the same staining qualities as the connective-tissue matrix in the layer of circular muscle-fibres of the body-wall. The typhlosolar vessel does not seem to possess a definite wall like the capillaries of the external plexus in Pheretima or the typhlosolar vessel of Lumbricus, but is only a part of the blood-sinus like the longitudinal capillaries, being, like them, in communication with the two pairs of transverse channels in each segment. We can therefore think of these transverse channels as circular ring-vessels which collect blood

[1] I have called these channels as they are thicker than the longitudinal capillaries.

from the longitudinal capillaries and the typhlosolar vessel (which we may regard as a specialized longitudinal capillary lying in the mid-dorsal line), and convey it to the dorsal vessel by means of the two pairs of dorso-intestinals in the same way as the ring-vessels of the oesophagus convey its blood to the supra-oesophageal vessel there (vide infra). It would be interesting to note here that, although the typhlosole is absent in the segments fourteen to twenty-six, there is a prominent blood-vessel in the mid-dorsal line of the gut-epithelium, the vessel corresponding to the typhlosolar behind, with which it is directly continuous.

(3) The blood-plexus in the third region (last twenty-three to twenty-eight segments).

In the last twenty-three to twenty-eight segments of the worm where the typhlosole in the gut is absent, and which region Beddard (**3**, p. 18) has referred to as the 'rectum', the intestinal plexus is different from what we have seen in the first two regions. The whole of the plexus is external, i.e. lies outside the muscular coats, there being no internal plexus. The regular and rectangular arrangement of capillaries in the typhlosolar (second) region at once changes into a branching tree-like plexus as shown in fig. 3. There is only one pair of dorso-intestinals in this rectal region in place of two pairs in the first two regions. Since there is no internal plexus the dorso-intestinals change their connexions and communicate in this region with the external blood-plexus.

The blood coming to the rectum from the ventro-intestinals and septo-intestinals goes to the external plexus, from where it passes to the dorsal through the dorso-intestinals, the part of the course involving the internal plexus having been cut out (vide infra).

(*c*) The Commissural, Integumentary, and Nephridial Vessels.

1. The Commissural Vessel.—As already mentioned, there is a pair of commissural vessels (parietal vessels) in each segment connecting the dorsal with the subneural vessel

(figs. 1 and 2). The commissural lies in the most anterior position in each segment, since the posterior face of a septum, on which this vessel lies, forms the anterior boundary of a segment. In its ventro-lateral part each commissural vessel is joined by a 'septo-intestinal' branch (figs. 1 and 2) which puts the commissural vessel in communication with the intestinal plexus, so that the commissural joins the dorsal and subneural vessels at its two ends, while in its ventral third it gives the septo-intestinal branch to the intestinal blood-plexus. It is interesting to note the Y-shaped places of junction (fig. 2) one comes across in sections, where the three limbs of the Y represent the branches of the commissural going to the dorsal and subneural vessels and the intestinal plexus respectively. All along its length the commissural vessel is joined by branches coming from the septal nephridia and the body-wall. In segments sixteen to twenty-one the commissural vessel also receives the efferent capillaries from the prostates which get their blood-supply from the branches of the ventro-tegumentaries. As shown in fig. 1, I could count in one preparation as many as eight branches entering the commissural, each of these branches being formed by the union of several branchlets.

The commissural vessel of Pheretima is a very interesting structure when we compare it with similar structures in other earthworms. Bourne (1) describes in Megascolex two vessels, which he calls 'intestino-tegumentary' and 'dorso-tegumentary', as follows: 'The main portion of the intestino-tegumentary vessel lies closely adherent to the body-wall just behind a septum, i.e. in the anterior portion of a segment', and 'the dorso-tegumentary arises in all segments regularly from the dorsal vessel immediately posterior to the septum which forms the anterior boundary of the segment in which it lies'. It is clear from this description and also from his diagram (Pl. IX, fig. 7, in his paper) that these two vessels of Megascolex run in the same transverse plane, and would thus correspond exactly to the commissural vessel of Pheretima minus its small ventral portion, since the commissural

also lies in exactly the same position. Its dorsal part with its connexions with both the dorsal vessel and the body-wall would correspond to the 'dorso-tegumentary', and its lateral part together with the septo-intestinal having connexions with the body-wall on the one hand and the intestinal plexus on the other would correspond to the 'intestino-tegumentary' of Megascolex. There being no subneural vessel in the latter genus, there is nothing in its blood-system corresponding to the ventral part of the commissural of Pheretima.

Again, the 'dorso-tegumentary' of Moniligaster (2) and Lumbricus (8) corresponds to the commissural vessel of Pheretima minus the septo-intestinal. Unlike Megascolex, these two genera (Moniligaster and Lumbricus) possess a subneural vessel like Pheretima, and we have a loop or commissural vessel connecting the dorsal with the subneural, which has been described by Jaquet (9) in Lumbricus as the 'branche dorso-sous-nervienne', a term adopted by Bourne for the same structure in Moniligaster. Jaquet also describes a 'branche tégumentaire' from the dorso-tegumentary; but I have examined the tegumentary (commissural or parietal) of Lumbricus and do not find a special 'branche tégumentaire' as Jaquet makes out. Of course, there are several branches from the body-wall (tegumentary branches) joining the commissural all along its course as in Pheretima, to which the term 'branche tégumentaire' can be applied; but the real point in which the commissural of Lumbricus and Moniligaster differs from that of Pheretima is that in the former two genera it has no connexion with the intestinal plexus, there being nothing corresponding to the 'septo-intestinal' of Pheretima.

From the comparisons made above it seems reasonable to deduce that the commissural vessel of Pheretima is a compound vessel which combines in itself the 'dorso-tegumentary' (commissural or parietal) of Lumbricus and Moniligaster (the dorso-tegumentary of Megascolex corresponding only to one of the tegumentary branches joining the commissural

in the other earthworms) and the 'intestino-tegumentary' of Megascolex. The probable homologies are set out in the following table :

1. Lumbricus	Branche tégumentaire	Branche dorso-sous-nervienne	Absent
2. Moniligaster	„ „	„ „	„
3. Megascolex	Dorso - tegumentary	Only partially represented by the tegumentary part of the 'intestino-tegumentary'	Intestinal part of intestino-tegumentary'
4. Pheretima	One of the capillaries from the body-wall joining the dorsal portion of the commissural	Commissural vessel	Septo-intestinal.

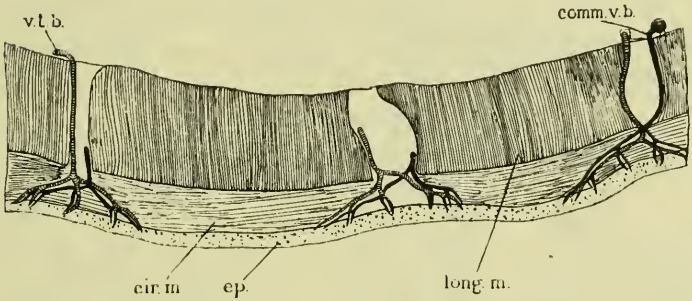
In describing the 'ventro-intestinals', of which there is a pair in each segment in Moniligaster (**2**, 1894, p. 330), Bourne remarks : 'They are the sole afferent vessels of the intestinal walls. There are no such vessels in Megascolex coeruleus, their function being performed by the "intestino-tegumentary" vessels.' In Pheretima we have both the 'intestino-tegumentary' (represented by the septo-intestinal) as well as the ventro-intestinal vessel in each segment ; and if both are afferent vessels of the gut-wall, as I believe they are, there is a double source of supply of blood to the gut in Pheretima.

As I shall discuss later on, I believe that the course of blood in the commissural is towards the dorsal vessel. The blood from the subneural goes to the intestinal plexus through the septo-intestinal, and the branches joining the commissural all along its course bring blood into it from the body-wall and the septal and integumentary nephridia.

2. The Integumentary Vessels.—The body-wall, consisting of its muscular layers, and the epidermis receives its supply of blood from the ventro-tegumentary branches, a pair of which comes off from the ventral vessel in each segment. I have already stated that these ventro-tegumentary branches

supply the body-wall of the segment succeeding the one in which they arise from the ventral vessel (e.g. the ventro-tegumentary arising from the ventral in the fortieth segment runs along and supplies the body-wall of the forty-first segment and so on). The ventro-tegumentaries give off numerous branches backwards and forwards (fig. 1), which are distributed over the body-wall and also supply blood to the integumentary nephridia (vide infra). The ventro-tegumentaries grow thinner and thinner along their course towards the mid-dorsal line near which they end in the body-wall.

Text-fig. 4.



A diagrammatic reconstruction of three serial sections showing the close parallelism of ' arterial ' and ' venous ' capillaries in the body-wall. *ep.* = epidermis ; *cir.m.* = layer of circular muscle-fibres ; *long.m.* = layer of longitudinal muscle-fibres ; *vt.b.* = a branch of the ventro-tegumentary vessel ; *comm.v.b.* = a branch of the commissural vessel.

The efferent vessels of the body-wall are the paired branches of the subneural in each segment and the numerous branches joining the commissural vessel in each segment.

The afferent and efferent capillaries run side by side in the substance of the body-wall, and can always be followed from the coelomic epithelium through the muscular layers to the epidermis. I can confirm for Pheretima Bourne's statement (2) with regard to the peripheral capillaries in Monili-gaster, that 'the most striking feature of these networks (he is speaking of capillaries in the body-wall)

is the strict parallelism which obtains throughout between "artery" and "vein"'. In serial sections it is very interesting to follow pairs of parallel capillaries in the body-wall, and one can invariably trace them to their afferent and efferent vessels. Fig. 4, reconstructed from three sections of 6 μ thickness, serves to illustrate the parallelism obtained in sections, while fig. 4 A gives an accurate camera lucida drawing of part of the body-wall mounted flat after the removal of longitudinal muscles. The strict parallelism between an 'artery' and a vein together with the capillary loops connecting them are very clearly displayed.

3. The Nephridial Blood-system.—The blood-supply of the three kinds of nephridia in Pheretima has already been described by me elsewhere (**3**), and I have nothing further to add here.

(*d*) The Dorso-intestinals and the Ventro-intestinals.

The Dorso-intestinals.—I have referred to these vessels already in describing the dorsal vessel. The dorso-intestinals form, so to speak, the efferent vessels (veins) of the intestinal blood-plexus, as all the blood in the intestine is returned to the dorsal vessel through these dorso-intestinals. There is a single pair of them in the fourteenth segment and in all the segments of the rectal (post-typhlosolar) region, while in the remaining large part of the intestine we have two pairs to each segment. We have already noted that the dorso-intestinals communicate with the external plexus in the rectal region but with the internal plexus in the first and second regions. At the place where the dorso-intestinal leaves the gut, it also receives a branch from the typhlosolar vessel (fig. 2).

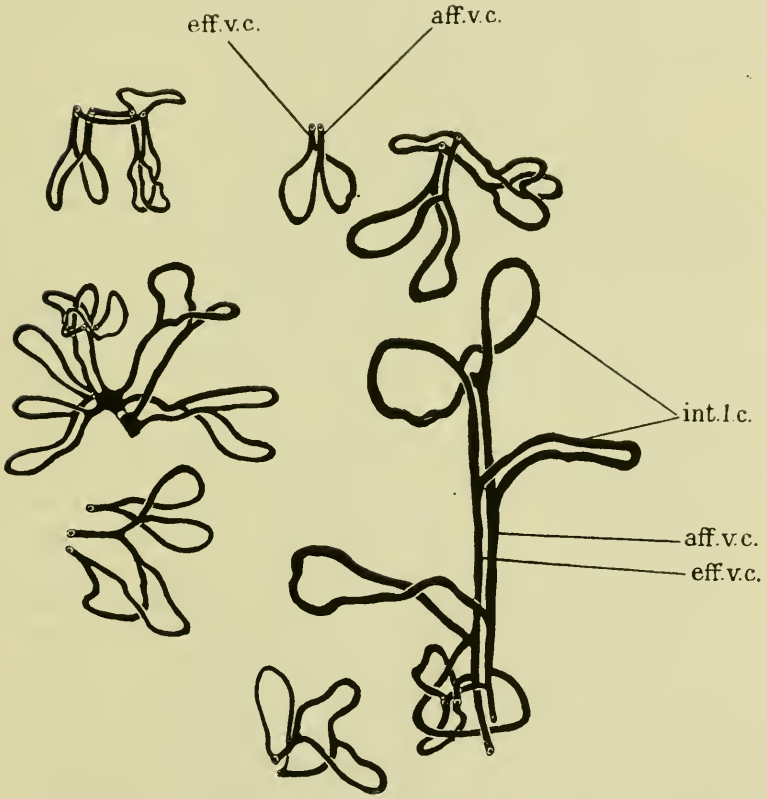
The Ventro-intestinals.—These single unpaired vessels in each segment have also been referred to above. They form the afferent vessels (arteries) of the gut, and are present in all the three regions.

3. The Blood-system in the First Fourteen Segments.

In the first fourteen segments the blood-system is highly modified on account of the cephalization of this region, and differs a good deal from the system in the general body-region.

Amongst the longitudinal trunks the subneural as such is

Text-fig. 4 A.



Disposition of blood-capillaries in the body-wall from a whole mount of a portion of the body-wall treated with caustic potash, showing how a 'venous' capillary passes into an 'arterial' one. *aff.v.c.* = capillary of the afferent vessel; *eff.v.c.* = capillary of the efferent vessel; *int.l.c.* = capillary loop connecting the afferent and efferent vessels.

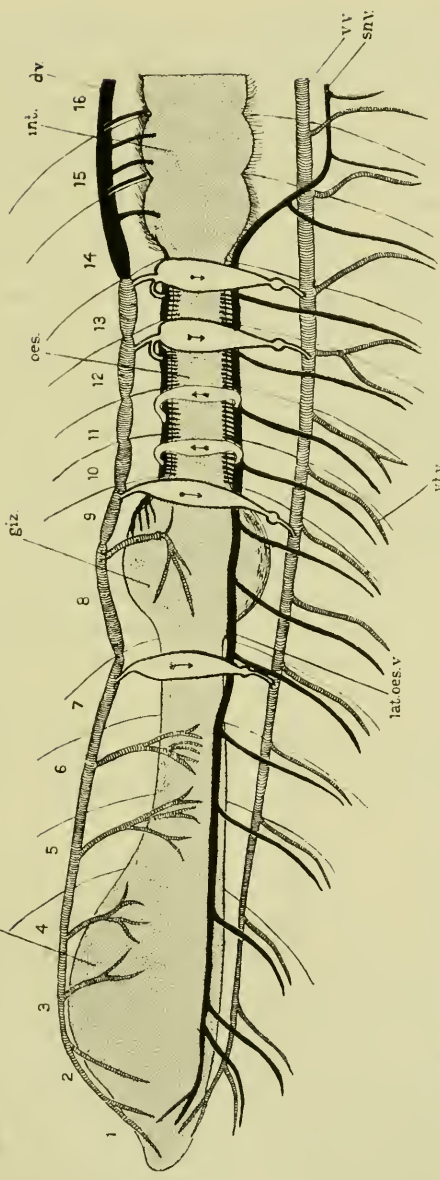
absent ; it bifurcates in the fourteenth segment, and the two branches curve round (fig. 5) the nerve-cord to be continued into the two lateral oesophageal vessels. A new large vessel in this region limited in extent is the supra-intestinal vessel, which is closely attached to the oesophagus in the mid-dorsal line and communicates freely with the blood-plexus of the oesophagus. Besides these there are the big pulsating ' hearts ' in many of the segments of this region, by means of which the dorsal vessel pumps out all the blood it receives either into the ventral vessel to be distributed by it or directly to the various organs in this part of the body.

(*a*) The Longitudinal Trunks.

1. The Dorsal Vessel.—The dorsal vessel continues in front up to the third segment, where it divides into three branches near the cerebral ganglion, these branches being distributed over the pharyngeal mass and the wall of the buccal cavity. While in the region of the intestine the dorsal vessel lies close upon the gut, being connected with it by two pairs of dorso-intestinals ; in this anterior region it is removed considerably away from the oesophagus. Except in the fourteenth segment, where the dorsal vessel is connected by a single (not two) pair of dorso-intestinals, there are no such venous branches at all in the anterior cephalized region. Since there is no subneural vessel in this region the commissural vessels connecting the dorsal with the subneural in the intestinal region are absent in this anterior region. However, the dorsal vessel here gives off, in many segments, pulsatile vessels called the ' hearts '. These structures I shall describe separately below.

The intersegmental valves present in the posterior part of the dorsal vessel are present here also, and have the same structure and disposition, making the blood flow in the anterior direction. But the valves at the orifices of the dorso-intestinals and commissurals into the dorsal (*vide infra*) in the posterior region have no counterpart here ; in their place there are other valves away from these orifices, leading the blood outwards from the dorsal vessel.

TEXT-FIG. 5.



A semi-diagrammatic representation of blood-vessels in the first sixteen segments of Pheretima. *d.v.* = dorsal vessel; *giz.* = gizzard; *int.* = intestine; *lat. oes.v.* = lateral oesophageal vessel; *oes.* = oesophagus; *ph.* = pharynx; *sn.v.* = subneural vessel; *v.v.* = ventral vessel; *vt.v.* = ventro-tegumentary vessel. The 'latero-intestinal' hearts are in the twelfth and thirteenth segments, the 'lateral' hearts in the seventh and ninth segments, while the tenth and eleventh segments contain the 'anterior loops'.

2. The Supra-intestinal Vessel.—The supra-intestinal vessel, which is confined to the oesophageal region behind the gizzard, occupies the same relative position with regard to the gut as the dorsal vessel does in the region of the intestine. It lies beneath the dorsal vessel rather closely attached to the dorsal wall of the oesophagus, while the dorsal vessel itself is removed considerably away from the gut. It is usually double along its whole extent, but the two halves come together and communicate with each other at several places. The supra-intestinal vessel extends from the tenth to the thirteenth segment. In the tenth and eleventh segments it communicates with the lateral oesophageal vessels by large commissural vessels or 'loops' that go round free from the wall of the oesophagus; while in the twelfth and thirteenth segments it communicates with the ventral vessel through the 'hearts'. The vessel ends anteriorly by breaking up into capillaries in front of the tenth segment, and these capillaries are distributed over the walls of the oesophagus and the gizzard. Posteriorly the vessel ends by joining the posterior pair of 'hearts' in the thirteenth segment, although a slender branch very often continues backwards on the mid-dorsal line of the gut for a segment or two.

The supra-intestinal is the efferent vessel for the gizzard and the oesophagus, and all the blood brought in it from these structures is no doubt carried into the 'hearts' of the twelfth and thirteenth segments.

3. The Ventral Vessel.—The ventral vessel extends anteriorly up to the second segment, and in each segment gives off a pair of ventro-tegumentary branches as in the posterior region, with the difference that the branches from a particular segment are spread over and distribute blood to the body-wall, the septa, and the nephridia in the same segment and not the succeeding one, as they do behind. All the special organs in this part of the body, e.g. the spermathecae, the seminal vesicles, the ovaries, and the oviducts are supplied with blood by little branches from the ventro-tegumentaries. The vessel ends anteriorly in a pair of branches

in the second segment. There are no ventro-intestinals in this region of the body.

4. Lateral-oesophageal Vessels.—These are a pair of fairly large vessels in the first fourteen segments of the animal situated on the ventro-lateral aspect of the oesophagus. They are always found full of blood and can be easily seen. Behind the gizzard, i.e. in segments ten to thirteen, they are very intimately attached to the wall of the oesophagus and, as can be seen in sections, communicate with the oesophageal ring-vessels throughout these four segments by as many branches as the number of ring-vessels. In the region of the gizzard and in front, however, they are free from the wall of the gut, but receive a branch in each segment from the wall of the gut.

The lateral oesophageals receive in each segment a pair of branches that bring back blood not only from the body-wall and septa of this region but also from the seminal vesicles and the spermathecae. They thus function here like the branches of the subneural and commissural vessels behind, which collect blood from the body-wall, the nephridia and other organs in coelom like the prostates.

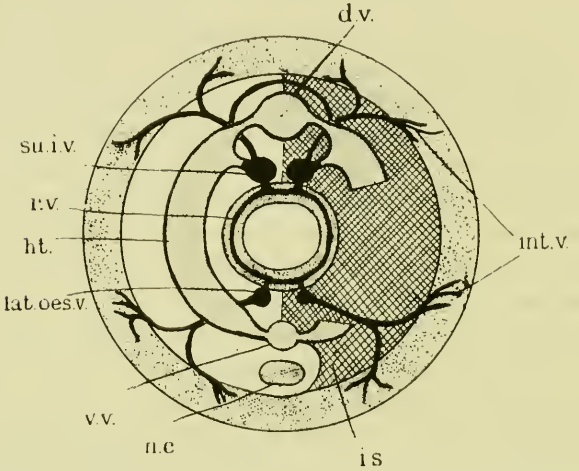
It only remains to be added that the lateral oesophageals are a continuation forward of the subneural vessel. In the fourteenth segment the subneural vessel forks into two, and each of the two branches loops round the nerve-cord and comes to lie dorsal to it and is continued forward along the ventro-lateral aspect of the oesophagus as the lateral-oesophageal vessels.

(*b*) The 'Hearts' and the Anterior Loops.

It will be seen from what we have described above that there is no direct communication between the dorsal and ventral vessels in the region of the body behind the thirteenth segment, but in the anterior thirteen segments the dorsal vessel communicates directly with the ventral through the 'hearts' in the seventh and ninth, and twelfth and thirteenth segments. It is only these four pairs of 'hearts' that are connected with the ventral vessel; but, besides these, there are other 'hearts'

which are also pulsatile but supply blood to some of the organs directly, e.g. the gizzard and the pharyngeal nephridia. I have adopted Bourne's suggestion (**1**, p. 64 n.) of naming all rhythmically contractile, circularly disposed vessels as 'hearts', which term thus includes even the anterior branches of the dorsal vessel which do not join the ventral vessel.

TEXT-FIG. 6.



A diagrammatic transverse section of the earthworm through the region of the 'latero-intestinal' hearts. In the right half is shown the intersegmental septum just behind the 'heart'. *d.v.*= dorsal vessel; *ht.*=latero-intestinal heart; *i.s.*=intersegmental septum; *int.v.*=integumentary vessels taking blood (venous) to the lateral oesophageals and the supra-intestinals; *lat.oes.v.*= latero-oesophageal vessels; *r.v.*=a ring-vessel in the oesophagus; *su.i.v.*=supra-intestinal vessel; *v.v.*=ventral vessel.

Again, Bourne (**1**, p. 64 n.), following Perrier, distinguishes 'lateral hearts' from the 'intestinal hearts' according as they are connected dorsally with the dorsal or supra-intestinal vessels. The 'hearts' in the twelfth and thirteenth segments in Pheretima communicate dorsally with both the dorsal and supra-intestinal vessels and are therefore 'latero-intestinal' hearts, while the 'hearts' in the seventh and ninth segments belong to the category of 'lateral hearts'. Coming to the 'loops' of the tenth and eleventh segments, we find that they

communicate dorsally with the supra-intestinal vessel, while ventrally they are connected with the lateral-oesophageal vessels. They might have been called ' intestinal hearts ' but for the fact that these ' loops ' do not pulsate, have non-muscular walls unlike those of the ' hearts ', and I believe that the flow of blood in them is from the lateral oesophageals to the supra-intestinal, a fact which I refer to again below. On these considerations I exclude these vessels from the category of ' hearts ' and call them ' anterior loops ', since they have nothing in common with the so-called ' hearts ' and ' anterior loops ' in greater detail below ; they are shown in fig. 5.

Thirteenth and Twelfth Segments.—In each of these two segments there is a pair of ' latero-intestinal ' hearts. In systematic accounts of the genus Pheretima it is only these two pairs that are described, and no mention is made of the anterior pairs of ' hearts '. Even if the term ' hearts ' be restricted to those commissures which communicate with the ventral vessel below it should include the ' hearts ' of the seventh and ninth segments. This diagnostic character for the genus Pheretima is thus generally erroneously described, and the genus should be recognized to possess at least four pairs of ' hearts ', two ' lateral ' and two ' latero-intestinals '.

The ' hearts ' of the twelfth and thirteenth segments (fig. 5) are situated in the posterior parts of these two segments, and their walls are intimately attached to the septa behind them. They have thick muscular walls and a spacious cavity, and at their dorsal ends communicate anteriorly with the supra-intestinal and posteriorly with the dorsal vessel. At the places where the branches from the dorsal and supra-intestinal meet to enter the ' heart ', each has a pair of valves leading to the ' heart ', and similarly there is a pair of valves at the ventral end of each ' heart ' just above the place where it joins the ventral vessel (fig. 11). The dorsal valves prevent the blood from going back to the dorsal or supra-intestinal vessels during systole, while the ventral valves prevent the blood from entering the ' heart ' from the ventral vessel during diastole.

Eleventh and Tenth Segments.—These two segments contain no ' hearts ', but each of them has a pair of commissural vessels connecting the supra-intestinal with the lateral oesophageal of each side. These vessels lie in the posterior parts of these segments near their posterior septa, and are partially covered by the latter. Unlike the ' hearts ' these ' loops ' of the tenth and eleventh segments are thin-walled, their walls being non-muscular, and they have no valves anywhere along their length.

The blood, by means of these ' loops ', flows from the lateral-oesophageals into the supra-intestinals. The latter collect blood from the gizzard and oesophagus and also receive blood in these two segments directly from the lateral oesophageals. All this blood they carry into the ventral vessel through the ' hearts ' in the twelfth and thirteenth segments.

We may note here that the lateral oesophageals in Lumbricus pour their blood into the dorsal vessel in the tenth segment and into the large parietal in the twelfth.

Ninth Segment.—In the ninth segment there is a pair of ' lateral hearts ' connecting the dorsal with the ventral vessel. This pair of ' hearts ' is generally asymmetrical, the left ' heart ' being large and well developed as compared with the small thin-walled and ill-developed one of the right side, which, however, sends a branch to the oesophagus in this segment. The ' heart ' on the left side has valves pointing downwards along the greater part of its length, and there is also a pair near the point of opening of the ' heart ' into the ventral vessel. There are altogether four pairs of valves and their position and arrangement is illustrated in fig. 11 A.

Eighth Segment.—In the eighth segment the dorsal vessel gives off a pair of large thick-walled branches which do not join the ventral vessel but on account of their contractility are still called ' hearts ' ; each of them presents a bulb-like dilatation at some distance from its origin and immediately forks into two (fig. 5), the posterior branch going to the septum and body-wall, and the anterior dividing and distributing blood over the wall of the gizzard in a large number of capil-

laries which run longitudinally parallel to one another. These branches of the dorsal vessel have a series of paired valves along their length between the point of their origin and the place where there is the bulb-like dilatation. The bulb-like dilatation which occurs at the distal end of all the ' hearts ' contains a pair of thick valves pointing away from the dorsal and towards the ventral vessel, as shown in fig. 11.

The blood to the gizzard, therefore, is supplied from the dorsal vessel by the pair of branches in this segment ; while the capillaries of the supra-intestinal vessel, which has its beginnings here, collect blood from the gizzard and take it into that vessel.

Seventh Segment.—In the seventh segment there is a pair of ' lateral ' hearts, each of which is joined below both with the ventral and the lateral oesophageal vessels, which latter are themselves joined together by a cross channel. In its upper part each of this pair of ' hearts ' is thick-walled and has valves leading blood outwards, but in its ventral part each ' heart ' is thin-walled and has also no valves in it. There is no doubt that the blood flows from the dorsal to the ventral vessel ; but it seems probable that the supply of blood in the ventral vessel, which is very thin in this region and contains little blood, is also replenished from the lateral oesophageals, which are always large and full.

Sixth, Fifth, and Fourth Segments.—In the sixth segment, and also in the fifth and fourth, there is a pair of branches given off from the dorsal vessel each of which has a pair of valves leading outwards near its origin, and supplies blood to the masses of pharyngeal nephridia in each of these three segments. These branches are also pulsatile and can therefore be named ' hearts '.

Third Segment.—In the third segment before the dorsal vessel breaks up anteriorly, it gives off a pair of branches to the pharyngeal mass behind the cerebral ganglion. These branches also possess valves near their origin which direct the flow of blood outwards.

(*c*) The Blood-vessels of the Gut in the first Fourteen Segments.

In segments ten to fourteen there are in the oesophageal wall a series of very definite and striking transverse vessels, about twelve pairs per segment, joining the supra-intestinal above and the lateral oesophageals below; the breadth of these vessels is at least equal to the intervals between them. They are not united by longitudinal connexions and are continuous across the mid-ventral line. These ring-vessels (fig. 6) are very characteristic of the oesophagus behind the gizzard, and are situated inside the muscular coats of the oesophagus. In this region both the lateral oesophageals and the supra-intestinals are intimately attached to the oesophagus, and the blood flows from the former into the latter through these transverse ring-vessels, the latter receiving no supply at all from the ventral vessel.

In the eighth and ninth segments the gizzard receives its supply of blood from the 'hearts' of the eighth segment, the branches of which divide and run along the outer wall of the gizzard in about fourteen parallel longitudinal capillaries. There is a second set of parallel capillaries which collect blood from the gizzard and join the supra-intestinal vessel.

In front of the gizzard, i.e. in the first seven segments, the pharynx and the oesophagus get their supply of blood from the 'hearts' of the dorsal vessel, and branches of the lateral oesophageals collect blood and take it to the latter from this part of the gut.

4. Comparison with the Blood-system of the Lumbricidae.

In main outline the arrangement of blood-vessels in *Pheretima* resembles that of *Lumbricus* and *Allolobophora*, the latter more than the former. The main longitudinal trunks—the dorsal, the ventral, and the subneural—are the same in the three genera, but in *Lumbricus* there are also in addition the two lateral neurals which are absent in the

other two genera. Moreover, while in Lumbricus and Allolobophora the subneural goes right up to the anterior end of the body, in Pheretima it passes into the lateral oesophageals in the fourteenth, as it also does in Moniligaster (2), being absent in the first thirteen segments. The venous branches of the dorsal vessel bringing blood into it behind the 'hearts' are the 'dorso-intestinals' and the 'commissurals'. The latter, while they lie completely in one segment in Pheretima, occupy two segments in Lumbricus and Allolobophora. In these the ventral portions of the commissurals lie on the posterior face of a septum in one segment, while the dorsal portions lie on the anterior face of the same septum in the segment in front. In this way, while the commissural vessel enters the dorsal vessel in front of a septum, it enters the subneural immediately behind that septum; but in Pheretima, both the ends of the commissural and, in fact, the whole of the commissural, lies on the posterior face of a septum.

The ventro-tegumentaries in all the three genera arise in the segment anterior to the one they supply; but while in Pheretima and Allolobophora the ventro-tegumentary runs along the middle line of a segment (fig. 3), it runs very near the anterior septum alongside the commissural in Lumbricus. The parallelism between an artery and vein shown in fig. 4 in Pheretima in the body-wall is not found in Lumbricus, in which the arterial branch lying inside the muscular layers of the body-wall takes a dip towards the epidermis, runs beneath this layer for a short distance, and runs back to the muscular layers to be continued as a venous branch to the commissural into which it enters (6).

As regards the blood-vessels in connexion with the gut we may notice the absence of septo-intestinal vessels in the Lumbricidae, whereas in Pheretima the gut has a double source of blood-supply (the ventro-intestinals and the septo-intestinals); in the other two genera it gets all its blood from the ventral vessel only. The typhlosopar vessel of Pheretima, unlike that of the Lumbricidae, is only

a specially developed mid-dorsal portion of the gut-plexus, and has no definite walls of its own, nor does it communicate directly with the dorsal vessel as it does in Lumbricus.

In the anterior cephalized region of the body besides the differences in the number and position of the 'hearts', there is the presence in Pheretima of an additional 'supra-intestinal vessel' which receives all the blood from the lateral oesophageals and pours it into the 'hearts'; while in the other two genera, the blood from the lateral oesophageals goes directly to 'hearts', and there is no 'supra-intestinal' vessel.

5. The Course of the Circulation of the Blood.

All observers are agreed upon the fact that the blood-current in the dorsal vessel has a forward direction. I have already stated that just in front of each septal plane, where the dorsal vessel is very much constricted and has the narrowest lumen, there are forwardly-directed valves which, when the vessel contracts, prevent the flow of blood backwards. These inter-segmental valves, as we may call them, form an incomplete circular ridge on the internal wall of the vessel at their point of origin; but it can easily be seen that the valves consist of two large dorso-lateral valves, while there are small dorsal and ventral ones (figs. 7 and 10). These valves are more or less continuous with one another, so that we can regard them as constituting one valve with small dorsal and ventral lobes and large lateral lobes. The large dorso-lateral lobes project forwards into the lumen of the vessel for some distance, and are seen as two masses lying free in the dorsal vessel in transverse sections. Fig. 10 (*a*, *b*, and *c*) shows the disposition of this intersegmental valve in serial sections. In Lumbricus, on the other hand, there are two large lateral valves, as shown by Johnstone (**9**), in the same position and having the same function.

The dorsal vessel receives two pairs of dorso-intestinals and one pair of commissurals ('parietals' or 'dorso-sous-nerviens') in each segment behind the fourteenth. The

question is, what is the course of blood in these two kinds of vessels? Does the blood come into the dorsal from both or from only one? According to Bourne (**1**, p. 74) and Vejdovsky (**11**, p. 115), the blood flows from the intestinal capillaries into the dorsal vessel through the dorso-intestinals, and in this I agree with them. In recently-killed worms I have cut these dorso-intestinals to see from which of the cut ends the blood flows, and I have invariably found blood oozing out from the side of the intestinal capillaries. Moreover, the arrangement of valves which I refer to later confirms this view. With regard to the course of blood in the commissural vessel ('dorso-tegumentary' of Bourne in Moniligaster), I believe with Perrier (as quoted by Bourne in **1**) and Benham (**1**, p. 255) that blood enters the dorsal vessel from these commissurals. Bourne (**1**, p. 75), however, believes that blood leaves the dorsal vessel by the dorso-tegumentaries. But later on in his paper on Moniligaster, after discussing the point in an elaborate manner (**2**, p. 335) and concluding that Benham's view is incorrect and that blood flows outwards from the dorsal by the dorso-tegumentaries, he adds (**2**, p. 336), 'the peripheral capillaries in the region of the body behind the hearts are also supplied, to an extent which probably varies from time to time and is, I expect, never very great, from the dorsal vessel by means of the dorso-tegumentary vessels.' Further on in the same paper (p. 350), while generalizing on the vascular system of earthworms, Bourne refers again to the course of blood in the dorso-tegumentaries (commissurals) and says, 'I have again and again returned to the course taken by the blood in these vessels (dorso-tegumentaries). I cannot help thinking that primitively they are efferent vessels, and that both they and the dorso-intestinal vessels bring blood to the dorsal vessel. In this case they can only have, in worms otherwise well provided with a venous system, the function suggested above for Moniligaster grandis of regulating the pressure in the peripheral capillaries, and have practically no flow in them in one direction or the other.' Bourne here seems to give away his case

for the course of blood in the dorso-tegumentaries, and I am convinced that his statement with regard to the primitive condition that I have quoted above holds for adult Pheretima, and in fact all earthworms. Both by a study of the disposition of valves, and by cutting the commissurals and observing from which of the cut ends the blood flows, I am convinced that blood flows into the dorsal vessel from the commissural vessels as it does in the case of the dorso-intestinals. In fact I believe that the dorsal vessel all along the body of the worm behind the first thirteen cephalized segments is a channel only for collecting blood and propelling it forwards. It gives out no blood at all behind the thirteenth segment as it receives none in the first thirteen segments; so that we have two clearly marked divisions of the dorsal vessel—the large posterior division of it behind the 'hearts' being the collecting channel, and the anterior short division of the first thirteen segments being the channel for distribution of all the blood collected behind.

As regards the disposition of the valves situated at the entrance of the dorso-intestinals and the commissurals into the dorsal, they are easily seen in transverse sections projecting into the lumen of the dorsal vessel. In two lucky preparations of the dorsal vessel, in which the latter was torn open and fixed with the valves projecting out into the open lumen, I have been able to see the valves displayed in an admirable manner. They are shown in fig. 7. The valves are seen in two conditions, i.e. either protruding inwards into the lumen of the dorsal vessel or flush with the wall of the vessel. In the former condition they are more or less conical in shape, the blunt apex of the cone forming the projecting end into the dorsal vessel, and the base being continuous with the wall of the vessel; in the latter condition there is nothing projecting into the lumen of the dorsal vessel, and the valves look like closed sphincter muscles in the wall of the vessel, the actual valves being contained in the upper ends of the dorso-intestinals or commissurals. There can be no doubt that these two conditions of the valves represent them as they are during the

diastole and systole of the dorsal vessel projecting inwards when the dorsal vessel is filling and the blood is coming in through both the dorso-intestinals and the commissurals, and lying flush with the wall with the apertures closed when the dorsal vessel contracts.

Bourne (**2**, p. 334) says, 'In Moniligaster as in Megascolex, while there are valves which would mechanically

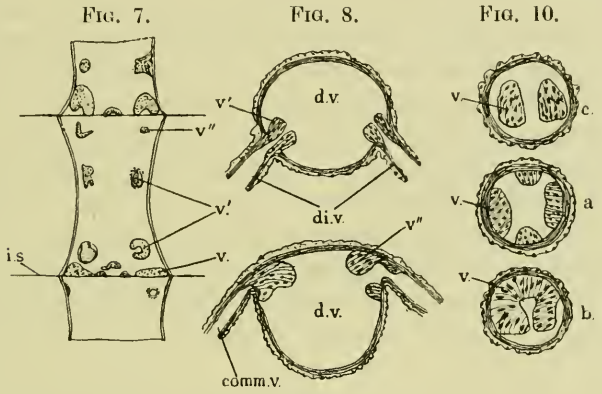


Fig. 9.

Text-Fig. 7.—Portion of the dorsal vessel cut open along its median dorsal line showing the valves in its lumen. *v.*=the valves at the intersegmental septa; *v'.*=valves at the entrance of the dorso-intestinals into the dorsal; *v''.*=valves at the entrance of the commissural vessels into the dorsal.

Text-Fig. 8.—Section of the dorsal vessel passing through the region where the dorso-intestinals enter the dorsal vessel showing the valves at the entrance. *d.v.*=dorsal vessel; *di.v.*=dorso-intestinal vessel.

Text-Fig. 9.—Section of the dorsal vessel showing the valves at the entrance of the commissural vessels into the dorsal. *d.v.*=dorsal vessel; *comm.v.*=commissural vessel.

Text-Fig. 10.—Three sections of the dorsal vessel showing the intersegmental valves. *a.*=about the place of origin of the valve; *b.*=a little in front; *c.*=still further forward.

prevent blood flowing into the dorso-intestinal vessel from the dorsal vessel, there are no such valves where the dorso-tegumentary vessels join the dorsal vessel. I have, however, observed in Moniligaster and some other worms a sphincter muscle in the wall of the dorso-tegumentary vessel close to its

origin.' As a matter of fact the valves at the point of entrance of both the dorso-intestinals and the commissural vessels (dorso-tegumentaries) look like sphincter muscles when they are not in the protruding position and are flush with the wall of the dorsal vessel. It is not unlikely that the sphincter muscles seen in Moniligaster by Bourne are really the valves in the closed condition, which, like those of the dorsal vessel, have the form of circular ridges. In transverse sections of Pheretima they are seen as small club-shaped structures, attached to the inner wall of the commissural vessel just where the latter narrows to join the dorsal vessel, and having their broad ends projecting freely into the cavity of the dorsal vessel (fig. 9). Johnstone (**8** and **9**) describes a similar disposition of valves in Lumbricus both in the dorso-intestinals and the commissurals, and I have verified it from my sections of Lumbricus. The disposition of valves and the course of blood-flow in these two vessels are therefore similar in both the worms (Lumbricus and Pheretima) and probably in all earthworms.

Another fact, which confirms my view with regard to the flow of blood into the dorsal vessel from the commissural (dorso-tegumentary) and not vice versa, is that in dissections of the fresh worm when the flaps of body-wall are pinned down after a mid-dorsal incision, the commissural vessels are almost always torn off from the dorsal vessel near their point of entrance into the latter, and the blood oozes out not from the dorsal vessel or the portion of the commissural left attached to it, but always from the cut end of the commissural near the outer edge of the flaps. This shows that the direction of blood is towards the dorsal and not away from it. If the flow of blood were from the dorsal to the commissurals, we should see the dorsal emptying itself through the upper cut pieces of the commissurals, especially since the dorsal vessel keeps pulsating for some time after the worm is opened in the salt solution. As a matter of fact no blood oozes out of the dorsal, which remains full.

Moreover, leaving aside the question of valves and the

flow of blood from cut ends, I think Bourne's view that blood in the commissural vessel comes out of the dorsal and flows towards the subneural is untenable even on theoretical grounds. He is agreed on the fact that branches joining the commissural vessel are veins bringing blood to it from the body-wall and the nephridia, and shows them as such in his diagrams (Pl. 26, fig. 34, 2); but he believes that all the blood is collected in the subneural and passes forwards along the lateral longitudinals (lateral oesophageals) to enter the posterior pair of 'hearts'. Assuming for a moment that Bourne's view is correct (although I do not agree with it) and that the blood from the subneural goes all the way to the hearts, why should any part of this blood come from the dorsal in each segment via the commissurals? If the commissural is a collecting channel for all the blood from the body-wall and the nephridia, why should it get any blood at all from the dorsal vessel? There is no meaning in the blood coming from the dorsal into the subneural in each segment and then entering the 'hearts', while it could do so by going into the 'hearts' straight along the dorsal vessel. It is to obviate this difficulty that Bourne takes the view that the commissurals have practically no flow in them in one direction or the other and that they regulate the pressure in the peripheral capillaries—a supposition which is easily disproved by cutting the commissurals and seeing that blood *does* flow in them towards the dorsal vessel.

As a matter of fact, so much blood leaves the dorsal vessel anteriorly through the 'hearts', of which there are four in *Pheretima* connected with the ventral vessel and others supplying the organs directly, that it is difficult to conceive on *a priori* grounds that any blood leaves the dorsal vessel at all behind the thirteenth segment.

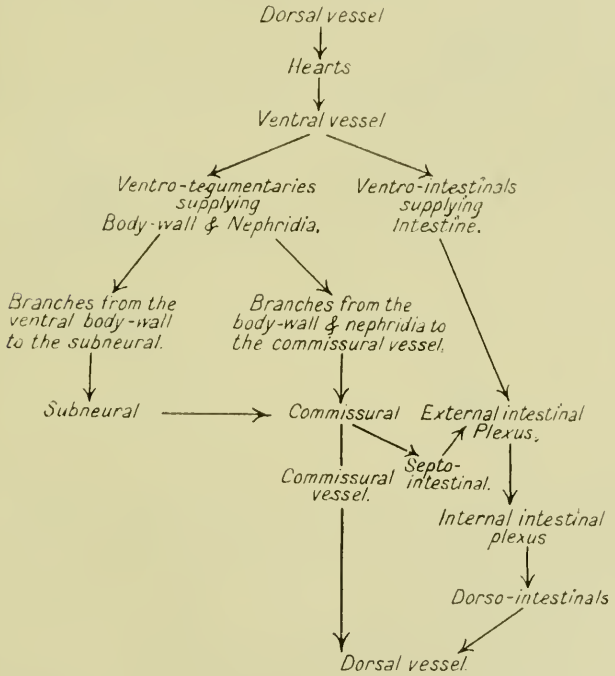
Having decided that the dorsal vessel all along the body behind the thirteenth segment is only a channel for collection and propulsion forwards of the blood which enters it from the intestinal network and the commissural vessels, the rest of the circulation in the worm becomes easy to follow.

The ventral vessel is the chief distributing channel and, so to speak, the arterial trunk of the body. All observers are agreed that blood flows backwards in this vessel in the region of the body behind the 'hearts', and that the blood is distributed to the body-wall and the other organs lying in the body-cavity (nephridia (septal and integumentary), nerve-cord, prostates, &c.) by means of the pair of ventro-tegumentaries in each segment, and to the gut by means of a single unpaired ventro-intestinal. Every structure in the body region in fact gets its supply from the ventral vessel.

The subneural vessel collects blood from the ventral part of the body-wall and the nerve-cord by means of a pair of small branches it receives in each segment. All this blood goes into the commissural vessels, from which part of it goes to the intestine through the septo-intestinal and the rest to the dorsal all along the commissural, the latter receiving the greater part of its blood-supply from the capillaries that enter into it from the body-wall and the nephridia all along its length. The flow in the subneural is therefore from in front backwards. This can be easily seen by pinching or cutting the vessel in a narcotized worm and watching the direction of blood-flow.

It should be noted that the intestine has a double supply—one from the ventral through the single ventro-intestinal, and the other from the subneural through a pair of septo-intestinals in each segment; this is what we should expect considering the large amount of blood in the extensive network of capillaries on the gut-wall. In Lumbricus the only source of blood for the gut is the ventral vessel; but there the gut receives two or more ventro-intestinal branches in each segment, while in Pheretima, there being only one unpaired ventro-intestinal vessel in each segment, the amount of blood supplied to the gut from the ventral vessel is comparatively small, and I suppose it is to supplement this that we have blood brought to the gut by the septo-intestinals. Both the ventro-intestinals and septo-intestinals bring blood to the external intestinal plexus from which the blood passes into the internal intestinal plexus. From the internal plexus

he blood finally passes into the dorsal vessel through the two pairs of dorso-intestinals in each segment. In the posterior region of the gut—the post-typhlosolar or the rectal region, however, the blood brought to the external plexus passes directly into the dorsal vessel through a single pair of dorso-intestinals in each segment, which, as already mentioned, communicate with the external plexus, the internal plexus being absent in this region. The course of blood in the intestinal region can be shown diagrammatically as follows :—



It will be seen that the ventral vessel and its branches, the ventro-tegumentaries and ventro-intestinals, form the arterial vessels, while the subneural, the commissurals, the dorso-intestinals, and the dorsal vessel itself are the chief veins (using the word in an anatomical sense) in the worm. The blood in the dorsal vessel in a certain segment must go to the ' hearts ', and return by the ventral vessel into that segment

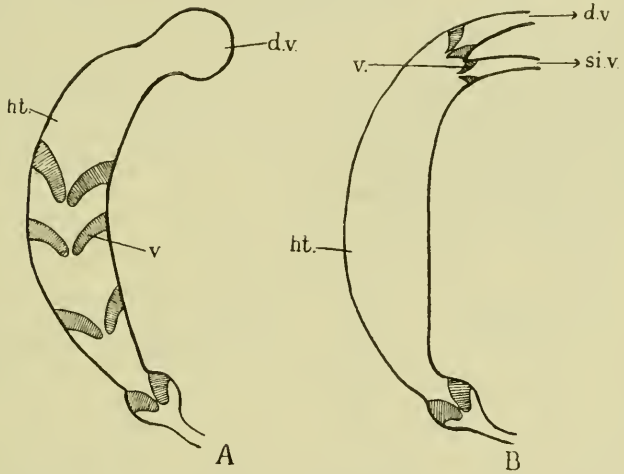
again—so that the blood-flow is not self-sufficient in one segment ; the blood must circulate in the whole body.

In the first thirteen segments (fig. 5) the blood-system is different, and so is the course of blood. The dorsal vessel is no longer a receiving channel ; it has no dorso-intestinals and commissurals opening into it and feeding it with blood—in fact it receives no blood at all, but behaves instead as a great arterial trunk, pumping out all the blood it has received in its posterior region. Of course the greater part of its blood, together with the whole of the blood in the supra-intestinal vessel, is pumped into the ventral vessel through the two pairs of ' latero-intestinal hearts ' in the twelfth and thirteenth segments. But a quantity of blood flows forwards anteriorly and this is pumped into the ventral vessel by means of the ' lateral hearts ' of the ninth and seventh segments, and is supplied to the gizzard and the pharyngeal nephridia by the ' hearts ' in the eighth and fourth, fifth and sixth segments, until the dorsal vessel ends by branching on the pharyngeal mass. In accordance with the change of function of the dorsal vessel we have the change in the disposition of the valves. In this region there are no valves projecting into the lumen of the dorsal vessel ; on the other hand, the valves are present in all the ' hearts ' at a little distance away from their origin from the dorsal vessel. These valves point in the direction away from the dorsal vessel, and lead the blood from the dorsal vessel outwards, preventing any blood taking the reverse course. There are also valves at the distal ends of the ' hearts ' (fig. 11) which allow blood to flow out of ' hearts ' during systole, but do not let the blood come back during diastole. The dorsal vessel is therefore a distributing channel here ; most of its blood it pumps out into the ventral vessel for distribution, but a small quantity it distributes itself to the gizzard, the pharyngeal nephridia and the pharynx.

With regard to the flow of the blood in the ventral vessel, I agree with Bourne (**1**, p. 77) in thinking that the blood coming from the ' hearts ' flows both forwards and backwards. There are no valves in the ventral vessel preventing blood from flowing anteriorly, and in addition to the ' hearts ' of the twelfth and

thirteenth segments there are 'hearts' in the ninth and seventh segments also to take blood into the ventral vessel. I also agree with Bourne (1) when he says, 'All the blood which enters the ventral vessel comes from the "hearts", and that all the ventro-integumentary branches—those anterior to the "hearts", as well as those posterior to them—are efferent vessels. So far as the ventral vessel is concerned, they carry blood away from it.' The ventral vessel, therefore, here as in

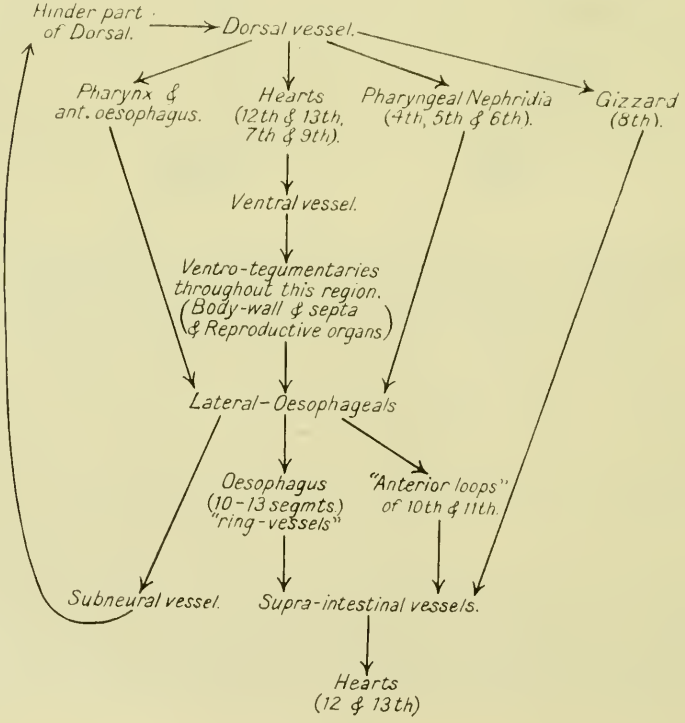
TEXT-FIG. 11.



Semi-diagrammatic representation of 'hearts' in longitudinal sections. A is one of the 'lateral' hearts of the ninth segment with the valves in its lumen and a bulb-like dilatation at its ventral end before it joins the ventral vessel. *d.v.* = dorsal vessel; *ht.* = heart; *v.* = valves; *si.v.* = supra-intestinal vessel.

the region of the body behind the thirteenth segment, is the distributing vessel and supplies blood through the ventro-tegumentaries to the body-wall, the integumentary nephridia as well as the spermathecae and seminal vesicles, the ovaries, and the oviducts. But it does not supply blood to the gut as it does in the hinder region; there are no ventro-intestinals here, and the function of supplying blood to the gut here is taken over partly by the dorsal vessel which supplies blood to the gizzard in the eighth segment and the pharynx and oesophagus in front, and partly by the lateral oesophageals. These vessels

in this region of the body are the counterpart of the subneural, the commissural, and the septo-intestinals of the hinder region, and bring blood from the periphery to the main stream and to the gut. They receive a pair of branches bringing blood from the body-wall, the septa, and other organs of the body, e.g. the nephridia (pharyngeal and integumentary) and the reproduction organs. The part of the oesophagus behind the gizzard is supplied with blood by the lateral oesophageals which lie intimately attached along the ventro-lateral aspect of the oesophagus. The blood from the oesophagus (ten to thirteen) (' ring-vessels ') and the gizzard is collected by the supra-intestinal vessel, which also receives blood directly from the lateral oesophageals through the ' anterior loops ' of the tenth and eleventh segments, and is conveyed to the hearts in the twelfth and thirteenth segments. The course of blood can be represented as follows :—



6. Summary.

1. The typical arrangement of the blood-system in Pheretima occurs in the region of the body behind the fourteenth segment, the first fourteen segments forming the cephalized region. The main longitudinal trunks are the same as in Lumbricus, except that the lateral neurals are absent as in Allolobophora. The dorsal vessel receives two pairs of dorso-intestinals and one pair of commissurals in each segment behind the cephalized region.

2. The intestinal blood-plexus is both an external and an internal one, and three regions can easily be distinguished. The first is internal, and extends from the fourteenth to the twenty-sixth segment; the second is both external and internal, is co existent with the typhlosole, and extends over the larger part of the gut; and the third is only external, and is confined to the rectal or post-typhlosolar part of the gut (last twenty-three to twenty-six segments).

3. The commissural vessel of Pheretima is a compound vessel, and represents both the 'dorso-sous-nervien' of Lumbricus and the intestino-tegumentary of Megascolex. The capillaries of the integument are not like those of Lumbricus but like those of Moniligaster, and there is a close 'parallelism' between an 'artery' and a 'vein' in the body-wall, in which the two pass into each other through a number of capillary loops.

4. There are four pairs of 'hearts' which connect the dorsal with the ventral vessel, and five pairs which supply blood directly to the various organs in the cephalized region. There are two pairs of non-contractile 'anterior loops' connecting the lateral oesophageals with the supra-intestinals, these loops being the counterpart of the connexions of the lateral oesophageals with the dorsal and the parietal in the tenth and twelfth segments respectively of Lumbricus. The subneural vessel is absent in the first fourteen segments, and is continuous with the lateral oesophageals of the anterior region.

5. As regards the course of circulation of the blood, the chief

fact is that the dorsal vessel is wholly ' venous ' behind the ' hearts ' and wholly ' arterial ' in the region of the ' hearts ' and in front (the whole of the cephalized region). The examination of valves and experiments by cutting and pinching the blood-vessels in Pheretima confirm the results of Johnstone for Lumbricus as regards the course of blood in dorso-intestinals and commissurals and make Bourne's theory untenable. The ventral vessel is the arterial trunk throughout, while the venous function of the dorsal and subneural behind is taken up by the lateral oesophageals in the cephalized region. The thin-walled and non-contractile ' loops ' of the tenth and eleventh segments must be distinguished from the thick walled and contractile ' hearts ' of the other cephalized segments, the ' loops ' being the channels for conveying blood from the lateral oesophageals to the supra-intestinals.

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The Development of the Ovary and Ovarian Egg of a Mosquito, Anopheles maculipennis, Meig.

By

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(From the Zoological Laboratory of the University of Birmingham.)

With Plates 17–20.

Contents.

Introduction.

The examination of mosquito ovaries was first commenced with the idea of finding out at what period the ovaries of the hibernating females commence to develop, so that an accurate knowledge of the time at which the mosquito lays the first eggs of the season might be determined. From an examination of sections of the ovaries, it soon became evident that the oocyte nucleus behaved in a somewhat unusual manner during the period of yolk formation. I therefore decided to examine this in detail and at the same time observe what might be termed the grosser anatomy of the developing ovary and oocyte. An immense amount of work has been done on the oogenesis of insects, but most of this has been confined to the detailed examination of the complicated nuclear changes which take place during this period. The mosquito, however, is peculiarly unsuitable for the study of the differentiation of the oocyte and of the prophases of maturation which takes place in the end chamber. As this is very small, in order to examine some of the stages, it would be necessary to cut very thin sections of ovaries containing oocytes with large yolk-masses and in some cases chorion as well. This is an operation which I found quite impossible to perform. The finer structure of the oocyte nucleus has therefore only been studied where it is rendered necessary in order to give a connected account of the development of the oocyte.

To the best of my knowledge the only references to the development of the ovarian egg of the mosquito are contained in two short papers by Christophers. In one of these (2) he gives a very general description of the ovary and egg-follicles, while in the other (3) he describes the development of the egg-follicle from the examination of fresh material. As the information in both these papers is of a very general nature it has been found necessary to repeat portions of it, as otherwise a connected account of the development of the ovary could not be given.

I will take the opportunity here of expressing my deep

indebtedness to Mr. A. J. Grove for the suggestion that I should take up this line of research, for much useful advice during the earlier stages of my work, and for my first supplies of material. For my later supplies I was entirely dependent on the kindness of Mr. R. F. Burton, to whom I wish to make grateful acknowledgement.

The work was done under the supervision of Professor F. W. Gamble, F.R.S., whom I have to thank for assistance in obtaining the very considerable, and not always easily accessible, literature of the subject.

Material and Methods.

The majority of the mosquitoes were taken during the latter part of their hibernating period and the first few weeks after they had regained their activity and had commenced to feed normally.

In order to eliminate the possibility of being misled by artefacts due to fixation, the following method was employed. Each batch of material was divided into three different parts; two of these were fixed in different re-agents, and in the case of the third the ovaries were dissected out in salt solution and one ovary of each insect was rapidly transferred to one fixative and the other to another. In this way the effect of different fixatives on ovaries in the same stage of development could easily be compared.

In the cases where the ovaries were not dissected out, the abdomen alone was fixed, and this was slit along each side with a fine needle in order to allow the easy entrance of the fixative.

It was found that in the case of the less-developed ovaries much the best results were obtained with those which were dissected out, but far less distortion was produced in more mature ovaries fixed while still in the abdomen. This was probably due to the fact that the surrounding tissues only allowed the fixative to reach the ovaries gradually and so prevented rapid osmosis.

A number of different fixatives were used, the principal of which were Flemming, both with and without acetic, Petrunke-

witsch, Zenker, and alcoholic Bouin. Of these, Petrunkewitsch was by far the most useful for general purposes as its penetration is very good, a most important consideration when dealing with oocytes containing a large yolk-mass, particularly when the egg-walls are present. For the finer cytological details Flemming with acetic gave the best results, though Flemming without acetic appeared to give a more perfect fixation, but the latter had the disadvantage that the chromatin did not stain as distinctly as it did with unmodified Flemming.

Another fixative of which I made considerable use was the modified Bouin described by Sheppard (27). This I used in conjunction with the method of staining described by the same author, i. e. bulk staining with carmalum and counter-staining with Grübler's light green. Using this method the fixation was excellent, and the double staining gave very beautiful preparations—yolk and chorion staining bright green and the protoplasmic structures red. This property was very useful in following the branching nucleus through the yolk-mass and in following the production of chorion by the epithelial cells. The fixative had the disadvantage, however, of making the material brittle.

The stain principally used was Grenacher's haematoxylin counter-stained with dilute Lichtgrün picric. For the latter the ordinary Lichtgrün picric solution (0·2 grm. Lichtgrün dissolved in 100 c.c. of a saturated solution of picric acid in absolute alcohol) was diluted with about ten times the bulk of 90 per cent. alcohol. The counter-staining was done under observation, as Lichtgrün appears to displace the haematoxylin and the reaction requires to be stopped when all the yolk has become green and the protoplasmic structures are still blue. Using this method the branching nucleus can easily be followed amongst the yolk granules.

Heidenhain's iron haematoxylin counter-stained with eosin or orange G was also extensively used and was particularly useful for the finer nuclear details, but as it stained the yolk-mass dense black it was not satisfactory for the more developed oocytes.

Most of the material was embedded in paraffin in the ordinary way, but this was not very satisfactory, as oocytes containing large yolk-masses broke up easily and it was difficult to cut uninterrupted series. Towards the end of my work I obtained much more satisfactory results using the double-embedding collodion and paraffin method described by Newth (21). Using this method uninterrupted series of thin sections were easily obtained.

Hibernating Mosquitoes and First Period of Egg Development.

During the winter, female A. maculipennis may be found in cowsheds, church towers, and in fact in almost any dry and comparatively warm place. They pass the winter in a semidormant state, but they are found to feed a little during this period, as occasionally an insect with a little blood in the abdomen may be observed. A microscopic examination of an insect at this stage shows that the fat bodies are relatively very large, the ovaries are always very small, in the 'resting stage', and the spermatheca is full of sperms. In cases where the first batch of eggs had already been laid and the second was developing, the spermatheca was seen to contain sperms, though they were not in such a compact mass as in the hibernating insects. As the first males of the season had not emerged at this period, it would appear that one fertilization of an insect is sufficient for more than one period of oviposition.

The period at which the ovaries of mosquitoes first commence to develop depends on the warmth of the season and also on the locality. Thus in 1919 the majority of the insects taken at the end of March in Kent showed considerable development of the ovaries, while a similar degree of development of the ovaries was not found till about three weeks later in the Shrewsbury district. On March 23, 1920, however, insects with the ovaries in the resting stage were only found with great difficulty in the Shrewsbury district. This is no doubt due to the early spell of fine weather in that year.

Warm weather acts merely as a stimulus to the activity of the insects and causes them to go out and seek food. The stimulus which gives rise to egg development appears to be a good meal of blood. Numerous experiments have been carried out to determine whether blood is necessary for the production of eggs in mosquitoes. To the best of my knowledge in only one case have mosquitoes been induced to lay when fed on any substance other than blood. S. K. Sen (**26**) succeeded in inducing Stegomyia scutellaris to oviposit by feeding with milk or peptone sweetened with cane-sugar, and in two instances was successful when the insect had fed on nothing but cane-sugar. I carried out a number of feeding experiments on A. maculipennis, feeding them on sugar and water, with and without the addition of peptone, and on dates, bananas, and other fruit, all of which the mosquitoes consumed very greedily, but in no case did any development of eggs take place. In all my sections of abdomens in which the eggs are developing, the gut is found to contain blood, with the exception of the final stage, in which the eggs are fully developed, when the gut is always empty. As all these specimens were collected in cowsheds, this does not prove that blood is always necessary for the production of eggs, but it appears to me certain that this normally is the case.

In hibernating mosquitoes the abdomen is very narrow and flattened dorso-ventrally, but when they take their first meal of blood in the spring the abdomen becomes almost globular and distended to its limits with blood. In an insect which has recently fed, the abdomen shows a large semi-transparent uniform mass of blood, while a small whitish mass is seen through the cuticle at the anal extremity. This consists principally of Malpighian tubules but also contains the ovary. On the second day the posterior portion of the blood is very dark red and opaque, while the remainder is as before. In sections the dark-red portion is seen to consist of partially-digested blood containing very distorted corpuscles, while the remainder appears to be quite fresh and might easily be mistaken for a fresh meal of blood. The white mass at the

anal extremity has enlarged somewhat owing to the growth of the ovary. By the third or fourth day the blood-mass is seen to be much reduced, and the whitish mass, the ovaries, about half fills the abdomen. The blood-mass is reduced to a mere spot or is entirely absent by the sixth or seventh day, and, if the weather is warm enough, the eggs are then laid during the night. In cold weather, however, the insects may wait several days before oviposition.

These observations were made on a number of insects collected in a calf-pen. At the time of collection their abdomens contained semi-transparent blood-masses and they had only recently fed. They were kept in jars in the laboratory and the eggs were laid though they received no further food.

The period elapsing between the time of feeding and oviposition appears to be about a week, which agrees very closely with Christophers's observations on A. rossi in India (**3**), in which case the period is given as six days.

I only succeeded in observing insects during the process of laying in two cases, and in both the eggs were laid within an hour of darkness setting in. The insects floated on the water by spreading their long legs over it and frequently dipped their proboscides into the water. When disturbed they flew off the water with ease and seemed in no danger of drowning. The actual oviposition I was unable to observe as the mosquitoes refused to lay in the light.

Female Genital Organs.

In the ' resting stage ' the genital organs of the adult female mosquito consist of two small ovaries lying ventro-laterally in the posterior portion of the abdomen. Each of these communicates posteriorly with an ovarian tube, and the two ovarian tubes unite to form a common duct, the gynaecophoric canal, which opens to the exterior at the posterior end of the eighth segment. A spermatheca, consisting of a thick perforated chitinous shell and surrounded by a layer of large clear cells, gives off a very thick-walled sperm-duct to the gynaecophoric

canal, which it enters a short distance anterior to the genital aperture. A mucous gland, which consists of very large goblet cells, also communicates with the gynaecophoric canal, close to the entrance of the sperm-duct (fig. 7).

The ovary is surrounded by two sheaths, an outer bag-like structure, the investing membrane, and an inner membrane, which is closely applied to the egg-follicles and fits them like a glove ; the fingers of the glove are the follicular tubes and the portion joining up the fingers encloses the lumen of the ovary. The investing membrane passes anteriorly into a tubular suspensory filament, which is fixed to the hypodermis at the junction of the fourth and fifth segments, in a dorso-lateral position. This filament is very long in the young ovary, but it becomes quite short when the ovary is fully developed.

The two sheaths are identical in structure, and consist of a structureless membrane, over one surface of which large nuclei are found. From these radiating muscle-bands pass over the membrane. These nuclei and muscle-bands are on the inside of the investing membrane and on the outside of the follicular tubes, and muscle-bands pass from the nuclei of the one to the other, thus traversing the cavity between the two sheaths and linking the investing membrane and the follicular tubes together, so forming a very complicated muscular system (fig. 24).

The muscle-bands of the sheaths are striped in the normal manner, thus differing from those of most insect ovaries (see J. Gross, **9**). They form broad bands close to the point of origin from the nuclei and taper away from here and branch, some of the finer branches appearing to consist of only a few, or even a single muscle-fibre, as the ' striations ' consist of bead-like, deeply-staining nodes on a fine thread (fig. 24).

It would probably be more correct, in many cases, to consider that the nuclei are placed at intervals on the muscle-fibres, rather than that they are the origin of the fibres. From an examination of fig. 28 it will be seen that many muscle-bands pass through the cytoplasm of the cells, and merely become slightly indefinite there. The ' striations ', though somewhat

distorted, are still placed at regular intervals. In other cases, however, the nuclei certainly appear to be the origin of the muscle-bands.

Over the greater portion of the surface of the investing membrane the muscle-bands radiate in the normal manner (fig. 28), but towards the junction with the oviduct they gradually become reduced to two laterally-placed bands which pass transversely to the long axis of the ovary (fig. 29). Finally, the investing membrane passes over the oviduct and the muscle-bands now form the circular muscles of the oviduct. In a similar manner the muscle-bands of the follicular tube membrane pass insensibly into the longitudinal muscles of the oviduct, inside which is found a layer of columnar cells surrounding the lumen of the oviduct.

If an ovary of a living insect is dissected out in salt solution, a vigorous rhythmatic peristaltic movement is noticed. This may be produced by the stimulus of the salt solution, but there is little doubt that this movement takes place in the living insect, at least when the eggs are being laid. The movement is undoubtedly due to the muscular system described, and the basket-work arrangement of the muscle-fibres is ideal for compressing the ovary and so pressing the eggs into the oviduct. The muscle-bands which pass from the investing membrane to the follicular tubes are probably of use in drawing the latter off the eggs, a process which takes place some time before the eggs are laid.

A number of very characteristic cells are found in the space between the two sheaths, and also between the follicular tubes and the egg-follicles ; one or more is almost always to be found in the region of each terminal chamber, between it and the follicular tube membrane (fig. 25). These cells consist of large nuclei embedded in a mass of very much vacuolated protoplasm, from which fibres are frequently seen to pass. The exact nature of these cells I have not been able to determine, but I am of the opinion that they have some relation to the tracheal system. The fibres seen passing from them are probably tracheal endings, but they are so fine that it is difficult to

determine their nature and they might equally well be protoplasmic strands. In several cases, however, I have succeeded in tracing some of these fibres to the bundles of tracheal endings, so that some at least are tracheal in nature.

It is possible that these vacuolated cells may be leucocytes as they agree in structure and size with Vaney's (31) description and illustrations of the leucocytes in the larva of Gastrophilus equi, but the fact that fibres enter them throws considerable doubt on this theory.

The tracheal system in the ovaries is very highly developed. Tracheae from the fourth and fifth segments go to the ovaries and branches of these penetrate the investing membrane. These tracheal trunks branch repeatedly in the space between the two ovarian sheaths. The final branches consist of exceedingly fine tubes, in which no spiral filament can be distinguished. These pass to the various parts of the ovary in bundles, the tubes being joined together by the tracheal cells which occur at intervals along the bundles. When such tracheal cells are cut transversely they appear to be very much vacuolated, owing to the numerous tubes passing through the cytoplasm. The individual tubes eventually become free from the bundles and end in the tissues of the ovary.

A moderately large tracheal branch passes into the base of each follicular tube and gives rise to numerous bundles of tracheal endings. In the young ovary these have a very characteristic appearance, and are seen as a prominent coiled mass at the base of each follicular tube. This allows for expansion when the ovarian follicles increase in size. The ultimate endings of these tubes are difficult to discover, but I have noted some entering cells of the ovarian follicles and isolated tracheal endings may be seen in almost any part of the follicular tubes.

Inside the follicular tube is an egg-string consisting of an end chamber followed by two or three egg-follicles in various stages of development. These follicles are joined together by cellular stalks consisting of a single row of cells (fig. 26). The last 'stalk' or funicle runs from the posterior and most-developed

follicle to the portion of the follicular tube membrane which invests the lumen of the ovary, with which it fuses.

The whole of the egg-string is invested by a thin structureless membrane, the tunica propria, which may also be regarded as the basement membrane of the follicular epithelium. I am here using the term 'tunica propria' in the sense defined by J. Gross (**9**). The term has been used by many authors as synonymous with 'peritoneal membrane', a practice which has led to much confusion. The peritoneal membrane is represented in the mosquito by the two ovarian sheaths.

Normally the tunica propria can scarcely be observed, as it is very closely applied to the follicular epithelium; but it frequently happens that the follicles degenerate, and then the tunica propria can easily be seen as a somewhat wrinkled, structureless bag surrounding the remnants of the follicle (fig. 24).

Each follicle consists of an oocyte and seven nurse-cells completely surrounded by a single layer of cubical cells, the follicular epithelium.

General Lines of Development of Ovary and Eggs.

Before giving a detailed description of the various changes which take place during the oogenesis of *A. maculipennis*, I will first give a general outline of the development of the egg-follicles and of egg formation, as a comprehensive view of the whole subject will render it more easy to follow the detailed descriptions of the different processes which together produce the mature egg, but which, for sake of clearness, have to be dealt with separately. Also a description of the anatomy of the mature egg will be given, as with a knowledge of this it will be possible to understand the object of the various processes.

The earliest stages of oogenesis are to be found in the end chamber. This consists of a central mass containing comparatively large nuclei, which often vary considerably in appearance but are not definitely divided into nurse-cells and oocytes, and of a peripheral layer containing smaller nuclei which give rise to the follicular epithelium.

At intervals a mass of cells is cut off from the end chamber and consists of seven nurse cells and an oocyte surrounded by a follicular epithelium. This follicle increases in size till it reaches the resting stage (fig. 26), which is characteristic of the ovaries of hibernating females. When the most-developed follicles of the ovary are at this stage the ovary is very small and quite transparent.

If the ovary of an insect which has just had a meal of blood be examined in a fresh condition, it will be found that a white opaque cloud is visible surrounding the nucleus of the oocyte. This consists of fine yolk. In living ovaries at a slightly later period it will be found that the whole oocyte is opaque white and occupies about half the follicle. In sections this opaque mass is found to consist of both coarse and fine yolk, and the oocyte nucleus is no longer spherical but sends out blunt processes into the yolk (fig. 18).

At a still later stage the follicles are elongated instead of almost spherical, and are quite opaque except for a small transparent cap, consisting of nurse-cells, and a thin investing layer of follicular epithelium. The nucleus has now become very much branched, branches passing throughout the yolk-mass and appearing to be in some connexion with the nurse-cells, which are evidently in a state of activity. A new structure has now appeared between the follicular epithelium and the yolk-mass. This consists either of globules or of a layer of gelatinous material, and is the commencement of the inner wall.

Shortly after this stage the nurse-cell nuclei are extruded from the yolk-mass and come to lie in the follicular epithelium, forming a cap over the anterior end of the egg. The oocyte nucleus has now reached its maximum condition of branching and shortly afterwards breaks down. The inner wall is thick but still gelatinous. The follicular epithelium becomes modified in two lateral areas and gives rise to the floats. The rest of the epithelium secretes the chorion over the whole surface of the egg, that portion which contains the extruded nurse-cell nuclei giving rise to the micropyle apparatus.

Finally, the follicular epithelium degenerates into a mere

membrane surrounding the fully-formed eggs; these lie in the lumen of the ovary, as the follicular tubes have contracted and merely cover the less-developed follicles and a small portion of the anterior end of the fully-formed eggs. The eggs, however, still lie in the position in which they developed (fig. 8).

When the eggs are being deposited they appear to break through the remains of the follicular epithelium and then pass down the oviduct to the exterior, the sperms entering the micropyle immediately before the eggs are laid.

Anatomy of the Mature Egg.

The mature egg is more or less cigar-shaped and is provided at each side with a float (fig. 9). It is, however, noticeably thicker at one end than the other, and I consider the thick end as anterior, as it is anterior in the ovary. The portion of the egg which is uppermost when floating I shall refer to as the dorsal surface.

The egg can be divided into three main parts—the outer wall or chorion, the inner wall, and the yolk-mass.

The outer wall of the egg of A. maculipennis appears to be identical in nature to that of other insect eggs, that is, it is formed of chorion, which closely resembles chitin, but differs from it in that it is soluble in warm KOH solution, whereas chitin may be boiled in concentrated caustic potash for hours without effect.

The structure of the chorion of the mosquito egg shows a high degree of specialization. It consists essentially of a thin envelope surrounding the whole egg, two floats placed dorso-laterally, and a very beautifully-formed micropyle apparatus situated immediately below the extreme anterior end of the egg.

The envelope is completely covered with processes of four kinds. The ventral surface is thickly covered with short knob-like processes (fig. 41), and some of these are slightly larger than the remainder and are so arranged that they divide the whole of the ventral surface of the egg into polygonal areas (fig. 9). These areas probably have some connexion with the form of the epithelial cells, but they appear to be too large to be produced by individual cells.

The dorsal surface and the portion of the envelope lying under the floats are covered with very different processes. They are longer, and thin sheets of chorion radiate from a central axis, so that in section the processes are star-shaped. At the top of each process a cap-like structure joins all the radiating thin sheets together (fig. 41).

At the line of division between the dorsal and ventral types of processes there is a single row of much longer processes which extend as a band from the terminations of the floats to the tips of the egg (fig. 9).

The fourth type of process only occurs in small numbers and is found at each end of the egg. This type is a comparatively large boss-like structure consisting of a solid mass of chorion, and seven or eight are found at the extreme anterior and posterior ends of the egg (fig. 40).

The floats consist of a single sheet of chorion attached to the chorion envelope along its ventro-lateral surface only. The sheet curves round till it almost touches the dorso-lateral surface so enclosing a considerable cavity. The whole of the chorion sheet which forms the float is highly corrugated (fig. 9).

The whole of these structures, with the possible exception of the 'bosses', appear to serve the purpose of supporting the egg on the surface of the water. The ventral processes enclose a film of air, which cannot be expelled by the water owing to its surface tension and the closeness of the processes to one another. The floats enclose a relatively large volume of air, and again surface tension prevents the entrance of water. The band of long processes, from the floats to the tips of the egg, probably helps to support the egg by making use of surface tension directly, i. e. by lying on the surface film of water. The comparatively long dorsal processes do not help to support the egg normally, but if an egg is sunk it will be found that the relatively thick film of air enclosed by these always causes the egg to regain the surface with its dorsal surface uppermost.

If a drowning mosquito lays its eggs under the water it is

found that they all sink, so it is obvious that the buoyancy of the eggs is entirely due to the entrapped air.

The micropyle apparatus consists of a very thin disk-like membrane surrounded by a thick supporting ring. The central portion of the membrane is produced into a funnel, which passes through the inner wall to the interior of the egg, and the cavity of the funnel is the micropyle (fig. 41).

The supporting ring is somewhat irregular on the outer side, but the inner edge is very regularly scalloped, and the top portion of the ring in each scallop is produced towards the micropyle so that it overhangs the rest and together with the disk forms a shallow pocket (figs. 40 and 41).

Radiating out from the region of the micropyle to the point of junction of each 'scallop' is a very fine ridge. These are thickenings of the disk corresponding to the divisions of the cells which give rise to the apparatus. These ridges, together with the 'scallops', mark the apparatus off into well-defined areas. There are normally eight of these areas, but I have found examples of the apparatus with from seven to ten.

The funnel continues right through the inner wall and ends at the inner edge of the latter. It does not, however, communicate direct with the cytoplasm of the egg, but is sealed up by a small globular portion of the inner wall which for convenience I shall term the stopper (fig. 41).

A consideration of the structure of the micropyle apparatus and of the genital aperture leads me to the following theory as to the function of the former.

While the egg is passing through the gynaecophoric canal it is no doubt considerably compressed by the muscular walls of this canal. This would cause the thin membranous disk of the micropyle apparatus to be forced outwards, and it would probably lie level with the top of the supporting ring. By the time the micropyle apparatus has reached the region where the spermathecal duct opens into the gynaecophoric canal, the bulk of the egg has left the genital aperture, and thus the pressure on the egg is released. The membranous disk is now able to resume its original position, and in so doing would probably

draw sperms into the saucer-shaped cavity of the apparatus. Here the sperms would be directed by the radiating ridges to the micropyle, and would then pass down the funnel and between the stopper and the inner wall to the protoplasm of the egg.

When an egg is freshly laid on water it is nearly white, but after a few hours it becomes grey, and by morning it is usually, if not always, quite black. The whole of this change of coloration is due to the inner wall, which is transparent when laid but later becomes opaque black. In several cases insects in captivity have laid their eggs on dry media instead of on water, and in none of these cases did the eggs become black : they merely turned dirty yellow. It would thus appear that water has something to do with the production of the dark coloration, though how the water gets to the inner wall is not clear.

Besides changing colour the inner wall changes in character after the deposition of the egg. If a freshly-laid egg is placed in strong acid or alkali rapid expansion of the inner wall takes place, and it is seen first to become rapidly wrinkled and finally to burst through the chorion with explosive force.

An egg which has become black, treated in the same way does not appear to be acted upon. Also if a freshly-laid egg is crushed under a cover-slip the inner wall is seen to be gelatinous, and oil-like globules may be broken off if a little pressure is applied. If treated with osmic acid these globules become brown, so that there may be a chemical, as well as a physical, resemblance between the inner wall and oil. If an egg which has become black is crushed it is found that the inner wall is no longer gelatinous, but is hard and somewhat brittle as it cracks with pressure.

The yolk-mass occupies the whole of the egg inside the inner wall. It consists of an alveolar protoplasmic mass in the vacuoles of which yolk granules of two kinds are found (fig. 11).

The more obvious form of yolk consists of comparatively large granules 0·003 mm. to 0·01 mm. in diameter, which are proteid in character, as the following reactions show.

If treated with copper sulphate solution followed by excess of

caustic potash, i. e. the ' Biuret Reaction ', the granules turn a beautiful violet colour.

Nitric acid turns the previously white yolk light yellow, and when excess of ammonium hydroxide is added the yolk turns a brilliant orange colour. (Xanthoproteic Reaction.)

With osmic acid the granules turn yellow or yellow brown.

These granules appear to be homogeneous and solid, as they can be broken by the pressure of a cover-slip.

The other type of yolk consists of small granules 0·001 mm. in diameter, and these are found surrounding the granules of coarse yolk (fig. 11). They are chemically quite different from the large granules as they do not respond to any of the above reactions. They are certainly not fat globules, as might be expected, as they are not coloured in any way by osmic acid, either alone or in the presence of chromic acid in the form of Flemming without acetic : a test described by Gatenby (7). I have not succeeded in making the fine yolk granules respond to any chemical reaction or stain in any way. In sections they appear as clear vacuoles, but they must be more than mere drops of watery fluid, as, if an egg is broken in water, they are scattered through the liquid as minute spheres which exhibit a very pronounced dancing movement. This movement comes to rest after a few hours, so that it is probably due to diffusion currents from the granules.

It may be noted here that the fine yolk is a definite constituent of the mature mosquito egg, and is not an intermediate substance produced during the formation of yolk, as in the case of the ' granules adipeux ' of Pholcus phalangioides according to Van Bambeke (1).

The protoplasmic portion is very inconspicuous in the mature mosquito egg. In sections it is seen as a network of fine threads, the meshes of which are occupied by the yolk granules (fig. 11). At the periphery the protoplasmic threads are slightly thicker than in the remainder of the egg, and they form an ill-defined cortical layer. This layer is thickest at the two extremities of the egg, in each of which it forms a small area of granular protoplasm free from yolk. Occasionally a few

disconnected fragments of the branching nucleus can be distinguished in the yolk-mass, even in eggs which have been laid.

The only other protoplasmic structure visible is a small mass of granular protoplasm situated about a quarter of the length of the egg from the anterior pole and in the centre of the yolk-mass. This appears to be the remains of the chromatin residue, but before the egg is laid no nuclear structure can be distinguished in it. A short time after oviposition, however, this mass is found to contain minute chromosomes.

Differentiation of Germ-cells and the First Period of Growth of the Egg-follicles.

The growth of the egg-follicle falls naturally into two periods. The first period is from the time when the follicle is separated off from the end chamber up to the formation of the ' resting stage '. Growth is arrested at this point and only recommences after the insect has had a good meal of blood, when the follicle enters upon the second period of growth, which culminates in the formation of the mature egg. In the second and later generations of eggs, however, the two periods run concurrently, i. e. while the primary follicles are undergoing the second period of growth the secondary follicles are undergoing the first, and when the former have formed the mature egg the latter have reached the ' resting stage '.

If an end chamber in an early stage of development be examined it will be found to consist of a central mass containing comparatively large nuclei surrounded by a layer in which smaller nuclei are found scattered somewhat irregularly. Cell divisions cannot be distinguished and the mass is probably a syncytium (fig. 24). The larger central nuclei are those of the oogonia and oocytes, and the smaller peripheral ones give rise to the follicular epithelium.

The nuclei of the oogonia vary considerably in appearance even in the same mass, but in the earlier stages of the end chamber I have been unable definitely to separate the true oocyte from the nurse-cells. Occasionally mitotic figures are found in the central mass (fig. 31). This is no doubt the stage

at which the oogonia divide to produce the oocytes, all but one of which become modified to form nurse-cells.

In many end chambers, where the foregoing process has no doubt already taken place, the proximal nucleus is quite distinct from the remainder. It is clearer and contains a well-defined spireme, while the other cells are somewhat darker, and, though they often also appear to contain a spireme, this is never so sharply defined and usually can only be made out with difficulty. This proximal nucleus is the true oocyte nucleus, while the cells lying above it are the nurse-cells, and at the distal end of the chamber the remaining oogonia are found.

The epithelial layer grows between the mass of nurse-cells and the oogonia, so that the former, together with the oocyte, are completely enclosed in a follicle. The fact that there are seven nurse-cells and one oocyte suggests that they are produced by three successive divisions of a single oogonium, seven of the daughter cells becoming nurse-cells and the eighth the true oocyte. Occasionally an aberrant number of cells are included inside the follicular epithelium: eight large cells, and at the distal end a number of smaller cells, apparently eight in number. In this case the epithelial layer has surrounded two masses of daughter cells instead of one. I have only found such follicles in young ovaries, so that the mass of smaller cells evidently does not take part in the development of the follicle and probably degenerates. This further supports the theory that the nurse-cells and oocyte are the daughter cells of a single oogonium.

When the follicles are first formed the follicular epithelium consists of a comparatively small number of cells. These multiply rapidly by mitotic division (fig. 32), and at this stage no clear cell divisions are visible. Also there is only a small quantity of cytoplasm, the epithelium consisting principally of a large number of closely-packed nuclei. This mitotic division takes place throughout the first period of growth till, when the resting stage is reached, the full number of epithelial cells is attained and also the nuclei have reached their full size. During the second period of growth the epithelium increases

very considerably in area, but I am convinced that this is entirely due to the increase in size of the individual cells. In no case have I seen any sign of mitotic division during this period. Further, counts were made of the epithelial cells in median longitudinal, and transverse sections of follicles in various stages of development, and the average number of cells visible in a section was found to be practically constant irrespective of the size of the follicle.

The ' funicle ' arises by the local proliferation of the epithelial cells of the septum between the end chamber and the young follicle. Shortly after the young follicle is cut off from the end chamber a number of nuclei are found closely packed one above the other in the form of a short rod. This rod-like structure is found in one side of the septum, and this asymmetrical position is retained throughout the growth of the follicle, as will be readily understood when it is considered that the micropyle apparatus is formed immediately under the ' funicle ', and this is not terminal but ventral in position.

The nuclei in this rod-like structure continue to divide, forming a long string. The rest of the septum splits and gives rise on the one hand to the follicular epithelium and on the other to the epithelial layer of the end chamber. The only portion of the septum which does not split is that which contains the rod-like series of nuclei, and this now forms the funicle.

When the egg-follicle is first formed the nurse-cell nuclei often contain a more or less indefinite spireme and there is very little cytoplasm. As the follicle grows the nuclear contents become arranged in much convoluted bands which appear to be directly derived from the spireme. These bands are somewhat peculiar in structure. They consist of a non-staining ribbon of linin, across which lie a large number of chromatin bands giving an appearance somewhat resembling that of a striped muscle (fig. 30). These convoluted bands lie round the periphery of the nucleus with the result that individual sections give a very wrong idea of the appearance of the nucleus. This is due to the fact that the nurse-cell nuclei are gigantic (0·02–0·03 mm.) in diameter, so that each nucleus is

cut into a number of sections and the idea of the continuity of the convoluted bands is lost.

Towards the centre of the nucleus a large irregular nucleolus is found imbedded in a mass of linin. This is joined to the convoluted bands by linin threads. The nuclear membrane is very thick and is always plainly visible.

When the follicle has reached the resting stage each nurse-cell nucleus is found to be surrounded by a large mass of cytoplasm which is limited by a definite cell-membrane. The cytoplasm is slightly granular and takes up rather more stain than that of the younger cells.

The earliest stages of the oocyte nucleus which I have been able to distinguish contain a number of deeply-staining chromatin loops all of which arise together from one side of the nucleus (fig. 12). This is evidently the ' bouquet stage ' of the prophases. Usually a small nucleolus is also seen, and in the few cases I have observed in which this is not visible it is possible that it was hidden by the chromatin threads. At this early stage the nucleolus is a spherical vesicle and only stains very lightly.

As the nucleus grows the nucleolus becomes relatively larger, takes up stain rather more readily, and soon several vacuoles become visible in it. While this is taking place the chromatin threads wind themselves round the nucleolus and invest it tightly (fig. 13). The nucleolus continues to grow at a more rapid rate than the rest of the nucleus, while the chromatin strands do not appear to grow at all. The result is that the enlarging nucleolus gradually pushes the investing chromatin strands off itself, and these are then seen as a small mass of closely-woven threads at one side of the nucleolus (fig. 16). These threads concentrate into a closely-packed mass in which the individual chromatin threads can no longer be distinguished, and the whole has the appearance of a small dark nucleolus at the side of the true nucleolus. For want of a better term I shall refer to this mass as the ' chromatin residue '. This is embedded in a mass of linin which also invests the nucleolus and thus holds the two closely together (fig. 17). This arrangement

persists until after the oocyte has left the resting stage. Frequently, however, the chromatin residue is very difficult to find in oocytes in the resting stage. Close examination reveals the fact that it is not only very closely applied to the surface of the nucleolus, but is situated in a shallow depression in the latter and thus does not disturb the spherical contour. Nuclei in which this arrangement exists appear on a cursory examination to contain one large nucleolus and nothing else but nuclear sap. This is the characteristic appearance of the oocyte nucleus in the resting stage.

We have noted that the nucleolus, which is at first vesicular and has little affinity for stain, soon becomes vacuolated and stains more deeply. As it increases in size the vacuoles increase rapidly in number and the affinity for stain becomes more and more marked, till, when the resting stage is reached, the whole surface is covered with vacuoles and the nucleolus stains as deeply as chromatin. Though the nucleolus has so great an affinity for chromatin stains I do not consider that it is formed of chromatin. I regard it rather as a composite structure, consisting of a plasmosome in which chromatin, or some similar basiphil substance, is present. This is indicated by the fact that when stained with eosin the nucleolus is stained bright red, whilst the rest of the ovary is hardly perceptibly tinted by it, but when stained with haematoxylin the red colour is completely masked. These staining properties are confined to a cortical layer, in which the above-mentioned vacuoles lie. This layer surrounds a large central cavity the contents of which appears to be nuclear sap. This structure of the nucleolus is easily seen as it is so large, about 0·015 mm. in diameter, that it may be cut into three or four sections with ease. In such sections the cortical layer appears as a deeply-staining ring surrounding a cavity which contains non-staining material, in which irregular strands of another substance, possibly linin, can be seen (fig. 17).

Though the nucleus normally contains only one large nucleolus this sometimes appears to undergo fragmentation. In the more usual cases of this, one or more small nucleoli

may be seen in the nuclear sap surrounding the large nucleolus, or even inside the central cavity of the latter. The smallest examples of these appear homogeneous, but the larger fragments show a similar vacuolation to that of the large nucleolus, and are evidently produced by the fragmentation of the cortical layer. In one ovary examined all the oocyte nuclei contained numbers of small nucleoli. In some cases, however, the large nucleolus was indicated by a sort of phantom, as if it had given up practically all its substance, but the very small quantity of material remaining still traced its original form. In the cases where the original nucleolus had completely disappeared it is obvious that the fluid which was in its central cavity must have mixed with the surrounding nuclear sap, but there was no indication of two different fluids inside the nucleus. It therefore seems probable that the nucleolus is merely a hollow sphere, with nuclear sap both inside and surrounding it.

The nucleus is surrounded by a thick nuclear membrane which is stained black by iron haematoxylin and frequently shows numerous local thickenings.

When the oocyte has reached the resting stage it has a thick layer of cytoplasm round the nucleus. This usually stains the same as the cytoplasm of the nurse-cells, but sometimes it appears slightly more granular.

Second Period of Growth of the Egg-follicles.

The second period of growth extends from the time when the egg-follicle leaves the resting stage up to the formation of the mature egg. During this period several different processes take place and it will be convenient to consider these separately. These processes may be divided primarily into the nutrition of the egg and the formation of the egg-walls. The complexity of the changes of the oocyte nucleus renders it advisable first to treat with these thoroughly and then to deal with yolk formation and the nutrition of the oocyte in general. The subject of the formation of the egg-walls will be divided into the production of the inner wall, of the outer wall, and of the micropyle apparatus.

I. Branching of the Oocyte Nucleus and Segregation of Vegetative and Germinal Parts.

During the second period of growth of the egg-follicle of A. maculipennis the oocyte nucleus undergoes a most remarkable development. I have not been able to find a detailed description of a similar development in the case of any other insect, but, as will be seen later, it is probable that this particular form of development of the oocyte nucleus is by no means confined to A. maculipennis.

After the mosquito has fed on blood the first indication of alteration in form of the oocyte nucleus is observed in the nuclear membrane. Previously this was spherical in form, but now it is seen to be somewhat irregular in outline. This irregularity becomes more and more marked as development proceeds, till the nuclear membrane is seen to send out a few blunt processes into the cytoplasm and the cavity enclosed by the membrane appears somewhat larger than it was previously.

While this has been taking place the nucleolus has also been altering somewhat in shape. It loses its spherical form, first becoming ovoid and later slightly flattened in a plane at right angles to the axis of the egg-follicle, at which stage it begins to send out blunt processes (fig. 18). The structure, however, is still the same as in the resting stage, that is, it is vacuolated and contains a large non-staining central mass.

The chromatin residue commences to separate from the nucleolus at this stage. Its subsequent history will be dealt with separately. From this point the more obvious nuclear changes are confined to the nucleolus and the nuclear membrane, the nucleolus and its products forming by far the greater part of the bulk of the nucleus.

It has been noticed that both the nucleolus and the nuclear membrane have begun to send out blunt processes. These processes rapidly elongate and take on the form of branches, which in their turn send out secondary branches. The branching of the nucleolus and the nuclear membrane is intimately connected, as the nuclear membrane surrounds the nucleolar

branches. The progressive stages of the branching are seen in figs. 1–6, which are reconstructions made from serial sections. In individual sections the branching nature of the nucleus cannot be seen, as only sections of the branches are found and these appear to be fragments of the nucleus, as described by S. R. Christophers (2).

Besides altering in form the nucleolus undergoes an alteration in structure. Shortly after the branching has commenced the vacuoles of the outer crust become indistinct, and the central mass, which up to this point has not taken up stain, now becomes darker and the whole becomes very granular (fig. 19). Later the whole of the products of the nucleolus form a homogeneous granular mass which readily takes up nuclear stains. The branches of the nucleus are entirely formed of this mass and, in the earlier stages at least, are surrounded by the nuclear membrane.

As the branching proceeds the branches become finer and finer, and pass throughout the whole of the rapidly enlarging oocyte. They have, however, a very definite arrangement. It will be seen in figs. 22 and 23 that the main branches occupy approximately a median position between the centre of the egg and the periphery, forming a cup-like structure roughly following the contours of the egg. It must be pointed out, however, that in these two sections the nuclear branches appear much more continuous than is normal, though indications of this arrangement can be seen in all sections of this and later stages. The thickenings of the ring-shaped nuclear mass in fig. 22 are the main branches cut transversely, and the thin portions joining them are smaller lateral branches; these appear to connect the larger branches together, so that probably the nucleus forms a reticular structure from which thin short branches pass towards the centre of the oocyte, while others go towards the periphery. The reconstructions do not show this reticular structure of the nucleus, but this may be accounted for by the fact that only the very finest branches appear to join the main branches together, and I found it impossible to reconstruct the course of such fine branches with

accuracy. They have, therefore, been omitted from the figures of the reconstructions. Thus fig. 6 only shows a large number of more or less longitudinally placed branches, but I consider that these were joined together by a number of much finer branches.

As the branching proceeds the nuclear membrane becomes less conspicuous, but it is easy to see in the earlier stages of nuclear branching. Later it becomes closely surrounded by yolk and evidently lies closely applied to this. The appearance of a membrane in this position can usually be observed, but this by no means proves that a membrane is present. I find that if a crack appears in the yolk-mass, the edges of the crack often appear to be limited by a membrane, and this I believe to be due to the refraction of the transmitted light by the spherical yolk granules. In such cases the apparent membrane always closely follows the contour of the closely-packed yolk granules. In cases of the branching nucleus, therefore, in which the appearance of a membrane can be observed in a position separated from the yolk-mass, I consider that this is actually the nuclear membrane, while, on the other hand, if there appears to be a membrane closely following the limits of the yolk-mass, it cannot be definitely stated that a membrane is, or is not, present. Bearing these considerations in mind, I find that portions at least of the nuclear membrane cover the branches up to a late stage, as when the nurse-cells are breaking down the nuclear membrane can still be seen in places. Whether it is continuous or not at this stage it is impossible to say, but I favour the view that it does not exist over some portions of the branches.

When the nurse-cells are breaking down large deeply-staining globular masses are found in the nuclear branches (fig. 21). These appear to be formed of substance derived from the degenerating nurse-cells. The globular masses are probably absorbed by the nuclear substance as they cannot be observed in later stages.

After the extrusion of the nurse-cell nuclei the main function of the branching nucleus, that of the nutrition of the oocyte,

appears to be completed. It does not immediately degenerate, however, but continues to branch, the branches becoming finer and finer till finally they merge imperceptibly into the cytoplasmic reticulum, when all trace of the nucleus is lost. Occasionally there are still some vestiges of the branches remaining when the egg is laid.

It will be seen later that all this complicated branching of the nucleus may be regarded as a mechanism for the transference of nutritive material to the egg. As has already been noted, this nutritive mechanism is mainly the product of the nucleolus, the nuclear sap and nuclear membrane participating but being only of secondary importance. The nucleolus may therefore be regarded as the vegetative portion of the nucleus. The chromatin residue does not take any part in the nutrition of the egg, but from it the female pronucleus and the polar bodies appear to be produced, so that it is the germinal portion of the nucleus. S. R. Christophers (2) refers to this chromatin residue as the 'female pronucleus', but as the polar bodies have not yet been separated from it this is obviously a misuse of the term.

When the chromatin residue first begins to leave the side of the nucleolus, it is found to be no longer a deeply-staining mass of chromatin, as only portions of it take up stain readily. Its appearance at this stage varies considerably, but it is usually formed of a non-staining matrix in which a deeply-staining round spot is found, and commonly several other parts take up stain often appearing to be portions of the coiled threads of which it was originally composed (fig. 19). The whole of this is embedded in a mass of lining from which radiating strands pass to various parts of the nucleus.

During the growth of the oocyte the chromatin residue travels progressively farther away from the nucleolus, and as it does so its staining properties decrease. The round spot mentioned above is the last portion to lose its power of taking up chromatin stains, but finally the chromatin residue can only be recognized as a small lightly-staining mass situated a little below the nurse-cells. This is the last stage I have been able

to discover, and it occurs when the egg-follicle is about a third of its full size. It now becomes lost in the yolk-mass from which stains will no longer differentiate it. It does not follow, however, that because it is no longer visible it has therefore ceased to exist as a separate entity.

A little later, after the nurse-cells have been extruded, a small mass of protoplasm which is free from yolk is found situated a little behind the anterior extremity of the egg, that is, approximately in the position in which the chromatin residue disappeared. The central portion of this mass is rather denser than the remainder, and this I regard as the derivative of the chromatin residue. Some eggs were sectionized which had been fixed about an hour after laying. In these a number of minute chromosomes were found situated in the denser central portion of the above-mentioned mass. It would therefore appear that the reconstruction of the chromosomes takes place shortly after the fertilization of the egg, a process which frequently occurs in insect eggs.

As the chromatin residue was derived from the chromatin of the spireme, and as after fertilization the reconstruction of the chromosomes takes place in the anteriorly-placed mass of protoplasm which is free from yolk, it seems a reasonable assumption that this mass contains the derivative of the chromatin residue.

II. Yolk Formation and the Nutrition of the Oocyte.

Shortly after the egg-follicle enters the second period of growth the oocyte commences to enlarge and soon occupies about half of the egg-follicle, the nurse-cells occupying the other half. At this stage yolk begins to make its appearance. First the cytoplasm is seen to contain a number of small globules which do not stain. These enlarge and are then recognized as fine yolk. Immediately after this granules of coarse yolk make their appearance, usually forming a zone midway between the nucleus and the periphery of the oocyte. These granules are very small, but they increase in size as the oocyte

grows, and more small granules or ' young yolk ' appear in the cytoplasm till this is completely filled with yolk. As the oocyte grows, therefore, it is natural that the young yolk should appear at the point where the cytoplasm is increasing most rapidly, that is round the periphery and more particularly at the proximal end of the oocyte. This actually is the case, as will be seen from fig. 27, in which the small granules of young yolk can be plainly seen around the periphery of the oocyte and a much larger mass is visible at its proximal end. Young yolk may also be observed amongst the larger and older granules in the central mass of the oocyte, and no doubt growth is by no means confined to the peripheral portion of the cytoplasm.

At the same time that this coarse yolk is appearing fine yolk is also being laid down in the cytoplasm, the production of the two substances thus taking place simultaneously.

As the oocyte is growing rapidly and large quantities of yolk are being laid down, the question arises as to how the nutrition of the oocyte takes place. The fact that most of the young yolk is laid down in a peripheral position might lead one to suppose that nutritive material passed by diffusion through the follicular epithelium. This probably does take place to some extent, but only in the earliest stages, as later the follicular epithelium begins to secrete the inner wall and then no doubt requires all the nutritive material which passes into it.

The greater part of the nutritive material undoubtedly reaches the egg through the medium of the nurse-cells, and these in their turn must receive it from the ' rosette cells ' as these are the only portion of the epithelium which is not secreting the inner wall. The inpushing of the rosette-cells and their close application to the nurse-cells (figs. 36 and 37) may assist in the transference of the nutritive material.

That the nurse-cells are in a state of activity during this period is indicated by the fact that the cytoplasm stains irregularly, more deeply on one side than the other (fig. 27), an appearance which seems to be characteristic of cells which are secreting.

Between the inner side of the nurse-cells and the branching nucleus a mass of cytoplasm is found which stains more deeply than the remaining cytoplasm of the oocyte. This forms a connexion between the nurse-cells and the oocyte nucleus, and I regard it as the path of the nutritive material from the former to the latter (fig. 27).

The branching nature of the nucleus, and the general arrangement of the main branches in a medium position between the centre of the oocyte and its periphery, form an ideal distribution system for carrying nutritive material to all parts of the oocyte.

The path of the nutritive material would therefore appear to be from the surrounding fluid to the rosette-cells, through these into the nurse-cells, which in their turn pass it to the branching nucleus through the medium of the above-mentioned more deeply-staining mass of cytoplasm. The branches carry the fluid to all parts of the oocyte, and the cytoplasm of this uses it in the formation of yolk granules.

When the oocyte is approaching full size the cytoplasm of the nurse-cells begins to disappear (fig. 37) till finally the nuclei are only surrounded by the cell membrane. Simultaneously large globular masses of deeply-staining material appear in the branches of the oocyte nucleus (fig. 21) and obviously have some connexion with the degenerating nurse-cells. These globular bodies are by no means confined to the region of the nucleus near the nurse-cells, but are found in all parts of the main branches, and it is therefore only reasonable to suppose that they have travelled along the branches. This gives considerable support to the view that the branching nucleus is a mechanism for the transference of nutritive material.

It should be noted that the nurse-cells degenerate when the period of nutrition is practically completed, and that in so doing part of their substance is used directly for the nutrition of the oocyte. This is further proof of the nutritive character of the nurse-cells.

As there is no longer any nutritive material for the branching nucleus to carry, it is obvious that if this is its only function

it should now degenerate. This it does, as we have seen, by continuing to branch till the final branches merge into the reticulum of the cytoplasm, when nutrition is completed.

III. Discussion concerning the Oocyte Nucleus and Nutrition of the Oocyte in A. Maculipennis.

I have unfortunately not found it possible to examine the whole of the literature dealing with the nutrition of insect eggs, but, in the literature I have consulted, I have not discovered a case in which the mechanism of nutrition is to my mind as clearly demonstrated as it is in the developing egg-follicle of A. maculipennis. I do not believe, however, that the insect under consideration is unique in having this particular mechanism of nutrition. From several series of sections which I cut of the closely allied insect Theobaldia annulata, I am convinced that the same mechanism is present here. Also Soyer (**28**) makes a short reference to the nucleus of the developing oocyte of a Staphylinid, and from his description it would appear closely to resemble that of A. maculipennis. He remarks, 'Le noyau, très irrégulier déjà à ses phases les plus jeunes, se ramifie et se déchire en une multitude de franges dans toute l'étendue du vitellus. Cette ramification finit par être poussée si loin qu'on n'a plus sous les yeux qu'une sorte de long filament avec quelques branches latérales, à peine visibles, dont les extrémités se ramifient et se perdent entre les vésicules lécithiques qui emplissent à ce moment la masse ovulaire'. According to Korschelt (**13**) Stuhlmann has observed the branching of the nucleus throughout almost the entire oocyte of Necrophorus vespillo and of Silpha sp.

Branched nuclei are by no means uncommon, particularly in insects. They are commonly found in nurse-cells, gland-cells, fat body-cells, and the cells of Malpighian tubules, in all of which cases they appear to have some relation to the secretory activities of the cells. Thus in many gland-cells the nucleus is only branched during the period of secretion. In only two

cases have I found references to branched nuclei in cells which are not obviously secretory in function, but both of these concern embryonal structures which are undergoing rapid growth and are therefore in a state of great activity. Korschelt (**13**) cites cases of branched segmentation nuclei, and Seeliger (**25**) describes the branching of the nuclei in the muscle-bands of young Oikopleura. In the latter case the branching reaches an extraordinary high state of development, becoming finally a complicated reticular network of very fine threads. Korschelt (**13**) regards the formation of nuclear branches as a method of increasing the surface of the nuclei to aid secretion. Thus, speaking of egg-cells, he remarks, 'Die Bildung der Fortsätze stellt eine Oberflächenvergrösserung des Kernes dar, vermöge welcher dessen Berührungsfläche mit der Zellsubstanz erheblich vergrössert wird. In ähnlicher Weise wurde die Bildung von längeren oder kürzeren Fortsätzen des Kernes bei secernirenden Zellen verschiedener Art beobachtet. Hier waren die Fortsätze nach demjenigen Theil der Zelle gerichtet, wo die Secretion stattfand.'

It will thus be seen that the form and position of the nucleus of the oocyte in A. maculipennis indicates that it is secretory in function and comparable to the nuclei of secreting cells. This similarity is further shown by the fact that during the process of branching the nuclear contents break down and form a granular mass, a process which normally takes place in secreting cells during the period of activity. The close relation of one end of the branching nucleus to the nurse-cells and the other to the area of maximum activity of the growing oocyte, i.e. the posterior end, together with the relatively deeply-staining mass of cytoplasm between the nucleus and the nurse-cells, renders it difficult to imagine that the branching nucleus can be other than secretory in function. It is from the somewhat similar arrangement in other cells that Korschelt (**13**) draws the conclusion that the nucleus takes an active part in the nutrition of a cell. Thus he observes, 'Das Aussenden von Fortsätzen und Annäherung des Kernes an diejenige Seite der

Zelle, von welcher derselben Nährsubstanz zugeführt wird, die Umlagerung des Kernes mit einer von fern her angezogenen Nährmasse,—diese Vorgänge konnten einzig und allein als eine Einflussnahme des Kernes auf die ernährende Thätigkeit der Zelle gedeutet werden.' Also Doncaster (**5**), in his recent work, makes the following assertion : ' The nucleus—in some way controls the metabolic activities of the cell, and its peculiar behaviour in the growing oocyte can only be ascribed to its activities in this connexion.'

Chubb (**4**), on the other hand, denies that the oocyte nucleus takes an active part in yolk formation. Thus he says, ' The actual formation of the yolk spherules must therefore be regarded as an automatic process, which commences as soon as the accumulated materials in the cytoplasm attain the requisite degree of concentration, and which does not entail either increased nutrition of the ovum or increased activity of the nucleus '. The amoeboid movements of the germinal vesicle described by various authors, e.g. Bambeke (**1**), which are considered as an indication of nuclear activity, Chubb regards as probably being artefacts due to fixation. He observed oocyte nuclei in Antedon which were apparently amoeboid, but he shows that these are purely artefacts as ' In the first place the nuclear irregularity shows no spatial relation whatever, either to the other cell structures, to commencing yolk formation or to the position of the nucleus in the cell. In the second place it is only in radial section that the nuclear irregularity presents the appearance of Pseudopodia; in tangential sections these nuclear " processes " are found to invariably resolve themselves into a coarse wrinkling of the nuclear membrane. Finally, the artificial nature of the nuclear irregularity is strongly indicated by the variable behaviour of the nucleus with varying fixation.'

It is very probable that this explanation does apply to many cases where amoeboid structure has been described, but it certainly does not apply to the oocyte nucleus of A. maculipennis. The high degree of branching of the nucleus in this case could not possibly be regarded as an artefact due to

fixation, and in addition the branching has a definite spatial relation to other cell structures, yolk formation, and the position of the nucleus in the cell. The branched appearance of the nucleus is not confined to any type of section and it is a perfectly constant character in no way dependent on the fixative. Also it is not possible to regard the branches as the result of the pressure of the yolk-laden cytoplasm, so that the only possible explanation is that the oocyte nucleus of A. maculipennis is in a state of great activity during the period of yolk formation.

It has been shown that the oocyte nucleus only commences to branch when the yolk begins to appear, and that when all the yolk has been produced and the nutrition of the oocyte is complete, the branching nucleus breaks down and its substance is absorbed directly by the cytoplasmic portion of the yolk-mass. Immediately before the final disappearance of the branching nucleus this structure rapidly loses its power of taking up stain. This is a further indication of the close similarity existing between the oocyte nucleus of A. maculipennis and the nuclei of secretory cells. Thus Bambeke (**1**), speaking of glandular cells, points out that after the secretion has lasted for a certain time the power of the nucleus to take up nuclear stains diminishes.

At this point it will be convenient to examine some of the various mechanisms which have for their object the nutrition of the rapidly-growing oocyte. In each case it will be found that the main object of the mechanism is to increase the surface in contact with the cytoplasm of the oocyte, in order to facilitate the passage of nutritive material into the latter.

The activities of the oocyte nucleus in Colymbetes fuscus as described by Will (**34**) are in many ways not unlike those of the insect under consideration. When the oocyte enters on its period of rapid growth the nuclear membrane becomes irregular and finally many small branches pass into the cytoplasm. Later these become separated from the rest of the nucleus, and are used directly as nutritive material by the cytoplasm. A fresh nuclear membrane develops behind

the separated branch, and the nucleus then produces more branches which in their turn become separated, so that 'der protoplasmatische Leib der Eizelle auf Kosten des Eikernes wächst'. This, however, is not a case of the degeneration of the nucleus, as it continues to increase in size while it is giving up these portions of its substance. Therefore this mechanism of nutrition is practically the same as in A. maculipennis, except that in this case the nucleus continually passes portions of its substance into the growing oocyte as nutritive material instead of merely conducting nutritive fluid to the oocyte.

In Calliphora erythrocephala Lowne (16) describes another manner by which the oocyte receives portions of the nucleus as nutritive material. He says that 'When the egg is enlarged to about two-thirds of its maximum size the granules in the largest nucleus appear to stream out, the nucleus itself shrivels and is ultimately lost, whilst the whole protoplasm of the cell assumes a granular yolk-like appearance, in which the nuclear granules can no longer be distinguished'. The 'largest nucleus' is evidently the oocyte nucleus, the remainder being those of the nurse-cells. A similar passage of granules from the nucleus has been observed in the oocytes of many insects.

A modification of this process of nutrition has been observed by Gatenby (8) in the oocyte of Apanteles. In this, minute solid chromatoid granules first appear, and later a nuclear membrane appears round each of these. These grow and a lirin network appears, and the larger nuclei so formed resemble the true oocyte nucleus to the smallest details. These secondary nuclei disappear when nutrition is complete.

In Rhizotrogus solstitialis Rabes (24) describes a very different mode of nutrition. In this the nutrition of the oocyte is not confined to the nucleus and nurse-cells, the follicular epithelium playing an important rôle. As the oocyte grows the epithelium forms folds which penetrate into the yolk-mass, often as far as the middle of the oocyte, an excellent example of the tendency to increase the surface of contact between the oocyte and secretory structure.

Finally, we may consider the cases in which 'yolk nuclei' form part of the nutritive mechanism. It is evident that this collective term includes several distinct types of structures, and I will only deal with one of these, the Corpuscles of Balbiani. The origin of this body is obscure in most types which have been examined, but Chubb (**4**) shows very clearly that in the oocyte of Antedon this body arises in the nucleolus as a series of deeply-basophile spherules which are passed into the cytoplasm. These form a mass just outside the nucleus, and eventually they fuse to form the yolk nucleus. McGill (**19**) describes a similar aggregation of granules close to the nucleus in the oocyte of the dragon fly, and this gives rise to the yolk nucleus. Though she has been unable to demonstrate the origin of the granular mass she shows that it is very probably nuclear in origin, and in support of this theory remarks that 'Hennegay (1893) believes that the Corpuscles of Balbiani in Vertebrates are either parts of the nucleolus or the entire nucleolus which passes through the nuclear wall into the cytoplasm'.

Similarly Bambeke (**1**) observes that the 'corps vitellin' of Pholcus phalangioides arises close to the germinal vesicle, and he considers that it is nuclear in origin. He shows that this grows into a large and somewhat branched structure which takes an active part in the nutrition of the oocyte. This structure bears a considerable superficial resemblance to the branched nucleus of A. maculipennis, and a careful consideration of Bambeke's very excellent paper has led me to the conclusion that the resemblance is not merely superficial but that the two structures are both morphologically and physiologically comparable. It should be noted here, however, that Chubb (**4**) considers that the yolk nucleus of Antedon has no connexion with yolk formation though it is almost identical in every respect with the yolk nucleus of Pholcus. He gives a perfectly simple physical explanation for the changes undergone by this structure, which he regards as waste material forming a purely passive body.

We have seen that the branched nucleus of A. maculi-

pennis is almost entirely the product of the nucleolus. Now Bambeke considers that the yolk nucleus of Pholcus is nuclear in origin, and other authors are of the same opinion with regard to other animals. Thus Korschelt (13) observes, ' Wenn man sieht, welche complicierte Gestaltung dem aus concentrischen Schichten gebildeten Dotterkern mancher Spinnen zukommt, möchte man ihn für einen bedeutungsvollen Bestandtheil des Kernes halten und ihn gewiss nicht mit dem soeben besprochenen " Dotterkern " der Amphibien zusammenwerfen.'

In further support of the theory of the nuclear origin of the Corpuscles of Balbiani, Bambeke remarks : ' Dès que la forme de bâtonnet a fait place à celle de bourrelet ou de cupule, la constitution du corps vitellin se montre très semblable, voire même identique, à celle de la tache germinative. . . . Cette frappante analogie entre la constitution de ces éléments ne fournit-elle pas un argument de plus en faveur de l'origine nucléaire du corps vitellin ? '

Having shown that the body with which he is dealing is probably nuclear in origin and is comparable to the nucleolus, Bambeke proceeds to give his reasons for believing that the body is a true ' corps vitellin de Balbiani '. These may be summarized as follows :

1. Situation near germinal vesicle.
2. Affinity for colours similar to that of the nucleolus.
3. Presence of vacuoles.
4. Constancy of the character.
5. Appearance at commencement of growth.
6. Final degeneration.

All these characters are also true of the branching nucleus in A. maculipennis except that in nos. 1 and 2 similarity of position and character is replaced by identity. The presence of vacuoles is only found in the earliest stages of the nucleus, but this is not actually an important difference from the yolk nucleus of Pholcus, as in the latter the vacuoles disappear before it degenerates, so that actually this is a further indication of the similarity existing between the two structures. Is it

not reasonable, therefore, to consider the yolk nucleus of Pholcus and the branching nucleus of A. maculipennis as being homologous structures which only differ in that the one passes to the outside of the nuclear membrane while the other remains inside ?

I have already shown that the branching nucleus of the oocyte in A. maculipennis can only be regarded as a structure the function of which is to carry nutritive material to the various parts of the developing oocyte. After an exhaustive consideration of the various theories as to the function of the yolk nucleus Bambeke comes to the conclusion that the only one which can be adopted in the case of Pholcus ' est celle qui considère ce corps comme centre de formation des éléments nutritifs du vitellus '.

For these reasons I have come to the conclusion already stated that the branching nucleus of Anopheles maculipennis and the yolk nucleus of Pholcus phalangioides are morphologically and physiologically comparable. These structures are homologous with other types of oocyte nuclei and Corpuscles of Balbiani respectively. It would therefore appear that the Corpuscles of Balbiani may be considered as portions of the oocyte nucleolus which have escaped through the nuclear membrane in order to carry on the nutritive portion of the nuclear functions.

In Pholcus the division of the nucleus into two portions, one nutritive or vegetative and the other germinal, is only partial, as the germinal vesicle itself appears amoeboid and evidently takes part in the nutrition of the oocyte.

In A. maculipennis it has been shown that from an early stage the nuclear contents are sharply divided into a vegetative and a germinal portion, the nucleolus and chromatin residue respectively. During the resting stage there may be an apparent fusion of the two, but actually they are only closely applied together, the chromatin residue lying in an identation of the nucleolus. A close parallel is found in the ovary of the dragon fly according to McGill (**19**). In this case the thick spireme of the young oocyte surrounds the

nucleolus, giving rise to a ' double nucleolus '. Later one side of the nucleolus is formed of chromatin and the other is the plasmosome.

Gatenby (**8**) shows that in Apanteles glomeratus the division of the oocyte nucleus into germinal and vegetative parts takes place in a very different manner. Secondary nuclei are produced, apparently arising from material which has escaped from the true oocyte nucleus, and these are found round the periphery of the oocyte. Then ' some time before the ovarian oocyte has become ripe the secondary nuclei disappear by a process of degeneration or chromatolysis '. The secondary nuclei are considered to influence the production of yolk. Discussing this subject Gatenby remarks: ' The egg nucleus of many insects, of which Apanteles is an example, becomes partly decentralized; this is to say, the nucleus, instead of influencing various processes of oogenesis from afar, sends pieces of itself into the furthermost regions of the egg, which carry on part of the vegetative functions at least of the chromatin of the ordinary nucleus.' This statement applies equally well to the oocyte nucleus of A. maculipennis, though the pieces sent ' into the furthermost regions of the egg ' remain attached to the rest of the nucleus.

It has already been shown that, though there is good reason to believe that the ' chromatin residue ' gives rise to the segmentation nucleus, there is a period in which no chromatin matter can be distinguished, and the oocyte of the mosquito then appears to be without a nucleus. A similar phenomenon has been encountered in the oocytes of other insects by many observers. Will (**34**) states that the oocyte nucleus of Dytiscus becomes a mass of fine granules from a small portion of which the ' definitive Kern ' is later produced. Lowne (**16**), speaking of Calliphora erythrocephala, remarks, ' In the ripe unimpregnated ovum I have entirely failed to find any nuclei or cellular elements of any kind, and I feel sure that if any such elements were present they would readily be distinguished in my sections '. Lubosch (**17**) states that this disappearance of the staining portions of the oocyte

nucleus for a certain period is the rule rather than the exception in animal eggs, and Doncaster (**5**) makes the following observation on the subject : ' Very commonly the chromosomes . . . disappear, and the chromatin becomes scattered through the nucleus in the form of fine particles, or for a time it may vanish altogether, at least in the sense that it ceases to take up stain.'

The production of the segmentation nucleus at about the period when the egg is laid is the normal occurrence in insect eggs, and it is quickly followed by the polar divisions. Doncaster (**5**) observes that ' in some animals the act of laying seems to be the stimulus and in others the polar division only occurs when a spermatozoon enters the egg ' ; but as in A. maculipennis oviposition and fertilization are simultaneous, it cannot be stated which acts as the stimulus.

In conclusion, the more important points with regard to the oocyte nucleus of A. maculipennis may be summarized as follows :

1. From the earliest stages separate vegetative and germinal portions can be distinguished in the oocyte nucleus.

2. During the second period of growth the nucleus branches throughout the entire oocyte.

3. The branching nucleus, in conjunction with the nurse-cells, takes an active part in the nutrition of the oocyte.

4. The branching nucleus is almost entirely the product of the nucleolus.

5. The branching nucleus is morphologically and physiologically comparable to the Corpuscles of Balbiani of other animals.

6. The germinal portion of the nucleus, the ' chromatin residue ', is the product of the condensation of the spireme threads.

7. The ' chromatin residue ' becomes invisible for a short period and reappears after oviposition as the segmentation nucleus.

IV. Development of the Outer Wall.

The first portion of the outer wall to appear is that which forms the floats. This is secreted between two layers of epithelial cells which come to lie one above the other by a very specialized form of folding of the epithelium.

During the earlier stages of the growth of the follicle the epithelium is of a typically cubical form, but later the cell divisions in two lateral areas become oblique, the obliquity being more marked towards the centre of each area. This process continues with further growth of the follicle (fig. 34) till one much elongated cell lies over the top of several (fig. 35). The underlying cells, however, do not lose their connexion with the tunica propria, but remain attached to it immediately in front of the end of the overlying cell. Finally, it is found that in the two lateral areas there are groups of very much elongated cells which lie almost parallel to the tunica propria. The float is secreted between the outermost of these and the one lying immediately under it (fig. 35). Each corrugation of the float is produced by the secretion of the chorion over the outer surface of one of the much elongated underlying cells.

It will be seen that this overlapping arrangement of the follicle cells is practically a fold of the epithelium. It is not an ordinary epithelial fold, however, as the basement membrane, i. e. the tunica propria, is not disturbed and does not take any part in the folding.

The remainder of the wall makes its appearance shortly after the commencement of the formation of the floats. It is first seen as a simple and very thin membrane lying immediately under the follicular epithelium. Soon local thickenings are found on this membrane (fig. 35). These are the commencement of the processes. The thickenings become larger and grow into the cytoplasm of the epithelial cells. Numbers of such thickenings are formed under each epithelial cell, and the shape of the processes cannot therefore be determined by the form of the secreting cells in the manner which frequently occurs, e. g. in the corrugations of the floats.

The thin membrane of the outer wall does not appear to increase appreciably in thickness, but the processes grow far into the cytoplasm of the epithelial cells till they reach their final size and form. The bosses, in spite of their large size, arise in exactly the same manner as the rest of the processes.

The epithelial layer now undergoes degeneration and becomes separated from the processes till it forms a layer lying over the top of these. Degeneration proceeds till only irregular masses of flattened nuclei can be seen attached to the inner side of the tunica propria (fig. 41), which forms a thin sheath round the whole egg.

V. Development of the Micropyle Apparatus.

The first indication of a special structure being produced for the formation of the micropyle apparatus appears when the egg is about a third of its full size, at the period when the inner wall is beginning to form as a definite layer. At this stage the epithelial cells immediately surrounding the point where the funicle of the secondary follicle joins the primary ovarian follicle become somewhat larger than their neighbours and protrude slightly inwards towards the nurse-cells (fig. 36).

As the egg increases in size this inward protrusion becomes more marked, particularly in the case of the peripheral cells of the group. Finally, the latter are pushed completely inside the epithelial layer and lie between the nurse-cells and the epithelium (fig. 37).

If examined from a surface view these extruded cells are seen to radiate from a common centre, in the form of a rosette, and for that reason I propose to refer to them as rosette-cells (fig. 38).

At this period the cytoplasm of the nurse-cells is seen to be rapidly breaking down and disappearing, and also the contents of the nuclei are degenerating. The chromatin strands lose their definite structure and gradually become a shapeless mass and the nucleoli undergo fragmentation (fig. 37).

The cytoplasm of the rosette-cells becomes very closely

applied to the nurse-cells and gives the appearance of ingesting them.

The nurse-cells, which consist merely of degenerating nuclei invested by the cell membrane, now pass into the epithelium, in which they lie till they become completely degenerated. It will be seen from fig. 39 that they have every appearance of being ingested by an epithelial cell, i.e. a rosette-cell, though I have been unable to demonstrate that they are completely surrounded by the cytoplasm of the rosette-cells. This is not surprising as, owing to the large size of the nurse-cell and the comparatively small size of the rosette-cell, the layer of cytoplasm of the latter surrounding the former would of necessity be exceedingly thin, and would be very difficult to distinguish from the nurse-cell membrane or from the surrounding epithelial cells.

Whether the degenerating nurse-cells are completely ingested by the rosette-cells or not, it is certain that there is a very intimate relation between the two, and the latter invest a considerable portion at least of the former. The degenerating nurse-cell nuclei would appear to form a general food reserve which is used by the rosette-cells while forming the micropyle apparatus.

The large size of the nurse-cells causes the radial arrangement of the rosette-cells to appear distorted, though indications of this arrangement can always be made out.

The micropyle apparatus arises under the rosette-cells at the same time that the rest of the chorion appears. The whole, with the possible exception of the narrow portion of the funnel, is secreted by the rosette-cells, and there is no obvious mechanism to account for the secretion of the thick supporting ring by part of the surface and the thin disk by another.

The bases of the epithelial cells which are surrounded by the rosette-cells pass as fine threads down the funnel, and it is probably these that secrete the funnel, though the bases of the rosette-cells certainly reach the top of the funnel and may pass down it (fig. 39).

As the stopper appears to be a definite portion of the micro-

pyle apparatus it will be convenient to describe its origin here.

When the rosette-cells are arising from the epithelial cells and are just protruding slightly towards the nurse-cells, globules of matter are appearing between the epithelium and the oocyte over the whole follicle with the exception of this one point. These globules are the commencement of the inner wall.

If the protruding group of cells is examined carefully it will be found that there are globules opposite the central cells of the group (fig. 36). These are the beginning of the stopper and are exactly the same as those which are giving rise to the inner wall. The only point in the egg, therefore, where this secretion is not taking place is a ring corresponding with the rosette-cells (fig. 37).

As the egg grows this secretion continues till a well-formed inner wall and a definite mass of similar matter, the stopper, has appeared.

After the extrusion of the nurse-cells the inner wall narrows the hole through which they have passed, only leaving sufficient room for the passage of the funnel, and in so doing passes over the stopper, so that this now takes up a position immediately beneath the micropyle (fig. 39).

A very similar process of development is described by Gross (**9**) for the micropyle apparatus of *Xanthogramma citrofasciata*. In this a special group of epithelial cells is detached from the anterior pole of the egg, and this travels between the nurse-cells and finally comes to rest immediately under them. The follicle epithelium grows inward and separates the group of nurse-cells from the oocyte except in the region of the detached group of cells. By the time this is completed the nurse-cells have passed most of their cytoplasm into the egg-chamber, so that a mass consisting practically only of nurse-cell nuclei lies over the anterior end of the egg. The group of cells secretes a ' polsterförmiges Gebilde ', and the rest of the follicular epithelium secretes the exo- and endo-chorion. This ' polsterförmiges Gebilde ' comes to lie immediately under the micropyle apparatus, and is perforated by the micropyle.

It is interesting to note the different manner in which the specialized group of epithelial cells are produced, and the degenerating nurse-cells passed out of the egg chamber in this insect and in A. maculipennis.

The 'polsterförmiges Gebilde' of Xanthogramma and the 'stopper' of A. maculipennis are probably homologous, as they are produced in a similar manner by a specialized group of epithelial cells, and they are also similar in appearance and position. There is one noticeable difference, however: in Xanthogramma the structure is pierced by the micropyle, while in A. maculipennis it appears to be solid, the micropyle terminating immediately above it.

VI. Development of the Inner Wall.

When the egg-follicle has reached about a third of its ultimate size small globules of matter are found between the follicular epithelium and the oocyte. These are deeply stained by haematoxylin and can be readily distinguished from the yolk granules. The globules increase in number and size and finally fuse, forming a coat investing the entire oocyte, with the exception of a ring-shaped area under the rosette-cells.

It has already been shown that the inner wall is gelatinous in nature till some time after the egg has been laid, and when in this state rapidly swells in the presence of acids. It is therefore not surprising that this structure becomes very much distorted during fixation. In fig. 36 the inner wall is shown as a fibrillar structure, the fibrils stretching across the space between the oocyte and the follicular epithelium. This is a very common appearance of the inner wall in follicles of about this stage of development, and I regard the fibrils as being produced from globules which adhere to both the oocyte and follicular epithelium and become stretched into threads when these become separated. In eggs nearing maturity the inner wall appears to be a thick homogeneous layer lying under the follicular epithelium and in it large vacuoles are frequently seen, but the layer does not show any signs of fibrillar structure.

I consider that the substance of the inner wall is no longer in globules but has formed a continuous gelatinous layer. Obviously a fibrillar structure could not be produced from such a layer in the manner described above.

When the egg is freshly laid the inner wall is still a thick gelatinous structure, but after some hours it hardens and in sections is seen to form a thin dark-coloured membrane lying immediately under the outer wall.

As the inner wall appears between the oocyte and the follicular epithelium the question arises as to which of these secretes it. The cytoplasm of the oocyte is already occupied in the production of yolk and the follicular epithelium secretes the outer wall at a later period, so that whichever of these structures form the inner wall is also capable of producing an entirely different substance.

Over the greater part of the egg it is impossible to determine whether the inner wall is secreted by the follicular epithelium or the oocyte; but the stopper, which is merely an isolated portion of the inner wall, is formed between the follicular epithelium and the nurse-cells. The inner wall must therefore be secreted by the follicular epithelium, and after this has been produced the epithelium changes its form of activity and secretes the outer wall.

Degenerating Egg-follicles.

The degeneration of a certain number of egg-follicles seems to be a normal occurrence in the ovary of A. maculipennis. Commonly this degeneration takes place when the follicles are just entering on the second period of growth, but not infrequently at a much earlier stage the primary follicles are found to be represented by a small mass of degenerated cells surrounded by a loose and much-folded tunica propria. The significance of this degeneration is not clear. I have been unable to detect the presence of any bacteria or other organisms, and the fact that degenerating follicles are almost invariably to be found in small numbers in ovaries, but that all, or even

a large part, of the follicles of an ovary have never been found affected, suggests that the phenomenon should be considered as one of atrophy or auto-digestion rather than as a disease. When an ovary is developing the follicles are very crowded and are obviously under compression, and it is probable, therefore, that the removal of several of the follicles from the more crowded parts would benefit the remainder. This may account for the degenerating follicles, but there is nothing but the above consideration to support the theory.

Fig. 33 shows part of the degenerating epithelium of a follicle which has just commenced to produce yolk. It will be noticed that the appearance of degeneration is confined to the epithelium. This is normally the case, and it is only after the epithelium has almost broken down that the central mass of cells degenerates. Each epithelial cell produces one or more large globular masses inside the inwardly-directed portion of its cytoplasm, so that it closely resembles a goblet cell. The masses are very variable in appearance as they stain very irregularly. They are commonly very granular but are otherwise structureless. The thin protoplasmic investment of the globules soon breaks down, so that the globules form a mass which penetrates amongst the nurse-cells.

The mass of cells and globules appears gradually to enter into solution, as it decreases in size till nothing but a few degenerating nuclei and a very loose tunica propria remain to indicate the position of the original follicle (fig. 24).

Presence of Sporozoa and Bacteria in Egg-follicles.

As has already been observed by S. R. Christophers (2), the yolk of a mosquito egg is frequently entirely displaced by a mass of sporozoa. These appear as transparent spherical cysts 0·005 mm. in diameter, approximating in size to the coarse yolk granules, in which eight small bodies which take up stain are found (fig. 10). In sections this number is not constant, but there are never more, and the reduced number is probably due to the removal of part of the cyst. This is the

only stage of the organism which I have observed and, though a number of insects were found affected, the cysts were only observed in mature oocytes.

The nurse-cells of the ovary of one insect were found to be heavily infected with diplococci. The follicles were nearly fully developed, and I could observe no harmful effect of the bacteria. The infection appeared to be entirely confined to the nurse-cells.

Summary.

1. The period at which the ovaries of A. maculipennis commence to develop depends on the season and locality. Normally this is from about the middle of March to the beginning of April.

2. A meal of blood appears to be necessary for the production of eggs.

3. One meal of blood is sufficient to cause eggs to be produced. After the lapse of a day the large blood-mass in the stomach shows two zones: a posterior partially-digested portion and an anterior portion of apparently fresh blood. This appearance has sometimes been taken as evidence that more than one meal of blood has been consumed.

4. The eggs are fully developed six days after the insect has fed on blood.

5. In the case of two insects which were observed at the time of oviposition the eggs were laid immediately after dark.

6. The muscle-bands of the ovarian sheaths are striped; not unstriped as is usual in insects.

7. A large number of vacuolated cells are found in the ovary. The nature of these is not clear, but they appear to have some relation to the tracheal system.

8. The chorion of the egg is highly specialized to retain air round the egg, and the buoyancy of the egg is entirely due to the entrapped air.

9. The floats are produced by a very specialized form of folding of the follicular epithelium.

10. The micropyle apparatus is produced by specialized cells of the epithelium, the ' rosette-cells '.

11. Immediately below the micropyle is a specialized portion of the inner wall, the ' stopper '.

12. The inner and outer walls of the egg, though formed of entirely different substances, are both secreted by the follicular epithelium.

13. The inner wall is first gelatinous in nature and transparent ; but, after the egg is laid, becomes brittle and dark in colour, causing the egg to appear black. This change in character only takes place when the eggs are laid on water.

14. The mature egg contains two distinct kinds of yolk, one of large granules which are proteid in nature, and the other of small granules the nature of which I have been unable to determine.

15. There are two distinct periods of growth of the egg-follicles, the first culminating in the ' resting stage ' and the second only commencing after the mosquito has had a meal of blood.

16. Each egg-follicle consists of a follicular epithelium surrounding seven nurse-cells and an oocyte. These appear to be the product of a single oogonium.

17. The cells of the follicular epithelium multiply by mitotic division during the whole of the first period of growth. In the second period, though the follicular epithelium increases greatly in area, this is due purely to the increase in size of the individual cells.

18. From the earliest stages separate vegetative and germinal portions can be distinguished in the oocyte nucleus.

19. During the second period of growth the oocyte nucleus branches throughout the entire oocyte.

20. The branching nucleus, in conjunction with the nurse-cells, takes an active part in the nutrition of the oocyte.

21. The branching of the nucleus may be regarded as a mechanism for the purpose of increasing the surface.

22. I have observed a similar method of branching of the oocyte nucleus in Theobaldia annulata, and it

probably also exists in Necrophorus vespillo and Silpha sp.

23. The branching nucleus is almost entirely the product of the nucleolus.

24. The branching nucleus is morphologically and physiologically comparable to the Corpuscles of Balbiani of other animals.

25. The germinal portion of the nucleus, the 'chromatin residue', is the product of the condensation of the spireme threads.

26. The 'chromatin residue' becomes invisible for a short period and reappears after oviposition as the segmentation nucleus.

27. The chromatin of the active nurse-cells consists of minute bars situated on a much convoluted band of linin.

28. Degeneration of a certain number of egg-follicles is normal during the development of the ovary.

29. Sporozoa are frequently found in the eggs, often completely replacing the whole of the yolk.

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EXPLANATION OF PLATES.

Reference Letters.

b. = 'Bosses'. *c.c.* = Central cavity of nucleolus. *c.l.* = Cortical layer of nucleolus. *c.r.* = 'Chromatin residue'. *c.y.* = Coarse yolk granules. *D.* = Dorsal surface. *d.* = Disk of micropyle apparatus. *d.p.* = Dorsal processes. *f.* = Float. *f.e.* = Follicular epithelium. *f.m.* = Follicular tube membrane. *fu.* = Funicle. *f.y.* = Fine yolk granules. *g.* = Gynaecophoric canal. *i.m.* = Investing membrane. *i.w.* = Inner wall. *m.* = Mucous gland. *m.a.* = Micropyle apparatus. *m.b.* = Muscle bands. *n.* = Nurse-cells. *n.m.* = Nuclear membrane. *o.c.* = Oocyte cytoplasm. *o.n.* = Oocyte nucleus. *o.t.* = Ovarian tube. *o.w.* = Outer wall or chorion. *p.f.* = Primary follicle. *r.c.* = Rosette-cells. *s.* = Spermatheca. *s.f.* = Secondary follicles. *s.p.* = Suspensory filament. *s.r.* = Supporting ring of micropyle apparatus. *st.* = 'Stopper'. *t.* = Tracheae. *t.p.* = Tunica propria. *V.* = Ventral surface. *v.c.* = Vacuolated cells. *v.p.* = Ventral processes. *y.* = Yolk.

Figs. 1–6.—Reconstructions of progressive stages of branching of oocyte nucleus. Same scale. Note.—Branches overlie, and do not enter nurse-cells.

Fig. 1.—Resting stage.

Fig. 2.—Nucleus becoming irregular.

Fig. 3.—Commencement of branching. Nucleus still vacuolated.

Fig. 4.—Later stage of branching, vacuoles have disappeared.

Fig. 5.—Branching nucleus in half-developed follicle.

Fig. 6.—Branching nucleus in full-sized oocyte, after extrusion of nurse-cells.

Fig. 7.—Adult female genital organs. Ovaries in resting stage.

Fig. 8.—Ovary containing full-sized oocytes.

Fig. 9.—Egg after deposition. Anterior end at top of figure. A. Dorsal view. B. Lateral view. C. Median transverse section.

Fig. 10.—Sporozoa from yolk-mass.

Fig. 11.—Section of yolk-mass.

Figs. 12–21.—Progressive stages of oocyte nucleus. 12–16 scale of 15. 17–21 scale of 17.

Fig. 12.—' Bouquet stage '.

Fig. 13.—Spireme surrounding nucleolus.

Fig. 14.—Nucleolus becoming free from spireme.

Fig. 15.—Nucleolus becoming vacuolated.

Fig. 16.—Spireme condensing.

Fig. 17.—Resting stage. Spireme condensing to form chromatin residue.

Fig. 18.—Commencement of second period of growth. Chromatin residue losing staining properties.

Fig. 19.—Slightly later stage. Chromatin residue separating from nucleolus which has practically lost vacuolated structure.

Fig. 20.—Portion of nuclear branch in half-developed follicle.

Fig. 21.—Portion of nuclear branch containing globular masses at period when nurse-cells are breaking down.

Fig. 22.—Transverse section of half-developed follicle, showing ring-like formation of branching nucleus.

Fig. 23.—Longitudinal section of follicle at same stage, showing position of nuclear branches.

Fig. 24.—Longitudinal section of secondary follicle and end chamber. Folded tunica propria left by degenerated primary follicle.

Fig. 25.—Longitudinal section of secondary follicle. One nurse-cell nucleus contains spireme.

Fig. 26.—Longitudinal section of follicle in resting stage.

Fig. 27.—Longitudinal section of follicle at beginning of second period of growth, showing denser cytoplasm between nurse-cells and oocyte nucleus, commencement of inner wall and yolk production.

Fig. 28.—Musculature of investing membrane.

Fig. 29.—Muscles of investing membrane and follicular tube membrane, showing transition to circular and longitudinal muscles of oviduct.

Fig. 30.—Nurse-cell nucleus in resting stage. Tangential section.

Fig. 31.—Transverse section of end chamber containing mitotic figure.

Fig. 32.—Mitotic division in follicular epithelium cells during first period of growth.

Fig. 33.—Degenerating follicular epithelium.

Fig. 34.—Early stage of epithelial folding for float formation. Somewhat distorted section chosen as it clearly shows limits of epithelial cells.

Fig. 35.—Later stage of folding. Float and outer wall with commencement of processes secreted.

Fig. 36.—Commencement of differentiation of rosette-cells and production of 'stopper'. Longitudinal section.

Fig. 37.—Later stage of same. Nurse-cells degenerating. Longitudinal section.

Fig. 38.—Transverse section of rosette-cells at same stage as 37.

Fig. 39.—Longitudinal section. Degenerating nurse-cell nuclei shown partially surrounded by rosette-cells.

Fig. 40.—Surface view of micropyle apparatus.

Fig. 41.—Longitudinal section of anterior end of egg, showing section of micropyle apparatus and position of 'stopper'.

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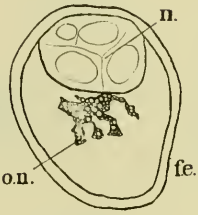

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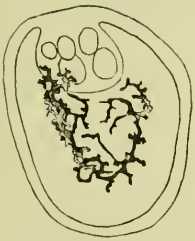

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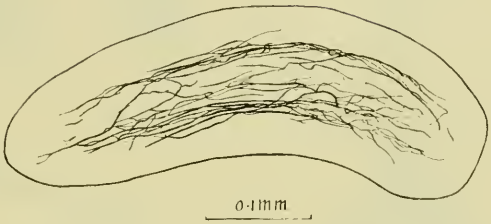

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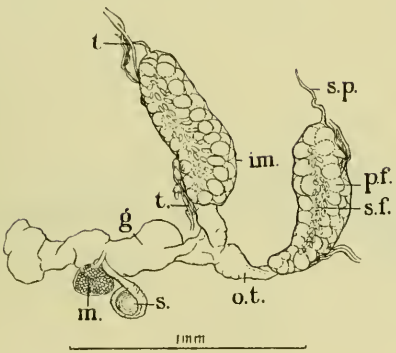

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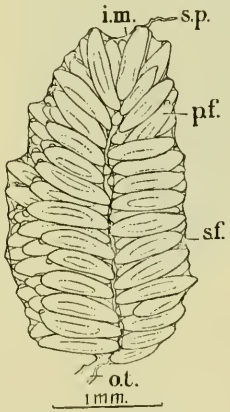

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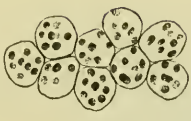

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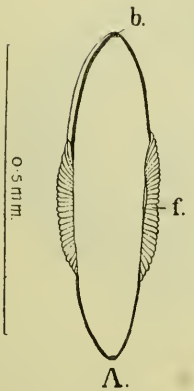

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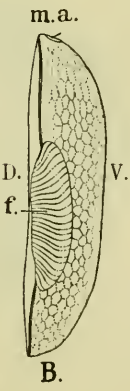

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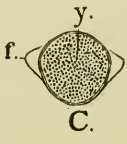

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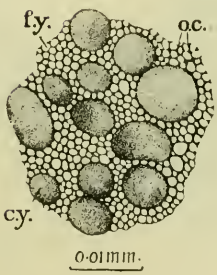

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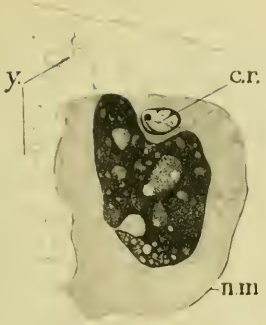

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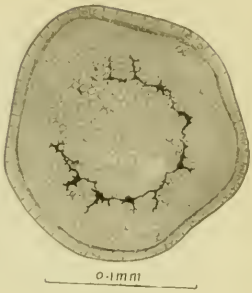

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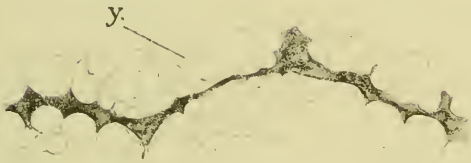

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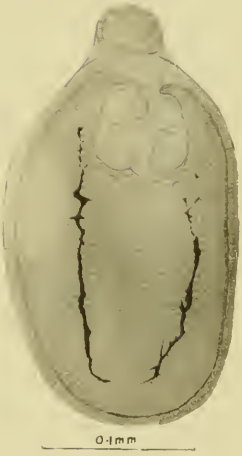

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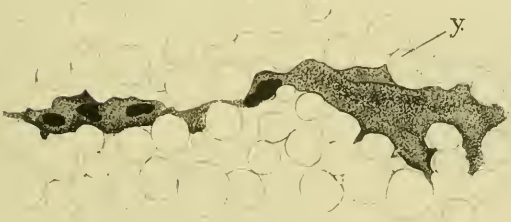

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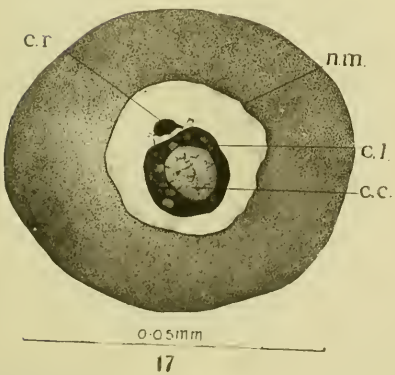

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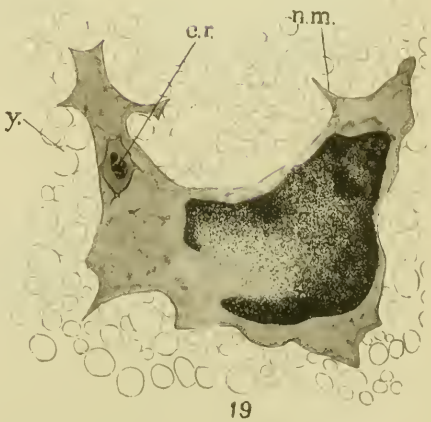

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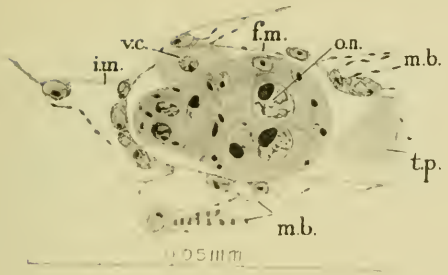

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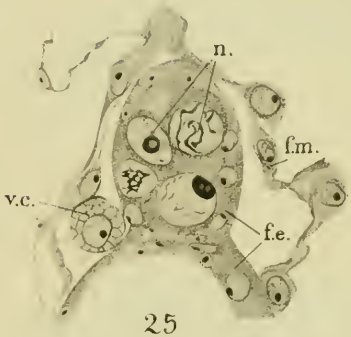

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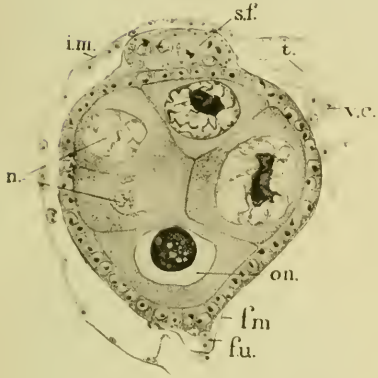

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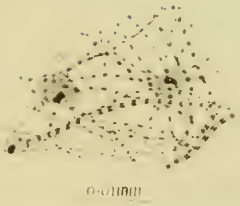

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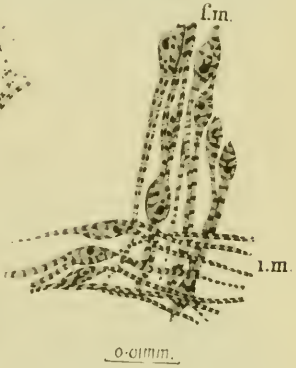

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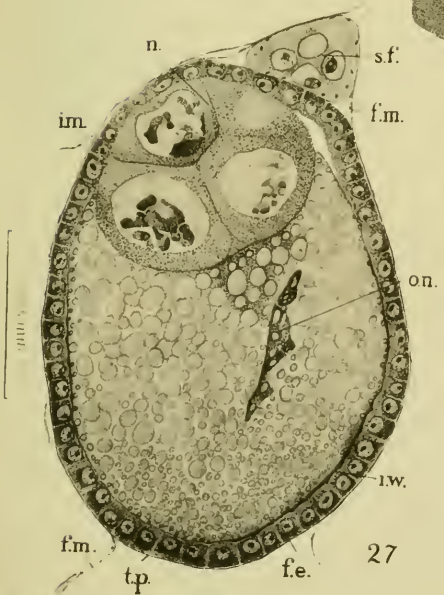

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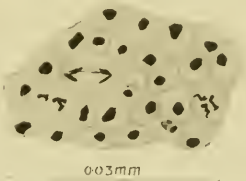

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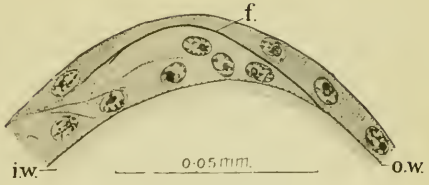

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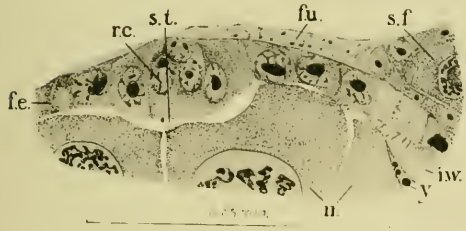

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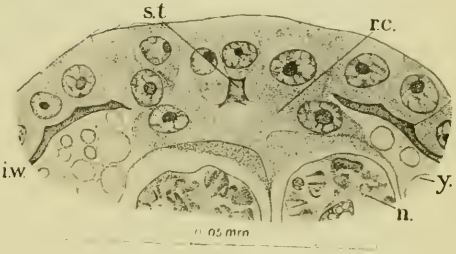

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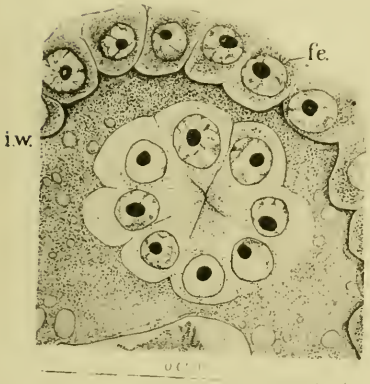

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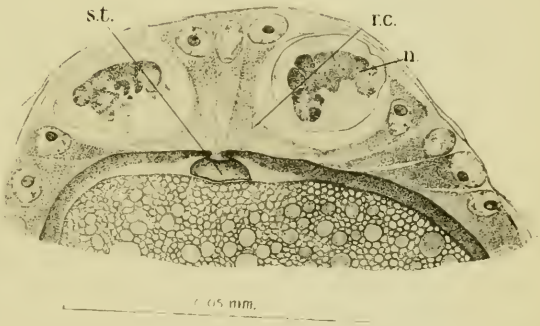

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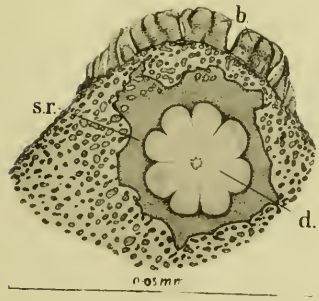

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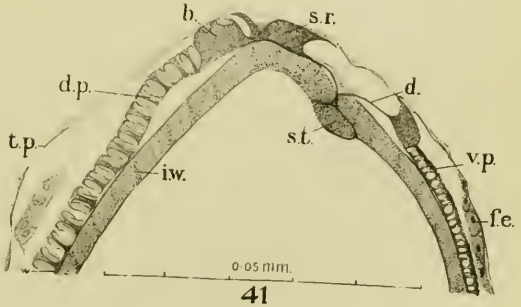

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On the Bionomics and Post-Embryonic Development of certain Cynipid Hyperparasites of Aphides.

By

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Research Fellow of Newnham College.

With 11 Text-figures.

Contents.

Introduction.

The biology of the entomophagous Cynipidae, which include the sub-families of Encoilinae, Figitinae, and Charipinae, has been little studied. The Encoilinae and Figitinae are known to be parasitic chiefly upon Diptera. The Charipinae have hitherto been reared from Aphididae, and occasionally from Coccidae; but no account of their development has been published, and systematic workers have described them indifferently as parasites or hyperparasites. It is probable that the latter view will prove correct for the majority of the sub-family.[1]

The following is an account of the bionomics of certain of these Cynipidae, of the genus Charips. This was formerly known as Allotria, but in 1910, Kieffer (**19**) reverted to the name originally given by Haliday in 1870, and his terminology has been followed here. The genus is divided into two sub-genera, Bothrioxysta, Kieff., and Charips, Hal. The majority of individuals reared from material collected in the field in the course of this work were of the species Bothrioxysta curvata, Kieff.; but a few examples of Charips victrix, Westw., and of another genus, Alloxysta erythrothorax, Hartig, were obtained. No distinction was observed between the larval forms, which is not surprising where the specific distinctions of the adults are variable and slight. It is even possible that certain forms, now ranking as species, may not be physiologically distinct; for in one instance, in captivity, a male of Alloxysta erythrothorax appeared to mate with a female of Charips victrix, which afterwards oviposited.

Hence throughout this work it has been thought most convenient to use the generic name, Charips, when speaking

[1] Silvestri (**23**), in a foot-note to his work on Encyrtus aphidivorus, remarks that Allotria (Charips) is a hyperparasite of aphides through Aphidius (Braconidae); and he adds that it lives upon the host internally, an observation which has been neglected by writers, both before and since.

generally, and to indicate the particular sub-genus or species where necessary.

I would here express my sincere thanks to Professor J. Stanley Gardiner for giving me facilities to carry out the work in the Zoological Laboratory at Cambridge; and my obligations to Professor J. J. Kieffer, who kindly determined the examples of Cynipidae submitted to him.

Material.

The material used was obtained in Cambridge in the summer of 1920. Charips (Allotria) has been reared from various Aphidiidae in different aphides, but throughout this work, Aphidius ervi, Hal., a parasite of Macrosiphum urticae, Kalt., was used, as the comparatively large size of the cocoons rendered them convenient for dissection. The parasite and its host were common and widely distributed round Cambridge in June and July. Moreover, the food plant of this aphid, the common nettle, usually grew in isolated patches along the roadside. This was an advantage, since the Aphidius, after parasitization by the Cynipid, is liable to secondary parasitization by certain ecto-parasitic Chalcids and Proctotrypids, which kill both the host and the first hyperparasite. Collections made from one spot showed that almost every Aphidius, whether attacked by a Cynipid or not, might bear one or more of these external parasites; while collections made fifty yards away were free from secondary infestation, and contained Cynipid larvae in all stages of development.

The rearing methods were the same as those used when studying Lygocerus (**10**). Camera lucida drawings and measurements were made from living specimens, mounted in salt solution or dilute glycerine. The larva, and the host when necessary, were also studied in serial sections.

Biological Note on the Host.

The development of the Braconid, Aphidius, within the aphid has been described by Seurat (**21**), Timberlake (**25**), and others.

The egg is deposited in the haemocoele of the host, and in the course of development a pseudo-serosa or trophic membrane of hypertrophied cells is formed round the embryo. The first larval stage is a transparent caudate form, which varies somewhat in different genera, the cauda of Aphidius being single, whereas, according to my observations, it is bifid in Ephedrus and Praon. This appendage diminishes in the succeeding instars, and the larva, which lies curved head to tail in the body of the host, gradually assumes the apodous maggot-shaped form usual among hymenopterous larvae. At first the presence of the parasite makes little difference to the aphid, which feeds and reproduces as usual; but, as development proceeds, degeneration of the host's tissues sets in. The embryos are affected first, and then the fat-body. The 'pseudo-vitellus' or symbiotic organ is not attacked until a later stage, and the nervous system and alimentary canal remain unchanged until just before the Aphidius transforms, when they, in common with the rest of the fluids of the body, are ingested by the parasite. The tissues break down into large globules, which in stained preparations appear as a vacuolated meshwork of connective tissue containing droplets of fat, while there is often a mass of degenerating nucleoplasm in the centre of the mass. By what means the parasite thus breaks down the surrounding tissues is not known, but although the larva possesses powerful mandibles, chemical rather than mechanical action seems probable.

As soon as the Aphidius has completely emptied the body of the aphid, it changes apneustic for peripneustic respiration, and weaves a cocoon inside the dry skin with silk secreted by the salivary glands.

The meconium is then voided and metamorphosis takes place.

Pairing.

In Bothrioxysta curvata, reproduction was either sexual or parthenogenetic according to whether a male was introduced into the rearing-tube or not. All observed ovipositions of Charips victrix took place after mating, but the ovipositions of Alloxysta were not determined.

Oviposition.

The female Charips oviposits in the Aphidius larva only while the aphid is alive. In this it differs from other hyperparasites, such as Lygocerus (Proctotrypidae) and Asaphes (Chalcidae), which insert their eggs only after the host has woven its cocoon. My observations in this respect are opposed to those of Gatenby (8), who says : ' The Cynipid parasitic forms associated with aphids apparently never attack live Aphidae, but seek out the dried skins of those already parasitized by an Aphidius.'

Subject to the condition that the Braconid larva shall still be bathed in the body fluids of its aphid host, the Cynipid has considerable latitude in its choice of a victim. The Aphidius usually selected is in the third or early fourth instar, but a second instar larva may be chosen (Text-fig. 3), though in such cases there is no evidence to show whether the hyperparasite can complete its development. The number of eggs laid by one female appeared to be about thirty. Only one egg is inserted at each oviposition, and others, when found, are probably the result of subsequent attacks.

The female Cynipid runs over the plant in an excited manner, vibrating her wings and tapping the aphides with her antennae. Healthy specimens are ignored, but the Charips seems to detect the presence of the primary parasite unerringly. When she finds an aphid containing a suitable host, she leaps on to its back, facing the head, and clings there firmly, despite its struggles, like a rider controlling a restless horse. Sometimes she is thrown off, but returns repeatedly to the attack until the aphid is exhausted into passivity. The actual insertion of the ovipositor takes from two to six minutes. This leisurely procedure is not surprising when it is remembered that the cuticle and body-wall of the aphid must be pierced before the probing for the host can begin, and as the Aphidius larva lies among the mass of aphid embryos its location can be no easy matter. Even when found the mesenteron is so distended with food that the body cavity is correspondingly reduced ;

and if the ovipositor of the hyperparasite were to be thrust the smallest degree too far, the egg would be inserted in the host's gut, and be lost at evacuation of the meconium.

The Egg.

The egg (Text-fig. 1) is an oval body, 0·010 mm. × 0·006 mm., with a short peduncle continuous with its long axis. The oogenesis was not observed, but immediately after oviposition a cloud of deeply-staining granules was visible at the posterior pole. This may represent the germ-cell determinant, or, as it has recently been termed by Silvestri, the oosoma. An oosoma in the eggs of phytophagous Cynipidae was first described by Weismann in Rhodites rosae as the 'Furchungskern'. Hegner (**12**) has demonstrated it in Diastrophes nebulosus, and Hogben (**14**) in Synergus. The latter says of the last-named species that the oosoma appears as 'a cloud of granules, more and more heavily staining, until the determinant resembles a spherical ball at the end of the egg'. On the other hand, an oosoma has so far not been seen in other forms, such as Rhodites ignota, Neuroterus, Andricus, and Cynips Kolleri.

The described eggs of Cynipidae are all pedunculated, and in certain gall-forming species the peduncle may be five or six times the length of the egg-body. Adler (**1**) first pointed out that the peduncle is situated at the anterior pole of the egg, which, according to him, differs in this respect from the eggs of other Hymenoptera Parasitica. He supposed that the function of the peduncle is respiratory, and he was supported in this view by Cameron (**3**), who observed that the species which have long peduncles are those which place their eggs where they cannot receive much oxygen from the plant, while in the spring generations of the same species, which oviposit in the leaves, it is usually short. Hegner considers the peduncle analogous to the two anterior processes of the egg of Ranatra linearis, described by Korshelt, which float out in the water from the plant-tissues within which the egg is placed.

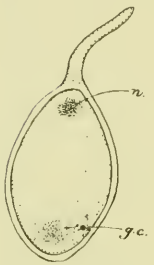
The observations of Riley, quoted by Sharp (**22**), suggest, however, that the peduncle may have another function. He found that in the ovipositions of Callirhytes clavula and Biorhiza nigra only the peduncle is inserted into the plant at first, and that the fluids collect at the posterior end of the egg. 'The fluids are then gradually absorbed from this exposed position into the inserted portion of the egg, and by the time the leaves have formed . . . the egg-contents are all contained within the leaf-tissue.'

Pedunculated eggs also occur in certain Chalcids. The egg of Leucospis gigas is furnished with a hooked process, whose purpose is evidently to suspend it from the cocoon of the Chalicodoma bee upon which the larva is parasitic. Imms (**17**) found that the egg of Blastothrix britannica, a parasite of Lecanium capreae, has a peduncle which protrudes through the body-wall of the host. The tip of the process disappears, thus putting the cavity of the chorion into communication with the outside air like a siphon. Timberlake (**26**) says that the egg of Microterys, parasitic upon Coccus hesperidium, is formed by two bodies connected by a hollow stalk. The stalk, together with the smaller body, projects through the body-wall of the host, and apparently serves for the respiration of the egg and of the larva in the early stages. The egg of Aphelinus mytilaspidis, parasite of Lepidosaphes ulmi (**16**), has also a process which, however, never projects outside the body of the host; and this is also the case with the egg of Comys infelix, a parasite of Lecanium hemisphaericum, described by Embleton (**4**) as possessing a bifid process. Howard and Fiske (**15**) state that the peduncles of the eggs of Schedius kuvanae protrude through the chorion of the eggs of the gipsy moth in which they are deposited. It may be remarked that four of these cases are parasites of Coccidae, sedentary animals whose metabolism and oxygen content must be low in comparison with that of other insects. Eggs approaching the pedunculated form occur in Encyrtus aphidivorus, Ageniaspis fuscicollis, &c., and here perhaps the

increase of the egg's surface, in proportion to its mass, may bear some relation to oxygen absorption.

There is no reason why the peduncle should not in some cases be respiratory, as supposed by Adler, and in others for attachment, as suggested by Riley. In certain instances it possibly serves both functions; but its reduction in Charips probably indicates that it has lost its use, whatever that may have been.

Text-fig. 1.



Text-fig. 2.

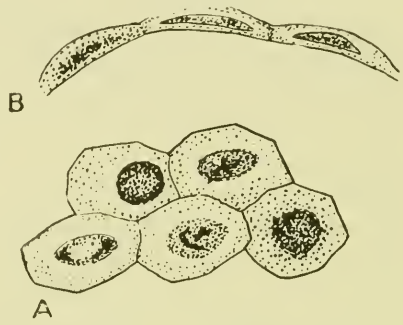


Fig. 1.—The egg immediately after oviposition. ×450. *n.* = nucleus; *g.c.* = cloud of granules.

Fig. 2.—Cells of the trophic membrane with degenerating nuclei. A from above; B in section. ×350.

The Embryonic Membrane.

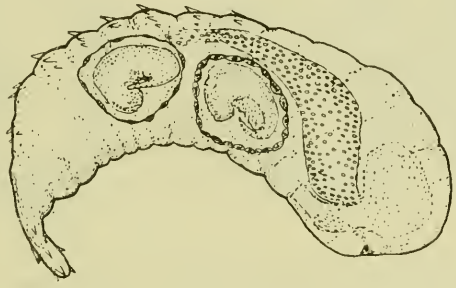
In Charips, as in certain other hymenopterous parasites, a trophic membrane or pseudoserosa is formed round the developing embryo as a globular sphere of large eosinophil cells, with definite nuclei and well-marked walls, polygonal in surface view and crescentic in section (Text-fig. 2). Membranes in this stage may be found up to the point of the hatching of the larva, after which they soon degenerate and disappear, though sometimes degeneration sets in at an earlier stage. A similar degeneration can be seen also in the membrane of the Aphidius host.

A membrane, resembling that described above, has been observed in certain Chalcids, but it does not appear to arise

in the same manner throughout the group. Silvestri (**23**, p. 67) has described its formation in Encyrtus aphidivorus, Mayr., where it originates as a delamination of the peripheral cells of the blastula. In the same work he gives an account of its origin in Oophthora semblidis, where at a certain point, the central protoplasm of the blastocoele breaks out through the blastoderm, bearing with it some free nuclei from the interior. This extruded protoplasm extends round the egg and forms the membrane.

In 1917 Gatenby (**6**) criticized the conclusions of Silvestri with regard to the latter species. Working on the development

TEXT-FIG. 3.



Larva of Aphidius containing two embryos of Charips. ×70.

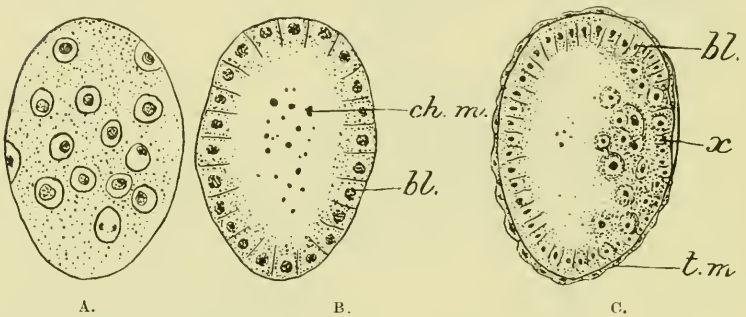
of Trichogramma evanescens, a form which he later recognized as con-generic with Oophthora, Gatenby showed that during the formation of the blastula small masses of nuclear matter are extruded into the blastocoele. Later, these, with the surrounding cytoplasm, move towards the periphery and ultimately stream out through the blastoderm. If the chorion is ruptured, the mass floats out into the host and soon perishes. If the chorion remains intact the extruded mass is flattened and extended by its pressure, until it surrounds the embryo, and the nuclei which it contains give it a fictitious cellular appearance.

Owing to the limited material at my disposal I originally intended to make no reference to the embryology of Charips;

but in the course of this work three stages in the formation of the blastula were observed (Text-fig. 4), and therefore a partial description of them is now given.

A shows the egg soon after segmentation has begun. B represents the blastula already formed, and comparison with the figures of Silvestri and Gatenby shows no essential difference, save that in Charips the germ-cells are indistinguishable from the rest of the primary layer. In C the egg is seven hours old, and it will be seen that the nucleoplasmic masses

TEXT-FIG. 4.



Early stages in the segmentation of the egg. ×900. *ch.m.* = extruded chromatin; *bl.* = blastoderm; *t.m.* = trophic membrane.

in the blastocoele have disappeared, and that there has been considerable displacement of the nuclei on the right-hand side. Certain nuclei are arranged in a manner that suggests that we have here a stage similar to that which Gatenby has indicated as the first appearance of the endoderm. Moreover, an involucre, apparently of cellular structure, surrounds the egg, and contains nuclear staining elements distinct from the degenerating chromatin masses shown in the previous figure. As intermediate stages are lacking it is impossible to say with certainty how this involucre arose.

Nearly all my available material was in the stage figured as B, but the membrane did not appear in it and there was no sign of the delamination described by Silvestri in Encyrtus.

Moreover, the arrangement of the cells does not suggest that they have arisen by division from the peripheral nuclei. The disappearance of the chromatin masses seems to indicate that there has been a recent escape of the contents of the blastocoele, but this matter does not appear in the involucre. It may be represented by a small mass found in the host's tissues opposite the point marked *x* in the figure. In any case, though Gatenby's explanation accounts for the appearance of the membrane in his own and in Silvestri's figures, it does not seem possible that the extruded matter could, under compression of the chorion, take an outline such as that shown in Text-fig. 4 c.

The data are too scanty to permit of our forming a definite opinion on the origin of the involucre in these Cynipidae, but I hope to pursue this subject later when more material is available. Gatenby, however, remarks that in some cases living nuclei are carried out with the extruded material: 'Curiously enough these fragments seem to live a good while, and nuclear changes, such as those undergone in the blastoderm, take place in some cases.'

Without hazarding an opinion on the different views of these observers as regards the Trichogrammatinae, a suggestion may be made that if the expulsion of live nuclei were to be carried further in Charips than it is in Trichogramma, these might by division give rise to the membrane. But either this division must be very rapid, to develop the involucre in the space of two or three hours, or else the initial expulsion of the living nuclei must be larger than it appears to be from an examination of the material.

The First Instar (Text-fig. 5).

Dimensions, $0{\cdot}38 \times 0{\cdot}13$ mm. The embryonic membrane is ruptured two or three days after oviposition. The newly-hatched larva is heavily armoured with dark segmental plates of chitin, which render it easily visible through the tissues of the host. It possesses a distinct head and thirteen body-segments, the last of which terminates in a caudal appendage.

In the living larva the twelfth segment is somewhat telescoped into the eleventh, so that only twelve segments appear to be present. The mouth parts are produced into a proboscis, within which lie two long slender mandibles. The head bears three pairs of chitinous nodules on the ventral side, and, in addition, a fourth pair dorsally. These processes are each furnished at the extremity with a transparent spot which

TEXT-FIG. 5. TEXT-FIG. 6.

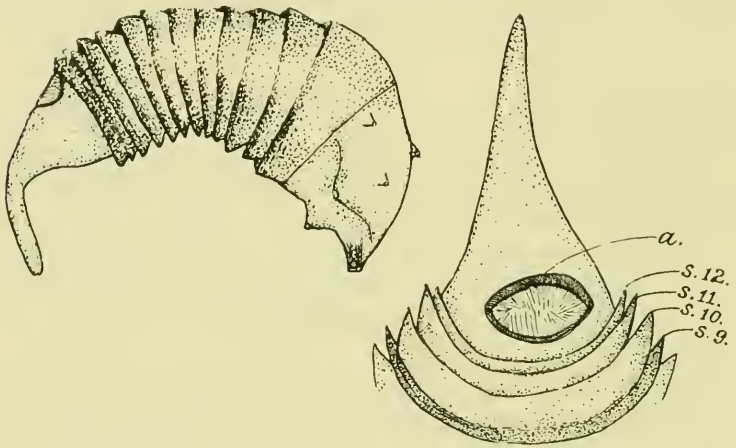


Fig. 5.—Larva of the first instar. ×150.
Fig. 6.—Anus and caudal appendage of the newly-hatched larva. ×350. *a.* = anus; *S.* 9–12 = chitinous plates of segments 9–12.

is possibly sensory in function. The body-segments diminish in diameter from the thorax posteriorly. Each appears as a circular band of chitin, somewhat overlapped by the one immediately preceding it. This overlap is so pronounced on the ventral side of the thorax in some examples as to give the effect of short processes; and as the latter actually appear after the first ecdysis it is possible that they may already exist under the chitinous plates, but at this stage it is not possible to demonstrate their presence definitely. The anus, which lies dorsal to the cauda, is a large and conspicuous structure surrounded by a chitinous ring (Text-fig. 6). From the

periphery transverse bands of chitin extend into the lumen, and give it a spiracle-like appearance. Owing to the opacity of the chitinous coat the internal organs cannot be seen, but the outline of the gut, which already contains food globules, is faintly visible by transparency.

The larva is curved ventrally with the tail bent round to form an angle with the abdomen. Its usual position is between the nerve-cord and gut of the host, either in the anterior or posterior third of the body. Owing to the manner in which the Aphidius lies in the aphid these are the parts most accessible to the ovipositor of the female Charips, and thus the earliest larval stage is presumably found where the egg has been deposited. The chitinized stage persists for a variable time. In one case observed the skin had been cast and left behind when the larva emerged from the trophic membrane. In others it lasted from two to four days. In the later stages the chitin can be found among the host's tissues. In ecdysis the skin usually splits transversely across the thorax, and the larva slips out. I have occasionally found examples in the second instar in which the moult had been incomplete, and the body of the larva was still encircled by one or more of the chitinous bands, like a rolled napkin enclosed by a ring.

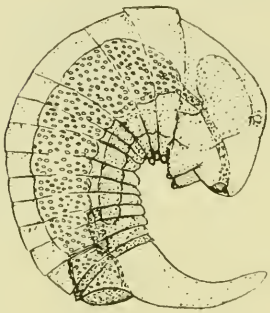
The Second Instar (Text-fig. 7).

The second instar resembles the first in size and general form, but is white and transparent without thickened chitin. The mouth is transversely oval, and furnished with two large simple mandibles. Below it is a pair of ventro-lateral lobes surmounted by sensory papillae. Each of the three first body-segments bears a pair of protuberances on the ventral surface, and the segmentation of the body is less marked.

The internal structure is visible through the transparent integument. The salivary glands lie latero-ventrally on either side of the midgut as two straight tubes. The nerve-cord appears as a broad unconstricted band. The two Malpighian tubules are very short, and immediately behind their orifices

the proctodaeum is much enlarged with a bulb-shaped lumen, communicating with the exterior by the wide anus. In some examples newly removed from the host a transparent membranous substance was seen extruded from it. When larvae at this stage were stained with carmine or methylene blue, it was found that the stain readily entered through the anus, and was taken up by the lining epithelium of the hind-gut before any other part of the body was affected.

Text-fig. 7.



Text-fig. 8.

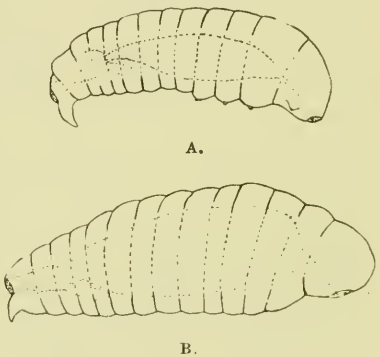


Fig. 7.—Larva of the second instar. × 150.
Fig. 8.—Intermediate stages of the larva. × 50.

Intermediate Stages (Text-fig. 8).

As the larva increases in size the tail and cephalic papillae become reduced, and the thoracic processes disappear. It was not ascertained whether there was a moult between this and the previous stage, or whether the change of form was due merely to growth and absorption of the appendages; but it is probable that there was at least one ecdysis about this time, though it was not actually observed. The body becomes much distended as the gut is filled with food matter, until the tail and processes finally vanish. After the disappearance of the cauda the anus gradually shifts back until it is at last terminal, and at the same time it becomes proportionately smaller.

The egg, as previously mentioned, is usually deposited in

the ventral side of the Aphidius at either extremity of the body. The chitinized larva, and subsequently its cast skin, are found in the same position, and orientated indifferently in any direction, but the later stages invariably lie along the dorsal side of the gut of the host with the head towards the head of the latter. Hence at some intermediate stage the hyperparasite must change its position. How this takes place was not observed, but, in view of the fact that the cauda of analogous forms is sometimes regarded as locomotory, it may be remarked that in Charips the first tailed larva does not move at all, while at some later stage, when the cauda is reduced, a definite, and frequently elaborate, change of position occurs.

The Full-grown Larva (Text-fig. 9).

When the larva is full grown it makes its way out behind the head of the host, whose remains it devours within the next few hours. The gut may then be evacuated and metamorphosis ensue speedily, but frequently there is a resting period of several days. Thus, in one case, eleven days elapsed between emergence and transformation, and in another case, eight.

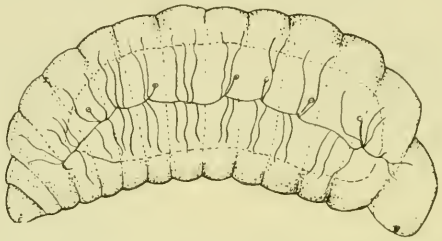
The full-grown larva is an apodous form measuring 1·70 × 0·90 mm. The body of thirteen distinct segments tapers somewhat to the anus. The skin is smooth, and there are no appendages except to the mouth parts. The crescentic labrum is furnished with eight small papillae. The mandibles are large, bidentate, and strongly chitinized. Each maxilla bears a disk, upon which are three papillae, one of which terminates in a short seta; and the labium, which is large and oval, bears laterally two pairs of papillae (Text-fig. 10).

The salivary glands, which in this form never secrete silk, extend forward from the seventh segment on either side of the gut ventrally. Each gland is a long straight tube composed of polyhedral cells, and, in the first segment, enters a duct which immediately behind the head unites with its fellow of the opposite side to form the short dilated common salivary duct

opening on the floor of the mouth under the U-shaped hypopharynx.

The mid-gut is shut off from the oesophagus by a valve. The former, which is greatly distended, is lined with flattened polyhedral cells with large nuclei. As in other hymenopterous larvae at this stage there is no communication between the

TEXT-FIG. 9.



TEXT-FIG. 10.

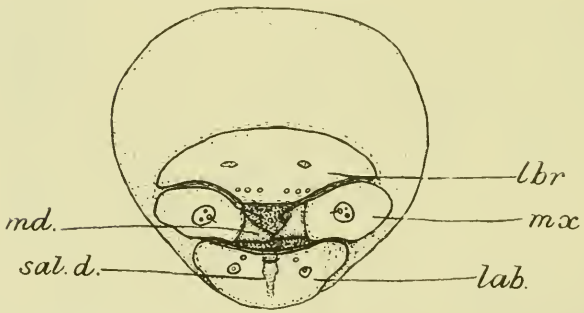
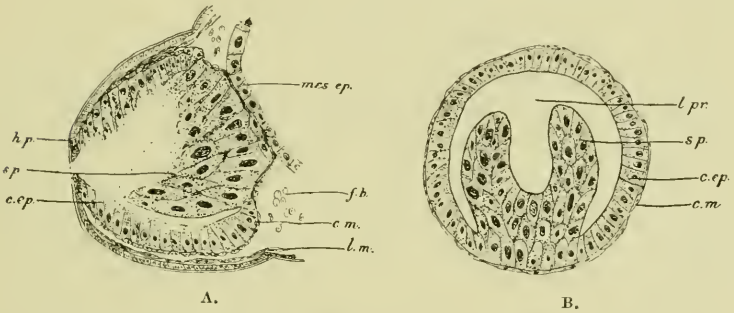


Fig. 9.—The full-grown larva. × 35.
Fig. 10.—Head of full-grown larva. × 100. *lab.* = labium; *lbr.* = labrum; *md.* = mandible; *mx.* = maxilla; *sal. d.* = salivary duct.

mesenteron and proctodaeum. The structure of the latter merits description. In larvae of the first and second instars the lumen is wide, and lined with a columnar epithelium of hypertrophied hypoderm cells with conspicuous nuclei. As development proceeds the anus becomes proportionately smaller, and an outgrowth from the antero-ventral wall of the proctodaeum projects backwards into the lumen. This outgrowth is shaped like a shovel, shortest on its dorsal aspect, and has

lateral expansions over-arching the cavity inside. In effect, it partly divides the proctodaeum into two compartments, one within the other, and the Malpighian tubules communicate ventrally with the inner of the two. The outgrowth or process itself is formed of two layers of elongated basophil cells, with well-marked nuclei, similar to those of the wall of the hind-gut, and in the later stages it almost fills the lumen. If it contained muscular fibres it would be easy to suppose that this outgrowth functions as a valve, shutting off the orifices of the Malpighian tubules from the general proctodeal cavity; but as the presence

TEXT-FIG. 11.



Proctodaeum of the young larva. A in sagittal, B in transverse section. ×350. *c.ep.* = columnar epithelium; *c.m.* = circular muscles; *f.b.* = fat-body; *h.p.* = hypoderm; *l.m.* = longitudinal muscles; *l.pr.* = lumen of proctodaeum; *mes.ep.* = epithelium of mesenteron; *s.p.* = process projecting into the lumen of the proctodaeum.

of muscular tissue cannot be demonstrated, its only purpose appears to be to increase the surface area of the columnar epithelium of the hind-gut (Text-fig. 11).

The two Malpighian tubules are exceedingly short. Each is composed of eight or nine large cells only, but these surround a lumen of considerable diameter. The nervous system appears as a broad slightly-constricted band. The supra- and sub-oesophageal ganglia, and the three ganglia of the thorax, are well marked; but those of the abdominal region are indistinctly separated, with the exception of the last two, which are fused and form a distinct bulb-like swelling.

The rest of the internal structure demands no particular comment.

The tracheal system becomes functional when the parasite leaves the host. The two main lateral trunks are united by an anterior and a posterior commissure. Dorsal and ventral lateral branches are given off in each segment 1–10. There are six pairs of open spiracles. The first is placed between segments 1 and 2, and the remainder on segments 3, 4, 5, 7, and 9. Of the considerable number of examples examined only two departed from this rule in possessing, in addition, a pair of spiracles on segment 8.

Pupation and Emergence.

Pupation lasts from twenty-two to twenty-six days, and at the end of this time the Cynipid gnaws an irregular hole on the dorsal side of the cocoon and creeps out. In captivity the adults lived from three to eight days. They fed upon the sap oozing from cut leaves and upon the honeydew of the aphides. They sometimes sipped the latter from the anus of the living animal, and were occasionally observed to scrape the dried sugar from empty skins with their mandibles.

It is not known how many broods may be reared in the season, nor how far these Cynipid hyperparasites are specific for different Aphidiidae, but as far as it goes the evidence suggests that they have a considerable range of hosts. Thus the number of broods is probably determined by the number of Aphidiidae available.

Also at present there is no evidence as to how the parasites and hyperparasites of Aphides pass the winter. I have found living larvae of Aphidius salicis, Hal., in Aphis saliceti, Kalt., in cocoons collected in July, and opened in the laboratory in January. This suggests that a few may pass through the winter in this stage; but, although I paid particular attention to this point, I could find no indication that Aphidius ervi had not all emerged by the end of August, for, of the considerable number of cocoons from different localities that were examined, all were empty.

Comparison of the Larval Characters of Charips with those of other Entomophagous Cynipidae.

Our knowledge of the larval forms of the other entomophagous Cynipidae is limited to three species.

In 1834 Bouché (**2**) described the full-grown larva of Figites anthomyiarum, Bouché, found in the puparia of Anthomyia (Diptera).

In 1886 Handlirsch (**9**) gave an account of the corresponding stage of another Figitine, Anacharis typica, Walker, parasitic upon Hemerobius nervosus, Fabr.

In 1913 Keilin and Pluvinel (**18**) described the post-embryonic development of an Encoiline, Encoila keilini, Kieff., parasitic upon the Dipteron Pegomyia.

In comparing the full-grown larva of Charips with these three forms, we find certain structural differences between them. Charips and Anacharis possess thirteen segments, whereas Figites and Encoila have but twelve. The tubercles of Anacharis are distinctive, and Encoila alone possesses simple mandibles. Figites, Anacharis, and Encoila all have nine pairs of spiracles, a character they share with the phytophagous forms. In Charips there are but six pairs of spiracles (exceptionally seven), and these are not arranged upon consecutive segments.

As regard the early stages, the only form available for comparison with Charips is Encoila. In the first instar the larvae are of the same general type, but Charips differs from Encoila in the absence of pronounced thoracic processes, and in the possession of a chitinized skin, mandibles, and an enlarged anus. The embryonic membrane does not seem to occur in Encoila, and, so far, has not been recorded in the Cynipidae.

Comparison of the Larval Characters of Charips with those of Parasitic Hymenoptera in General.

The early larvae of the hypermetamorphic Hymenoptera Parasitica may be referred to three main groups:

The first, or cyclopoid, type so far as has been found only in Platygaster (Proctotrypoidea), and is known chiefly through the researches of Ganin (**5**) and Marchal (**20**).

In the second type the last segment is furnished with an appendage, and thus may be called caudate. It includes, for example, such forms as Limnerium (Ichneumonidae) Aphidius (Braconidae), Comys, and certain Ageniaspids (Chalcidae) and Teleas (Scelionidae).

The third type was first observed by Wheeler (**27**) in the myrmecophagous Chalcid, Orasema, and has since been described by Smith (**24**) in another Chalcid, Perilampus. This larva, known as a planidium, is elongated and testudinate, furnished with imbricated plates of chitin.

The caudate type is the most frequent. The function of the tail has been supposed by different authors to be either locomotory or respiratory, but may possibly be both. In the early stages of such forms the tracheal system is apneustic and respiration is cutaneous. The cauda, by increasing the body-surface, may assist in the absorption of oxygen, and the thoracic processes of Encoila may have a similar function. At the same time the setae with which the cauda is furnished in some Aphidiidae suggest that it may sometimes serve for locomotion.

The first-stage larva of Charips is caudate, but I can find no other instance of heavy chitinization in this type. Indeed, the only parallel instance appears to be the planidium of Perilampus, whose life-history is somewhat different. Perilampus is hatched as a free living form, and later seeks out the caterpillar which contains the proper hymenopterous or dipterous host. It then lives as an endoparasite without growth or ecdysis for a variable time. After metamorphosis of the host, it emerges, sheds its chitinized skin, and completes development

as an ecto-parasite upon the pupa. Here presumably the chitin protects the larva during the search for the host. Charips is an endoparasite throughout larval life, but certain facts suggest that this may be a later adaptation, and that the chitinous armour may be a survival of a life-cycle not unlike that of Perilampus.

For instance, the chitin does not now seem to be of vital importance to the young larva, since it may either be thrown off at hatching and left behind in the embryonic membrane or persist for a variable number of days afterwards. Smith (24) suggests that the histolysis of the surrounding tissues is the stimulus that impels the Perilampus to change its mode of life and moult. Something of the kind may occur in Charips, though in this form metamorphosis of the host does not actually take place. The host larvae may be in different stages of development at oviposition, and yet those younger than the third instar could scarcely contain enough food material to enable the Cynipid to reach maturity. It is doubtful whether in such a case as that shown in fig. 3, where the gut is already displaced before the hyperparasites have left the embryonic membrane, the Aphidius can survive. But even in ovipositions in third-instar Braconids it would be fatal to the Cynipid if the development of the host were arrested too soon, for instance before the cocoon was woven. Thus it is possible that the chitinized stage is in some sort a resting phase, and I now regret that I did not pay more attention to this point in the material at my disposal.

Another point is that Perilampus is endoparasitic only in the first instar, whereas Charips lives internally until larval development is completed.

But a parallel may be drawn if the internal habit of the latter is a comparatively recent adaptation, and the demolition of the host's remains after emergence is a survival from a time when it made its way out of the host at an earlier stage and completed development as an ectoparasite.

The metabolism of Charips presents certain problems. The thick chitin must prevent cutaneous transfusion of oxygen

from the host's tissues. It is possible that the structure of the anus and proctodaeum is correlated with this, and that something analogous to rectal respiration exists in this form. The hind-gut has a large lumen enclosed by modified hypoderm cells. In the later stages the proctodaeum is proportionately smaller, and, when the chitin is cast off, respiration is presumably carried on through the cuticle, as in such forms as Aphidius, though mention should be made of the tongue-shaped process of large deeply-staining cells, which, like a typhlosole, projects into the lumen of the proctodaeum as development proceeds, and, if the view suggested here is correct, would increase the respiratory area.

A peculiar modification of the hind-gut occurs in the larvae of certain Braconids, such as Apanteles and Microgaster. The body terminates in a hollow bladder or vesicle of hypertrophied cells; and Gatenby (**8**), who has recently re-described this structure, makes the interesting suggestion that this is morphologically the proctodaeum, which has become everted for respiration. The enlarged, though uneverted, hind-gut of Charips may be intermediate between the highly-specialized structure found in these Microgasterinae and the unmodified proctodaeum of most hymenopterous larvae.

It is noteworthy that in these Cynipidae great development of chitin is associated with unusually short Malpighian tubules. If the chitin persisted throughout larval life we might be tempted to regard it as a means of disposing of such nitrogenous waste material as could not be dealt with by the tubules. But as the chitinized plates are lost early, while the tubules do not increase in size in the later stages, it is improbable that the two characters are correlated.

Reaction of the Host.

Aphidius reacts very differently to Charips and to Lygocerus. In parasitization by the latter, as described elsewhere (**10**), the host dies, and speedily deliquesces into a mass. Nothing of this kind happens where the Braconid contains a Charips larva. The Aphidius demolishes

the viscera of the aphid, and then secretes silk and weaves the cocoon as usual. The tissues retain their tone and colour, and irritation excites slight movement. On close examination, however, it can be seen that the body is somewhat contracted.

At this time the Cynipid larva, its head orientated with that of the host, lies above the mesenteron of the latter, which it constricts into a dumb-bell form. By some means the further development of the Aphidius is arrested, and always at the same point, namely, after the weaving of the cocoon. The meconium is never evacuated, and metamorphosis, which normally takes place soon afterwards, never occurs. The condition of the Braconid larva resembles in fact that of the prey that certain Hymenoptera store in their brood-cells.

Two explanations of this phenomenon suggest themselves. Either the female Charips at oviposition may inhibit the final changes of the host, possibly by injection of some secretion; or the Cynipid larva itself, during development, may affect the Aphidius by chemical or physical means.

The evidence is not conclusively in favour of either view. In support of the first one particularly marked instance came under notice.

A Charips female was observed to oviposit on June 26. The aphid was isolated, and four days later the Aphidius within began to spin silk. On July 4 the cocoon was opened in order better to follow the development of the hyperparasite, a plan that was adopted successfully in several instances. The Aphidius remained without change until August 7, a period of five weeks. The meconium was not voided, but beyond some contraction the larva looked healthy. In replacing it in the tube after examination it fell from the brush, and must have received some injury, for next day a discoloured patch appeared at the hinder end of the body. The larva was dissected carefully, but no hyperparasite could be found, and the organs showed little signs of histolysis. As oviposition had been observed, the facts suggest that some accident had prevented the development of the Cynipid larva, and this leads to the inference that the agent arresting the metamorphosis of the

host comes into force, if not at oviposition, at least at an early stage in larval life.

In support of the view that the larva itself may inhibit the development of the host is the parallel case of Perilampus. As the larva is hatched as a free-living form and subsequently enters the host, there can be no question of the inhibition dating from oviposition. Yet, according to Smith (**24**), 'The development of the host . . . invariably ceases at the time of exit of the planidium. Whether or no it is actually killed is not evident. In any case decomposition does not take place immediately, the host being left in a condition somewhat comparable to that of the prey of certain aculeate Hymenoptera.'

Perilampus differs from Charips in that metamorphosis has taken place before the exit of the planidium; but when the latter begins to live as an ectoparasite upon the newly-formed pupa, it is found that the growth of the head and appendages, with their setae and pigments, is arrested, and development is not completed.

Nothing resembling phagocytic reaction against the hyperparasite was observed, either as regards the living larva or the cast skin, which could sometimes be found unchanged among the host's tissues up to the time of emergence of the full-grown Cynipid larva.

Economic Status.

Charips checks the Aphidius in its destruction of plant-lice, and thus, from the economic standpoint, must be considered an injurious insect. But throughout its development it shares the vulnerability of its host to ectoparasitic Chalcids and Proctotrypids, and when secondary parasitization occurs it perishes with the Aphidius. From observations made in the course of this work it would seem that where the incidence of Chalcid and Proctotrypid hyperparasitization is high, the chances of Charips larvae attaining maturity are correspondingly reduced. For instance, if, of a hundred Aphidius, twenty-five are parasitized by Charips, and thirty-two

parasitized by such a form as Lygocerus (Proctotrypidae) by chance, 8 per cent. of the former should be destroyed; while where the incidence of parasitization by Chalcids, such as Asaphes, is as high as that of Lygocerus, this rate of mortality must be doubled. The above figure for Cynipidae is hypothetical, though, as it is based on examination of much material, it is probably not too low. That for Lygocerus was found to be the actual rate in certain instances (**10**). It is difficult to estimate the mortality accurately, because the host, if subsequently reparasitized, rapidly decomposes, and any endoparasite that it may contain soon becomes unrecognizable. Moreover, the bionomical relations of the different hyperparasites are so intricate that the chances of survival of any particular case are difficult to compute. Thus Charips actually lessens its own chance of survival, for the effect of its parasitization is to arrest the metamorphosis of the host, and thus maintain it in the optimum condition for oviposition by Lygocerus or Asaphes. Hence in the hypothetical case given above the number of Aphidius larvae parasitized by Charips and reparasitized by Lygocerus would probably be larger than that parasitized by Lygocerus only, and the mortality of the first parasite would actually be higher than the figure given. To this mortality from reparasitization I attribute the fact that from collections of parasitized aphides made in the field there were proportionately more Cynipid emergences in June than in July. Most of the hyperparasites obtained from later collections were Chalcids or Proctotrypids (Lygocerus); and the inference is that the later broods of Cynipidae suffered from a second parasitization of their hosts by other hyperparasites.

Summary.

1. Bothryoxysta curvata, Kieff., Charips victrix, Hartig, and Alloxysta erythrothorax, Westw., are hyperparasites of aphides through Aphidius (Braconidae).

2. Reproduction may be either sexual or parthenogenetic.

3. The egg is laid in the haemocoele of the host larva before

the death of the aphid, and post-embryonic development is internal.

4. A trophic membrane of hypertrophied cells is formed round the embryo.

5. The larva is, at first, hypermetamorphic; and exhibits greater development of the chitinous cuticle than is usual in endoparasites; but in the succeeding stages it approximates more closely to the general hymenopterous type.

6. The development of the Aphidius is arrested at a certain point, and metamorphosis does not take place.

7. The Cynipid, when ready to pupate, makes its way out of the Aphidius, whose remains it devours, and undergoes metamorphosis within the cocoon previously woven by the latter in the skin of the aphid.

8. These forms differ in certain particulars from the entomophagous Cynipidae previously described, and the chief differences are discussed.

9. Comparison is also made of the larvae of other Hymenoptera Parasitica, particularly of Perilampus.

10. Certain problems of metabolism are pointed out, and it is suggested that respiration may be partly rectal.

11. These Cynipidae are economically injurious as they check the Aphidius in its destruction of plant-lice; but there is high mortality among the larvae owing to secondary parasitization of the Braconid by other hyperparasites.

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Notes on the Larval Skeleton of Spatangus purpureus.

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With Plate 21.

Although 'one of the very first Echinoderms of which artificial fertilization and rearing of the larvae were undertaken' (Mortensen, **6**, p. 14), the development and especially the structure of the larval skeleton of Spatangus purpureus have been rather imperfectly known. Krohn's descriptions and figures (**2**, **3**) are not quite satisfactory with regard to the skeletal structure, and, moreover, the larvae described in his second paper are doubtful as to their specific identification (Mortensen, **6**, p. 15). Through Mortensen's renewed observations on the artificially-reared larvae of this species (**6**, pp. 14–17) the external features of the larval development are now made clearer. As to the larval skeleton, however, he was only able to give some brief information owing to the unfortunately bad state of preservation of his specimens. Among other Spatangoids, Echinocardium cordatum and Brissopsis lyrifera were carefully studied by Macbride (**4**) and Mortensen (**7**, pp. 144–8), and the larvae of these three species have been shown to have such a striking resemblance to each other in early stages that it is desirable to ascertain some more minute diagnostic characters for each species. In such circumstances

it seems not unnecessary to put on record detailed descriptions of the skeletal structure of the larva of Spatangus purpureus.

The material on which my work is based consists of a series of larvae, reared and preserved by Mr. Elmhirst at Millport, and kindly handed over to me for study by Professor E. W. MacBride.[1] Although there are found several gaps in developmental stages, the changes undergone by the larval skeleton could be followed fairly satisfactorily. From the labels which were found attached to the vials we obtain the following chronological accounts.

The earliest stage which is represented by segmenting eggs is dated 16th May 1914. This is probably the day on which the eggs were artificially fertilized. The further stages with regard to the age in days are :

2nd day	May 17th . .	Blastula.
3rd ,,	,, 18th . .	Gastrula.
4th ,,	,, 19th . .	Young 2-armed pluteus.
5th ,,	,, 20th . .	Fully-formed 2-armed pluteus.
6th ,,	,, 21st . .	4-armed pluteus.
17th ,,	June 1st . . .	6-armed pluteus.
?	? . . .	8- or 10-armed pluteus.
24th ,,	June 8th . .	12-armed pluteus.

Thus in full accordance with the statements of Mortensen (**6**, p. 15) the larva reaches its last stage in the course of three weeks. It is to be regretted that those larvae whose skeleton was best preserved had been kept together in one vial, all the different stages being mixed up, and without any label, so that it is not possible to give a chronological state-

[1] The present work was done partly in the zoological laboratory of the Imperial College of Science and Technology and partly in the British Museum (Natural History). My cordial thanks are due to Professor E. W. MacBride of the College and to Sir Sidney F. Harmer of the Museum, for help and encouragement in various ways and for the privilege of the use of the laboratory and the libraries.

ment in most cases as regards the first appearance of a new calcification centre or its subsequent development, &c.

At the outset I may call attention to the fact that the latticed rods, viz. the postoral, postero-dorsal, and posterior unpaired (so-called aboral spike), are morphologically different from the other simple, though often thorny, rods which serve equally as the support of each corresponding arm. Théel (**11**, pp. 40–1) described very clearly the early development of the postoral rods of Echinocyamus pusillus as follows: 'they (the latticed rods) begin to arise during the gastrula stage as three small processes, one on each rod of the star close to its centre, Pl. iii, fig. 38. These processes stretch in length, run parallel and become connected by transverse beams'. The same is exactly true for the corresponding rods and also for the other latticed rods in Spatangus. In all of these a three-rayed 'star' is first laid down lying parallel to the surface of the body. From each of the rays or arms, very close to the centre, is given out a vertical process, directed towards the surface of the body. The latter, three in number if, as in most cases, all developed, give rise to a latticed rod. The postoral and postero-dorsal rods of Echinocardium cordatum are both stated by MacBride to be formed of only two parallel rods (**4**, pp. 475, 477). As compared with the table-like calcareous body, which is commonly met with in all classes of Echinoderms, the latticed rod corresponds to the spire, and the three-rayed portion to the base. Thus the above-named two-paired and one unpaired latticed rods are morphologically composite in structure and are from the beginning directed vertically to the surface of the body. On the other hand, those rods supporting the antero-lateral and postero-lateral arms are morphologically simple, being produced either as prolongations or branches of the three-rayed base, which were lying originally parallel to the surface of the body. The body-, recurrent, and horizontal rods are also either prolongations or branches of the basal part, which remained running along the surface of the body without, however, pushing out

to support arms. The dorsal arch consists only of the three-rayed portion, from which any vertical process fails to develop. The pre-oral and antero-dorsal rods also belong, according to this interpretation, to the simple type of the rods. In a similar manner, it seems to me, in Arbacia, Dorocidaris, Echinocyamus, &c., the posterior unpaired star fails to produce vertical processes, which would give rise to the aboral spike in the Spatangoid larva, the laterally directed basal arms being only developed as the postero-lateral rods. Prouho's discovery of an abnormal larva of Dorocidaris papillata which produced a well-developed aboral spike (**10,** pp. 349–50, Pl. xxv, fig. 9; cf. Mortensen, **5,** p. 75) is exceedingly interesting in this respect. Mortensen (**5,** p. 71) maintains that the statements of some authors, e.g. Kölliker's, who have described plutei with six to ten latticed rods must be wrong. My observations confirm this conclusion. Though in some abnormal cases those morphologically simple rods may be doubled or split, analogous to those I have observed, e.g. the dorsal horizontal rod of the right side (Pl. 21, figs. 7 and 8, *dh*) and the left recurrent rod (fig. 6, *re*), it is quite impossible that they should assume latticed structure.

Late in the gastrula stage a pair of calcification centres appear, which are bilaterally symmetrical in position. This state of affairs is so well known in other Echinoids that any detailed description is quite unnecessary. I may, however, point out that the body-rod represents one of the three-rayed basal arms, not simply a posterior continuation of the postoral rod, as might easily be wrongly inferred because they both run in an almost straight line (fig. 1, *br, po*). The other two arms of the base are represented respectively by the ventral horizontal rod (*vh*) and the recurrent rod (*re*), from which latter the antero-lateral rod (*al*) is given out later.

The third, unpaired calcification centre appears near the posterior end of the body (*ab*). This may appear as early as in the stage where the future postoral arms can as yet hardly be recognized as arms, viz. when the larva has formed a slight

concavity at the oral field and has begun to assume roughly a tetragonal shape. The star is situated in such a position that two of its arms lie bilaterally and the remaining one is directed dorsally. The former two ultimately give rise to the postero-lateral rods, while the third remains as a short but distinct spur-like process all through the larval life (figs. 3–8). From each of these arms a vertical process is produced, directing posteriorly. These three vertical processes form together the aboral spike (fig. 2, *ab*). Being robust in structure the transverse beams extend rapidly so as to obliterate the openings between them.

Hand in hand with the rapid growth in length of the post-oral rods the arms of the basal portion develop to assume their future position. The body-rods, which run straight postero-medially, are the most rapid in growth among them, and their posterior ends come to overlap each other (fig. 1, *br*.). In the corresponding stage as well as later, as figured by Krohn (**2,** Pl. vii, figs. 1–3, 6), the posterior ends of the body-rods are shown standing fairly apart. Except in a later stage where the rods begin to be absorbed at the posterior ends (figs. 5 and 7), I have never met with such a state as shown in his figures. The second arm, the recurrent rod (*re*), which is at first directed dorsally, soon bends posteriorly. In the meantime it produces a branch at its bent portion. This branch, which is the future antero-lateral rod (*al*), proceeds a little towards the median line, but soon bends anteriorly to run almost parallel to its fellows of the other side, though slightly approaching this as it runs. Its base is a little broadened and bears a few minute processes, as shown in Krohn's figure (**2,** Pl. vii, fig. 5, *c*) and confirmed by Mortensen (**6,** p. 15). The remaining arm of the first calcification centre runs along the ventral surface, almost transversely towards the median line, but slightly deviating anteriorly (fig. 1, *vh*). This is the ventral horizontal rod. The end soon comes in contact with that of its fellow of the other side, and they ultimately fuse, forming a characteristic thickened joint (figs. 2 and 3, *vh*). This feature is constantly

seen and lasts for a fairly long period, and it seems to me that this can be regarded as a specific character in identifying Spatangoid larvae. In many other Spatangoid larvae this is not the case ; these rods either stand apart or pass across, as in Echinocardium cordatum and its doubtful ally (Müller, **8**, p. 290, Pl. iii, fig. 2). In cases where both ends come very close together, as in Echinopluteus fusus (Müller, **9**, Pl. vii, fig. 2), E. solidus (**9**, Pl. vi, fig. 9 ; Pl. vii, fig. 1), and perhaps Brissopsis lyrifera also (Mortensen, **7**, fig. 2), they do not form any thickened joint. Only in Chadwick's figures of an unidentified form (**1**, Pl. ix, figs. 61 and 62) the similar state of the ventral horizontal rods is very clearly shown.

By the time when the two-armed stage is fully developed, when the post-oral arms have reached the length nearly equal to the body proper, whereas neither the antero-lateral arms nor the aboral process are as yet distinct, the following features are to be noticed : the post-oral rods are usually solid and three-ridged, and the margin of the ridges is not serrated. Exceptionally, however, some irregularly-scattered holes may be met with even near the proximal end of the rod, but owing to the very slight differences in the refractive indices between the thin, filmy skeleton and the surrounding medium, which consists of oil of cloves or Canada balsam, it is difficult to demonstrate the holes clearly. Krohn (**2**, p. 256) observed no fenestration in these rods in the corresponding stage. Further, in his figure (Pl. vii, fig. 1) he showed only the antero-lateral and body-rods besides the post-oral, whilst the ventral horizontal and recurrent rods are not represented. The star of the aboral spike should also have appeared in this stage.

The recurrent rod grows rapidly, and when its posterior end comes in contact with that of its fellow of the other side (fig. 2, *re*) fuses with it and increases in thickness, often being beset with some irregular short processes near the end (fig. 3, *re*). A little anterior to this end a branch is soon sent out ventrally, while about the same time the body-rod produces a branch dorsally, and these two branches meet

and fuse midway between the body- and recurrent rods (figs. 4 and 6, *c*). There are very often some irregular spines or branches from the dorso-ventral connexion thus formed. As the result of this connexion there is formed a rectangular framework as seen from side (cf. Mortensen, **5**, p. 75, Pl. ix, fig. 9). From the point where the antero-lateral rod diverges from the recurrent rod there is formed frequently a short process directed anteriorly (figs. 2 and 6). This seems to have no significance.

Lastly, at the end of the two-armed stage the body-rods fuse at the point where they have been overlapping each other, so as to form an oblique cross. Very often there is formed an accessory connecting-span between the two body-rods. This is a short transverse piece lying a short distance anterior to the crossing-point. Now the calcareous framework encircling the stomach has become fairly rigid. The body-, recurrent, and ventral horizontal rods of both sides are fused in the median plane with the respective fellow of the other side, while, on the other hand, the body- and recurrent rods are connected with each other near the posterior end on each side of the body.

After having reached this state the body-rod increases no longer in length, so that, as long as its posterior end remains unabsorbed, its length can be taken as unit in describing the dimensions of other parts. The length of the body-rod can easily be measured when the larva is laid with its ventral side downwards, so that the rod is seen in its real length without foreshortening. As expressed in terms of the ratios to the body-rods, the post-oral rod reaches during the two-armed stage a length more than twice as long as the body-rod, the antero-lateral rod more than one-half, and the aboral spike about one-third.

The aboral process and the antero-lateral arms become discernible almost simultaneously. It may now be called the four-armed stage (figs. 3–5). The change which takes place during this stage is the enormous increase in lengths of the post-oral and antero-lateral rods and of the aboral spike.

The post-oral rods grow up to four or five times the length of the body-rod, while the antero-lateral rod and the aboral spike reach more or less twice the length of the same (fig. 5).

The post-oral rods seem in most cases to be devoid of fenestration in their proximal half or one-third, whereas the unfenestrated portion of the aboral spike is generally much shorter. In an extreme case in the latter the fenestration begins close to the proximal end (fig. 6, *ab*), exactly as the feature seen by Krohn in an unidentified form (**3**, p. 210). The distal parts of these rods are fairly regularly serrated. The serration seems to begin roughly at the point where the fenestration also begins (fig. 5, *po*, *ab*). The posterior ends of both the body- and recurrent rods show towards the end of this stage signs of degeneration, being gradually absorbed. The dorsal arch makes its appearance near the end of this stage, on the mid-dorsal line at the level where the oesophagus opens to the stomach (fig. 5, *da*). The two arms of the star, which lie symmetrically and are directed antero-laterally, increase rapidly in length, while the unpaired, posteriorly-directed arm remains very short, sometimes even obliterated.

Krohn's figure (**2**, Pl. vii, fig. 2) corresponds to the early four-armed stage. It is the dorsal view, in which the ventral horizontal rods and the body-rods are not shown, while the descending rods, which I take as the recurrent, are not coming to meet each other at the posterior ends. The post-oral rods are shown as fenestrated on their distal three-fifths, while the aboral spike remains unfenestrated. Both these kinds of rods are, however, shown to have serrated edges along their whole length.

The next, six-armed stage, is characterized by the appearance of the postero-dorsal arms. Previous to the appearance of these arms the supporting skeleton, which is called the postero-dorsal rod, is formed underneath each of them (fig. 6, *pd*). The rod develops in the manner similar to that of the other latticed rods, and as described and figured by Théel in Echinocyamus pusillus (**11**, p. 44, Pl. vi, fig. 88, *y*). The arms of the star lie in such a position that one

is directed anteriorly, another postero-laterally, and the remaining one postero-medially. From the lack of adequate material the fate of the former two arms cannot be stated with certainty, though it seems probable that they do not develop much farther. The postero-medially-directed arm in the later stages continues to develop in a direction parallel to the dorsal surface, reminding one of the body-rod on the ventral side (figs. 7 and 8). Near the base of this arm a branch is sent out in an antero-median direction, reminding one again of the ventral horizontal rod. This is the dorsal horizontal rod (*dh*). From each of the arms of the star, close to the centre, is given out a vertical process, very often differing in the rate of development, but ultimately the three in all give rise to the latticed postero-dorsal rod.

Although from want of material, especially of the later part of this stage, no definite statement can be made, yet, judging from later specimens, it is highly probable that the post-oral rod increases in length during the six-armed stage up to nearly 6 times the length of the body-rod, the antero-lateral rod 3 times, the aboral spike nearly 3·5 times, and the postero-dorsal rod probably at least 1·5–2 times the length of the same.

In Krohn's figure (**2,** Pl. vii, fig. 3) is indicated the three-rayed base of the postero-dorsal rod (*e*). The buds of the pre-oral arms have already appeared (*d*), while the dorsal arch is still in a rudimentary condition, of which, however, nothing is mentioned. The fact that the pre-oral arms appear without any mechanical influence of the underlying skeleton is also seen in Echinocardium cordatum (**4,** p. 477. Pl. xxxiii, fig. 6). But both in MacBride's case of Echinocardium and my specimens of Spatangus the appearance of the pre-oral arms takes place much later than the stage as shown by Krohn, viz. even when the postero-dorsal arms have attained a fair length, there was as yet no sign of these arms found. Krohn gives some detailed structures in a somewhat advanced six-armed stage (Pl. vii, figs. 5 and 6). If the fig. 5 is really the dorsal view, as stated

by him, then the dorsal arch (*d*) should lie above the antero-lateral rods (*c*). The two arms of the base of the postero-dorsal rod (*f*, *g*) are shown very well developed, and that the serrated recurrent rods meet each other at the broadened posterior ends is also clearly drawn. His fig. 6, which is the ventral view, is somewhat difficult to understand. There are two sets of rods which seem to correspond to the ventral horizontal rods, both overlapping each other at the end. Whether it is really an abnormal case, as in the right dorsal horizontal rod in my oldest larva (figs. 7 and 8, *dh*), or due to his misrepresentation cannot be decided at present.

The further advanced stages are represented by a small number of eight- to ten-armed larvae with dissolved skeleton, and a single specimen of the twelve-armed stage.

The fourth pair of arms to appear are the pre-oral, which are supported respectively by the direct prolongations of each end of the dorsal arch. The fifth pair are the postero-lateral, supported by the lateral prolongations from the base of the aboral spike. From want of material showing any adequate stage I cannot decide whether the postero-lateral arms have from the beginning a skeletal support, as e.g. in Echinocardium cordatum (MacBride, **4**, p. 479), or not, as e.g. in Brissopsis lyrifera (Mortensen, **7**, pp. 147–8). Judging, however, from the fact that the arms soon develop to assume their typical shape, instead of remaining as ear-shaped lobes, I am strongly inclined to think that the arms in question of Spatangus purpureus do contain their skeletal support from their earliest stage.

Owing to the remarkable increase in size of the stomach during the eight to ten-armed stage, that skeletal framework which formerly encircled the stomach must have undergone corresponding changes. This can be judged from the state seen in the twelve-armed specimen (figs. 7 and 8). Both the body- and recurrent rods are shortened at the posterior ends, their side-by-side connexion being broken. The ventral horizontal rods of both sides are also separated from each other at the joint. This broken framework does not now encircle the

stomach, but has gradually been pushed posteriorly, and the angles between the body- and recurrent rods of one side and their fellows of the other side are much widened.

The twelve-armed specimen (figs. 7 and 8) is much younger than the larva figured by Mortensen (**6,** fig. 14), the total length measuring only 2·1 mm. The pre-oral and postero-lateral arms are nearly equal in length, measuring 0·3 mm., a little shorter than the antero-lateral, which measure 0·35 mm. The antero-dorsal arms, which have appeared last, are only in the form of buds. The other arms and process are remarkably long, i.e. the posterior arms measuring 1 mm. in length, the posterior process 0·9 mm., and the postero-dorsal arms 0·8 mm.

A short distance anterior to the point where the antero-dorsal rod is sent out from the dorsal arch, the latter produces a short lateral branch. The same is noticed by Müller in Echinopluteus fusus (**9,** Pl. vii, fig. 3) and by Mortensen in Echinocardium cordatum (**5,** p. 103, Pl. ix, fig. 6). In a Spatangoid larva, which has been doubtfully identified by Mortensen (**5,** pp. 102–3) with Echinocardium cordatum, Müller described and figured a peculiar feature in that the median posterior branch of the dorsal arch fused with the tips of the dorsal horizontal rods (**8,** p. 290, Pl. iii, figs. 1 and 4, *d*). So far as I know such a case has never since been recorded by any other observers nor have I noticed it in my specimens (figs. 7 and 8, *da*, *dh*). The postero-lateral rod has no noticeable characteristics, being of a uniform thickness throughout and rather smooth, differing from the richly-serrated state as seen in Echinocardium cordatum (Mortensen, **5,** p. 103, Pl. ix, figs. 7 and 8; MacBride, **4,** Pl. xxxiii, fig. 11, *pla*).

The rectangle formed by the body- and recurrent rods as seen in some younger stages (figs. 4 and 6) can no more be found (fig. 8). The area roughly corresponding to the anterior half of the rectangle is now occupied by an irregularly-perforated calcareous plate, which is developed more strongly on the right side than on the left side. The bases of the post-oral (*po*) and antero-lateral rods (*al*) are incorporated into this

calcareous plate, and the recurrent rod is now hardly distinguishable. Although it is difficult to make out clearly, it seems highly probable that neither the bases of the postero-dorsal (*pd*) nor of the postero-lateral rods (*pl*) are fused with that plate. Similar features in the formation of calcareous plates are frequently met with in other irregular sea-urchins, e.g. Echinopluteus fusus (Müller, **9**, Pl. iv. fig. 7 ; Pl. vii, figs. 3 and 11), Arbacia pustulosa (Müller, **9**, Pl. iii, figs. 2 and 3), &c. Whether these plates have anything to do with the definitive skeleton of the young sea-urchin is still an open question, though it seems probable that they are absorbed altogether at the time of metamorphosis.

Summary.

1. The larva of Spatangus purpureus reaches its last stage, which is characterized by its possession of six pairs of arms, in the course of three weeks after fertilization.

2. The paired arms develop in the following order : post-oral, antero-lateral, postero-dorsal, pre-oral, postero-lateral, and antero-dorsal. The posterior process appears about the same time as the antero-lateral arms become distinct.

3. These six pairs of arms and the unpaired process are each supported by a calcareous rod. Of these calcareous rods one can distinguish two classes which differ morphologically from each other, viz. the simple and the composite.

4. To the class of simple rods belong the antero-lateral, pre-oral, postero-lateral, and antero-dorsal rods. They are either direct prolongations or branches of the three arms produced from one of the calcification centres. They are originally horizontal (parallel to the surface of the body) in position, and are homologous with the body-, recurrent, and horizontal rods.

5. The remaining rods, viz. the post-oral and postero-dorsal rods and the aboral spike (posterior rod) are composite. They are each composed of three parallel rods connected by transverse beams so as to give a latticed appearance. Each

of the parallel rods is a branch given out vertically from an arm of the calcification centre.

6. The larval skeleton of Spatangus purpureus is characterized chiefly by (*a*) more or less considerable length of the unfenestrated proximal portions in the latticed rods, (*b*) fusion of the tips of the ventral horizontal rods forming a thickened joint, (*c*) overlapping of the body-rods near their posterior ends, and subsequent fusion of this part so as to form an oblique cross, (*d*) rather simple appearance of the postero-lateral rods, and (*e*) formation of a calcareous plate on each side of the stomach in the oldest stage.

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EXPLANATION OF PLATE 21.

(All figures were drawn by means of a camera lucida and magnified 200 times.)

Fig. 1.—Dorsal view of a young two-armed larva, in which the rudiment of the aboral spike (*ab*) has just appeared. (An unusually long process is seen arising from the base of the right post-oral rod.)

Fig. 2.—Dorsal view of an old two-armed larva to show the fusion at the tips of the ventral horizontal rods (*vh*). (The posterior ends of the body-rods, *br*, are not overlapping here as normally.)

Fig. 3.—Dorsal view of a young four-armed larva to show the fusion of the posterior ends of the recurrent rods (*re*). The body-rods (*br*) are also fused with each other (hidden behind the aboral spike, *ab*).

Fig. 4.—Left-side view of the same specimen as shown in fig. 3. A rectangle is formed by the body- (*br*) and recurrent rods (*re*). (From the base of the aboral spike an additional process is given out ventrally.)

Fig. 5.—Dorsal view of an old 4-armed larva, in which the rudiment of the dorsal arch (*da*) has appeared and the posterior ends of the body-rods have begun to degenerate.

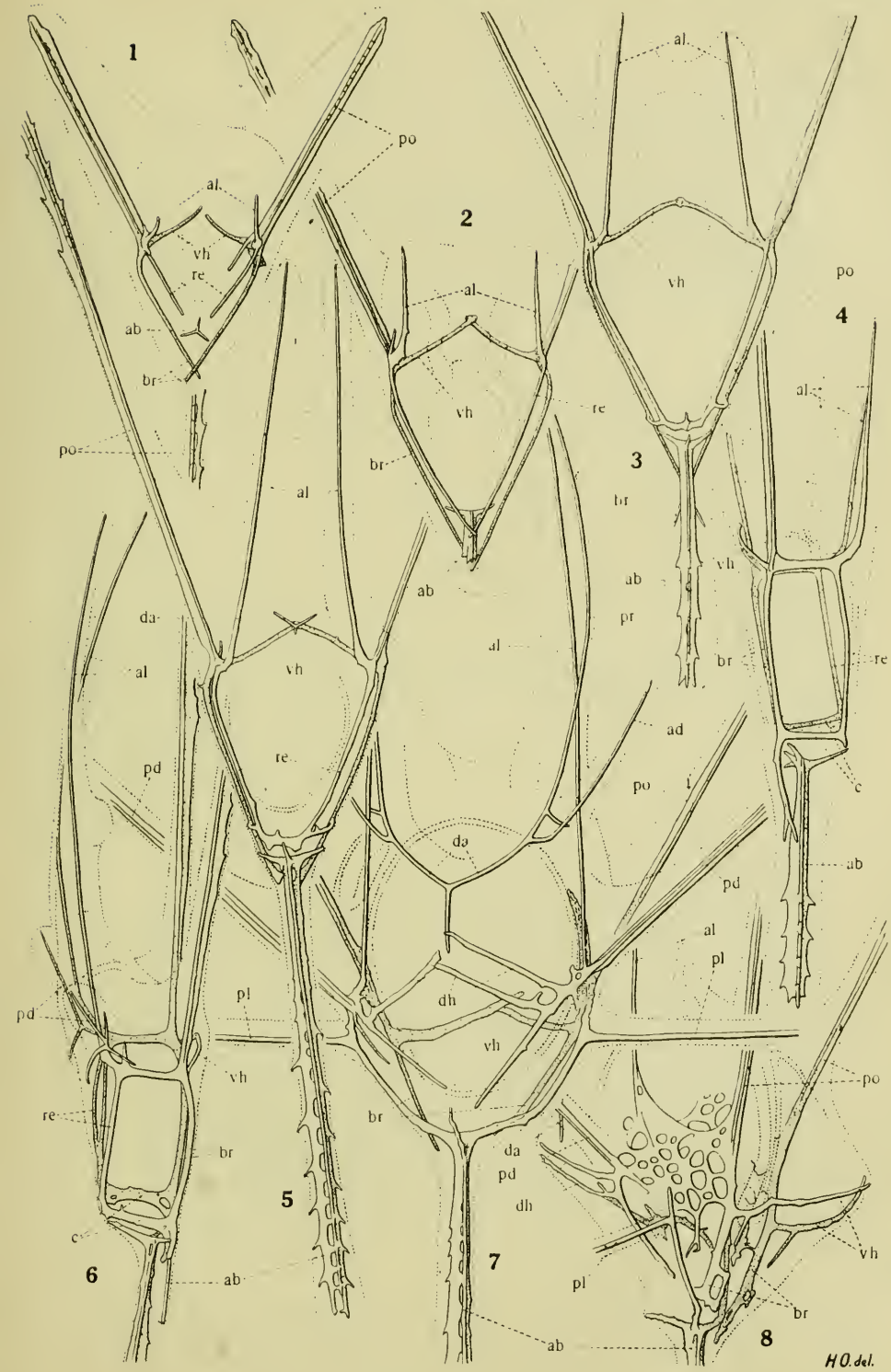
Fig. 6.—Right-side view of a young six-armed larva to show the early stage of the postero-dorsal rod (*pd*). (The left recurrent rod, *re*, is here seen abnormally split into two.)

Fig. 7.—Dorsal view of a twelve-armed larva. The body- (*br*), recurrent and ventral horizontal rods (*vh*) have all lost their connexion with the fellows of the other side. (The right dorsal horizontal rod, *dh*, is abnormally doubled.)

Fig. 8.—Right-side view of the same specimen as shown in fig. 7, to show the calcareous plate formed between the body- (*br*) and recurrent rods.

Abbreviations.

ab = aboral spike; *ad* = antero-dorsal rod; *al* = antero-lateral rod; *br* = body-rod; *c* = dorso-ventral connexion between body- and recurrent rods; *da* = dorsal arch; *dh* = dorsal horizontal rod; *pd* = postero-dorsal rod; *pl* = postero-lateral rod; *po* = post-oral rod; *pr* = pre-oral rod; *re* = recurrent rod; *vh* = ventral horizontal rod.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On the Classification of Actiniaria.

Part II.—Consideration of the whole group and its relationships, with special reference to forms not treated in Part I.[1]

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With 20 Text-figures.

Contents.

1. Introduction.

It has been necessary, on account of the length of the present paper, to confine Part II to discussions; the definitions of families and genera involved, on the lines of those already given in Part I, will be printed in another issue of this Journal as Part III, which will also contain a list of literature and an index to genera covering Parts II and III. The list of literature will be additional to that printed in Part I, and any numbers given in brackets in the following pages will refer to the two lists as one whole.

Part I dealt with a relatively limited and compact group of

[1] Part I was published in Vol. 64 of this Journal.

anemones in a fairly detailed way; the residue of forms is much larger, and there will not be space available in Part II for as much detail. I have not set apart a section of the paper as a criticism of the classification I wish to modify, as it has economized space to let objections emerge here and there in connexion with the individual changes suggested. Part I tried to clear the ground and discuss the method of attack, so that the arguments there given need not be repeated, and so that the general principle and method suggested there might be taken for granted in Part II. I should like to record here that in these papers on Classification there will be found points in contradiction to certain remarks in earlier papers—'Terra Nova' and 'Actiniaria collected off Ireland'—but the point of view is bound to become modified in some particulars as further experience opens new vistas. That the view-point should remain immovably fixed in the light of developing knowledge would more need apology than that it should march with necessity. Work on Part II has served only to strengthen and confirm the plan suggested in Part I of this paper.

Definitions to be given in Part III are based as far as possible on anatomically-described species, leaving the more doubtful forms to fit themselves in as knowledge of them increases. Consequently lists of species given include rather the better-known forms on which the definition is founded, than exhaustive enumerations. Even to identify an anemone from an old figure or description is very risky; to be sure of an old species one must obtain and re-describe the type-specimens if such exist. If there are none, it is guess-work—cf. Pax (**75**), p. 309, and others.

One result of working through all the Actinian genera (supported by a personal anatomical study of a large number of them) is the recurrence of impressions connected with the difficulty of species-identification of some of them from preserved material—and the unfruitfulness of the pursuit. It would seem that family and genus are fairly easily tracked down when once a certain number of data are gained, and that these are intelligible quantities. But when it becomes a matter

of species the variation of the different anatomical criteria of distinction may be so wide, and the limits of specific variation so little known, that to go beyond the genus is little more than guess-work ; especially when one thinks of the modification caused to certain characters by mode of preservation, degree of contraction or distension of the animal, age, reproductive condition, locality, and other things. Two paths there are here which need following. Firstly, a large number of anemones should be collected (some belonging to stable and some to unstable species, and representative of various families) in cases where it could be positively certified that all individuals collected for any one species were undoubtedly the same. These should be preserved in different ways and states, and a study made which would reveal the limits of specific variation—or it might prove that sometimes there are no limits. Even after this, many descriptions would need supplementing before a revision of species within the group could be attempted. The second path is the study of nematocysts ; it may prove that measurements of these will provide clear specific distinctions. I believe Professor Carlgren will bring forward a good deal of evidence in this connexion. I have not been able myself to give this point much attention, but what I have done rather suggests that the size of the cells is too variable and too similar in closely-related species to help us. Pax has a note on this in his paper on the ' Family Actiniidae ', pp. 80–2. At least it becomes evident that species-identification from preserved material, with certainty, is going to be extraordinarily laborious. It would probably better repay effort to take more notice of the living animals, for here one's experience suggests that species-identification from colour and habit in life would usually be easy and sure. Experience is leading me to the view that among these low and plastic forms a species may have its peculiarities of organic constitution at an early stage of the development of their expression, such expression having affected colour scheme and general facies of the living animal but not necessarily to any extent the internal anatomy which can be studied in preserved

specimens. If this idea can influence the study of anemones, it will turn the attention of some workers in the direction of refuting it by minute research and measurement; and others towards 'leaving it at genera' and looking into the matters of living form and broader group-problems, in any case resulting in better knowledge of the group. Special detailed studies of individual families should yield good fruit. In some cases at least further work would reveal interesting and instructive similarities and variations running through all the members of a given family, but of a kind beyond the scope of the short definitions to which a paper like the present is limited. It would also reveal which families are more and which less homogeneous, and help to clear up ideas of relationships. I have made a preliminary study of the Chondractiniidae, for instance, which promises to be interesting in this sense.

Once more I wish to record hearty thanks to several friends who have given me their aid in one way or another, especially to Professor H. J. Fleure for much kindness, and to Captain A. K. Totton, M.C., for kind help with literature and specimens at South Kensington. I am also much indebted to Professor Stanley Gardiner for the loan of a collection of specimens without the aid of which it would have been very difficult to complete the paper.

Some of the illustrations in this paper are copied from other sources. Text-fig. 14, K, is copied from Plate 22, No. 2, in W. Saville-Kent's 'The Great Barrier Reef of Australia' (W. H. Allen & Co., Ltd., 100 Southwark Street, S.E. 1); Text-fig. 19 is from a photo by Saville-Kent in 'The Naturalist in Australia', p. 224 (Chapman & Hall, Ltd., 11 Henrietta Street, W.C. 2), and later on printed in 'Marvels of the Universe', p. 1135 (Messrs. Hutchinson, Paternoster Row, E.C.); Text-fig. 9 is copied from 'Journ. Mar. Biol. Soc.', N.S., vol. x, no. 1, 1913, p. 73; Text-fig. 8 is from 'Sci. Trans. R. Dublin Soc.', ser. ii, vol. iv, 1889, Pl. 35, fig. 1. I wish to acknowledge with thanks permission to print my versions of these figures, from Messrs. W. H. Allen, Chapman & Hall, and Hutchinson, Dr. E. J. Allen, the Science Committee of the

Royal Dublin Society, and the executors of the late Mr. Saville-Kent.

2. Brief Historical Section.

Unfortunately space forbids the inclusion here of even outline histories of all the families dealt with in the paper similar to those given for Sagartiidae and Paractidae in Part I. The number of families is far greater, and possibly the historical interest is less than in the previous case. The following details, therefore, are limited to an outline of the more usual classifications used up to date, and which it is the suggestion of this paper to modify.

G. C. Bourne's scheme is the following:

Class Anthozoa.

Sub-class I. Octactiniaria (Octocorallia, Carlgren).
Sub-class II. Ceriantipatharia (Hexacorallia, Carlgren).
Sub-class III. Zoanthactiniaria (Dodecacorallia, Carlgren).
Order 1. Zoanthinaria.
Order 2. Edwardsiaria.
Order 3. Dodecactiniaria.
Sub-order A. Madreporaria.
Sub-order B. Actiniaria.

The principle of his three sub-classes is that of Carlgren, Bronn's Thierreich, 1908.

The position of the Zoanthinaria and Edwardsiaria varies in different schemes. In Carlgren's 1900 plan, for instance, the Edwardsiaria go under his group Athenaria, and the Zoanthinaria stand away separately and rank equal to the Ceriantharia and Actiniaria. Bourne has recently shown (**9**) that the Edwardsiids must be clearly separated from ordinary Actinians, and it is his allocation of them which is to be accepted.

The subdivision of the sub-order Actiniaria will vary accordingly as one follows Carlgren or not. Carlgren's division, as used by him in 'Ostafrikanische Aktinien' (1900), for example, is as follows:

Sub-order Actiniaria.

Tribe 1. Protantheae.
Sub-tribe 1. Protactininae.
Sub-tribe 2. Protostichodactylinae.
Tribe 2. Nynantheae.
Sub-tribe 3. Actininae.
A. Athenaria.
B. Thenaria.
Sub-tribe 4. Stichodactylinae.

Other arrangements ignore the Protantheae and Nynantheae, dividing at once into Actininae and Stichodactylinae, in which case the Protactininae rank as Actininae, the Protostichodactylinae as Stichodactylinae.

The Protantheae are separated from the Nynantheae by the possession, usually, of an ectodermal muscle-sheet and nerve-layer in the body-wall and generally in the actinopharynx also; and in some of them by the absence of basilar muscles, and ciliated tracts on the mesenterial filaments. The Actininae and Stichodactylinae, and similarly the Protactininae and Protostichodactylinae, are marked off from each other by the fact that in the Actininae (and Protactininae) only one tentacle communicates with each exocoel and endocoel, at most, whereas in the other groups two or more tentacles grow out from at least the stronger endocoels.

This section may suitably contain a list of the more generally-used families, which will be convenient for reference later, assigned to their respective positions under Carlgren's main groups.

1. PROTACTININAE: Gonactiniidae, Ptychodactidae, Halcuriidae.
2. PROTOSTICHODACTYLINAE: Corallimorphidae.
3. ACTININAE:
   Athenaria: Ilyanthidae, Halcampidae, Halcampomorphidae, Andvackiidae, Halcampactidae.

THENARIA : Sagartiidae, Paractidae, Boloceridae, Actiniidae, Bunodidae, Aliciidae, Phyllactidae, Dendromeliidae, Minyadidae.

4. STICHODACTYLINAE : Discosomidae, Stoichactidae, Heteranthidae, Homostichanthidae, Aureliidae, Actinodendridae, Phymanthidae, Thalassianthidae.

This is, of course, the list as it stands without taking any account of the present paper, even Part I of it. The work of Part I was chiefly devoted to a revision of the Sagartiidae and Paractidae, taking those names in the old sense as used on this page.

3. DISCUSSION OF CHARACTERS TO BE USED IN CLASSIFICATION.

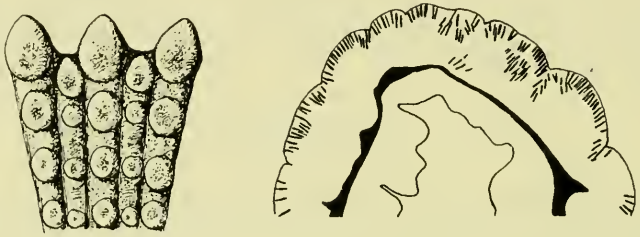
The characters already discussed in Part I, pp. 456–68, will of course be used here again, where they come in, but a few others remain to be mentioned.

In the families under discussion now, there are no mesogloeal sphincters save in Halcampa, but it has to be decided how far the character of the endodermal sphincter is to be trusted as a family feature. All grades of it exist, from very weak diffuse or very weak circumscribed to very strong circumscribed, through various degrees of diffuseness and circumscribed diffuseness (cf. Text-figs. 11 and 12). It may be quite absent. In some families the range is not more than from absent to weak diffuse. But in other cases there are so many grades that one can draw no line of demarcation anywhere ; and it must be admitted that the form and grade of development of the sphincter cannot be used as a family character except where it is fairly stable. The same thing really applies to mesogloeal sphincters, but here it has been less noticed because no one happens to have suggested an artificial distinction between diffuse and circumscribed mesogloeal sphincters.

It has long ago been realized that presence or absence of

verrucae and acrorhagi[1] cannot be used in limiting families, and this leads on to the question of vesicles. A certain number of forms develop, either all over their bodies or in certain parts only, various sorts of hollow vesicular outgrowths of the coelenteron (see Text-figs. 2, A, and 18). These may be slightly or very highly specialized. It may be argued that they are only verrucae which have gone farther, but in most cases they have gone a good deal farther, and really

TEXT-FIG. 1.



A. Small portion of the upper part of the body of Bunodactis alfordi, somewhat enlarged, to show the vertical rows of verrucae, three of them ending above in conical acrorhagi.
B. Half a transverse section of an acrorhagus of B. alfordi. Mesogloea black, ectoderm and endoderm white, the black strokes in the former representing nematocysts.

seem to constitute a definite and characteristic feature by which forms possessing them may be separated from those which do not. Since these forms also show an agreement among themselves in other ways, falling naturally into sets, we may fairly take ' presence of vesicles ' as a family character for use among others.

The presence or absence of a definite base seems a valid

[1] In this paper the term ' acrorhagi ' is used to cover ' marginal spherules ' of any sort, whether simple or compound, whether nematocyst batteries or not. There seems to be too much variation in their structure for it to be possible to maintain a serviceable distinction of them into acrorhagi, pseudo-acrorhagi, &c. A sketch of typical acrorhagi from Bunodactis alfordi is given in Text-fig. 1.

and useful distinction between the Ilyanthids and the (more or less) adherent forms, even though in special instances the Ilyanthid condition is partly retained or imitated by others. Text-fig. 7 shows the contrast between the two states. The conversion of the base into a definite float as in Minyas provides a third useful type.

Among the forms without acontia or mesogloeal sphincters one cannot make use of presence or absence of cinclides as might have been hoped. They have here excited so little interest that not much trouble has been taken to find them, and the range of their distribution is not really known. They are recorded in some forms such as Peachia and Harenactis, and I must record here that I have personally observed them very clearly in a species of Phymanthus—quite an unexpected find. It seems to me not unlikely, from noticing the ways of living anemones, that there may be discovered cinclides of some sort (even if only acrorhagial perforations) in some or even many families. A study of Actinia equina, Anemonia sulcata, Bunodactis gemmacea, and Tealia crassicornis in this connexion might reveal something quite interesting—and attention should be paid to the thin region just near the edge of the base, as well as to the rest of the body.

Among Stichodactylines we have to deal with characters of quite a clear-cut sort affecting form and arrangement of tentacles, and these provide simple and satisfactory family distinctions. (See Text-figs. 2, B, 14, 15, 19.)

Taking these remarks, together with the similar ones in Part I, we may list some of our more useful characters as follows:

Presence or absence of (i) a definite base, (ii) a float, (iii) cinclides, (iv) a distinction of the body into regions, (v) vesicles, (vi) a mesogloeal sphincter, (vii) acontia, (viii) mesogloeal disc-and-tentacle muscles, (ix) a division of the mesenteries into macro- and microcnemes, (x) macrocnemes over and above six pairs, (xi) perfect mesenteries over and above six pairs, (xii) more tentacles than one in connexion with some or

TEXT-FIG. 2.

A. Vertical section of a whole specimen of Phyllodiscus, to show two vesicles (*v*) and two tentacles (*t*) cut through. Mesenteries, &c., are omitted for clearness. B. Vertical section of a portion of the upper part of the body-wall and outer part of the oral disc of Cryptodendron. The section passes through many short tentacles (*t*), and although all do not belong to the same mesenterial chamber (mesenteries are omitted for clearness), there is not by any means only one tentacle to each chamber as at A. *s*, sphincter; *b*, body-wall.

all of the endocoels, (xiii) more tentacles than one in connexion with some or all of the exocoels, (xiv) permanent tentacle-bearing arms of the oral disc.

This is of course an incomplete list, but other characters not needing special mention here will reveal themselves in their respective contexts. None of the characters can be treated in an absolutely hard-and-fast way, and may need special consideration in special cases. Of those listed, nos. iv and viii affect genera more than families, but are interesting even if their presence or absence does not in itself determine the fate of a given form. No. vi has to be taken in connexion with the fact that sphincterless forms have to be included sometimes with forms which have a mesogloeal sphincter, sometimes with those possessing an endodermal one, or else alone, according to the sum of their other characters. Characters such as presence or absence of brood-pouches are not of much classificatory use.

There are many other things involved in classifying Anthozoa which will be pointed out in due course, but a few need special mention; they affect most, on the whole, groups larger than families. These may be taken one at a time.

(i) Presence or absence of ciliated tracts on the mesenterial filaments. These ciliated 'tracts' or 'pads' (Flimmerstreifen of German authors) are very definite structures, and their presence or absence seems to be one of the soundest indications we have of difference of tendency between one group and another. It forms also an easily-made-out character and one to which there is hardly any of the usual objection of intermediate conditions between presence and absence. Their loss, as I conceive it (or their non-development if it were that), by the corals and by certain anemones seems to constitute a very distinct evolutionary step, which may be seized upon for purposes of classification. Its usefulness both as a clue and as a sound distinction has been somewhat swamped by the amount of attention which the next character has absorbed; but I propose here to lay a good deal of stress upon it as being more valuable than no. ii. The

contrast between the kind of filament with ciliated tracts and that without may be seen from Text-fig. 17, where three of the four sorts of filament illustrated have the tracts (though not all the same kind of tract, in detail), and the fourth has none (c).

(ii) Presence or absence of ectodermal muscle in body-wall.—In this case we are dealing with a universal ancestral character which has been allowed to die out in most forms. It persists in those retaining most primitiveness, and is present, at least partially or as a vestige, here and there among more advanced forms, physiological causes probably accounting for its retention. It can therefore only be used in a limited way in a classification—useful in defining primitive groups, but not a criterion of relationship when it becomes a question of forms some of which have retained it, in greater or less degree, and others have shed it.

(iii) Presence or absence of spirocysts in ectoderm of body-wall.—This is another character about which a similar view may be taken to that developed in connexion with the last one.

(iv) Presence or absence of basilar muscles.—These muscles are natural developments correlated with the stabilizing of a well-marked basal disc. Their presence is certainly a good characteristic of the higher forms in general, but here again it may be misleading to think too much about them in connexion with transitional forms or forms of doubtful relationships. For purposes of family-definitions, it appears that the presence or absence of the base itself is the first consideration, basilar muscles or not.

(v) Presence or absence of any perfect metacnemes.—One set of forms (Gonactinia, Protanthea, and Oractis) seem well distinguished from others by virtue of the fact that they alone among Actinians (excluding Edwardsiids and odd individuals among Halcampas, Aiptasias, &c.) have the four couples of protocnemes (the eight 'Edwardsia-mesenteries') perfect, none of the metacnemes being so, with the result that there are no perfect

pairs. This, taken among other things, seems to mark them off pretty well from other primitives, and constitutes a character upon which one is inclined to lay more weight than has been done hitherto—it is another, though a less important one, the value of which has been somewhat overshadowed as in the case of the ' ciliated tracts ', by the discussion of ectodermal musculature. A diagram showing this type of mesenterial arrangement for comparison with others may be found in Text-fig. 16, B.

4. Special Discussions and Outline of New Scheme.

§ A. The Gonactiniidae.

This family has been made to include Protanthea, Gonactinia, Oractis, and Boloceroides. For purposes of this discussion we shall limit it to Gonactinia and Protanthea, with Oractis as a probable but insufficiently-known member. Boloceroides requires separate treatment. The Gonactiniidae, then, have in common a number of characters, most of them primitive. The smooth unspecialized body has a definite attachable basal end, but without any basilar muscles. The animal is small and delicate, and has both the inner and outer surfaces of the whole of its mesogloea covered by a weak generalized muscle-layer, not specially concentrated to form definite retractors or sphincters, and present in ectoderm of body-wall and actinopharynx as well as elsewhere. The body-wall ectoderm also shares the character of that of the tentacles in that it possesses spirocysts. The mesenterial filaments are without ciliated tracts, and only the first eight mesenteries to appear (i. e. the protocnemes, which arise as bilateral couples and not as pairs) are perfect (see Text-fig. 16, B). These undifferentiated forms seem to come nearer than any surviving thing to the probable ancestor of the Zoanthactiniaria (Text-fig. 16, A), which, whatever it was, must surely have had in common with them the small size and delicacy, the generalized musculature and generalized distribution of spirocysts, and the eight perfect mesenteries

only. Not only have the Gonactiniidae a good deal approximating them to this ancestor, but also there are no other forms of this grade which can fairly be placed in the same family with them. It seems that the family must be looked upon as one apart, and representative of past things; the remaining question, which will receive attention later, being the rank of the group to which it must be allocated.

§ B. Boloceroides.

This is a genus of uncertain affinities and needs unusually careful placing. Carlgren has thought of it as a Gonactiniid, and others as a Boloceroid. It certainly does not come within the Gonactiniidae as understood in Section A, nor even near it. The characters by which it may be defined, those which most affect us at the moment, are as follows. (i) There is a definite base, but (ii) no basilar muscles. The body is (iii) smooth with unspecialized margin. (iv) There is no sphincter. (v) There is ectodermal muscle in the body-wall. (vi) Spirocysts are present in the body-wall ectoderm. (vii) The tentacles are deciduous. (viii) Six pairs of mesenteries are perfect. (ix) The mesenteries are not divided into macro- and microcnemes. (x) There are ciliated tracts on the filaments, but (xi) no true siphonoglyphes.

Of these characters, the genus shares nos. i to vi and ix and xi with the Gonactiniidae. Character vii turns up also in Bolocera and Bunodeopsis, and need not trouble us, because it is a special feature which may be taken as a convergence—not necessarily a token of relationship with Bolocera, and certainly not with Bunodeopsis. Characters viii and x are the two of importance in which it differs from the Gonactiniidae, but they are rather fundamental. Boloceroides represents a different stage altogether, by its possession of ciliated tracts and its attainment of pairs of perfect mesenteries, although at the same time it retains several primitive traits. It shares five characters (i, iii, viii, ix, x) with the genus Myonanthus (a form which, as will be seen, requires special consideration), but

differs from it in six others. It becomes evident that if we treat the sum-of-the-characters principle woodenly and mechanically here, we shall run Boloceroides into the Gonactiniidae or near them; but that will not represent the truth. It is a case for weighing individual points, and the best we can do for the genus is to place it near Myonanthus. Opinion will differ as to the relative value of the various points, but taking the general line of this paper, nos. viii and x will count more heavily for its relationship (not close) with Myonanthus than all its points of similarity to the Gonactiniids. For, after all, most of those points may be summed up as aspects of one fact, the generalized nature of the structure; they are primitive features not shed, and these are more numerous than usual outside the Gonactiniidae. There are other forms with much clearer relationships which retain some of them, e.g. Bunodeopsis.

This means the inclusion of Boloceroides either in the same family as Myonanthus, or in a family to itself near the one containing the latter. Some of its differences from Myonanthus are of generic importance only (deciduous tentacles and lack of sphincter), and the question remains whether its ectodermal muscles and spirocysts in the body-wall, and its lack of basilar muscles and siphonoglyptes can separate it. Considering the fact that in other coherent families some at least of these things may be present or absent, it leaves the separation a matter of doubt. In the present paper, therefore, Boloceroides will be included in the Myonanthidae (see pp. 524, 545, 564, &c.), with the reservation that probably there would be no harm in having a separate Boloceroididae (under Endomyaria and next to Myonanthidae) if preferred. The genus is evidently a transitional one.

Any close relationship between Boloceroides and Bolocera seems a matter of doubt. Bolocera may well be a subsequent development of the same stock, which has attained larger size and, with this, numerous perfect mesenteries, retiring to deeper water and losing the primitive condition of body-wall, &c. This, however, is no argument for

placing Bolocera with Boloceroides, but is additional evidence for thinking of the former as an Actiniid, taking the view that will be developed below, that the Actiniidae are one of the next steps on from the Myonanthidae.

I am conscious that the arguments used in this section are rather dangerous, and that along some such line an attack might be developed upon the whole system of classification by summation of characters. But I feel that it is a special case, like one or two others, and that, as suggested in Part I (p. 470), the summation principle must not be used blindly like an arithmetical measure; looking upon it as useful typically, but needing modification here and there.

§ C. The Ptychodactidae.

Carlgren (1911) has shown clearly that two curious genera, very different in detail but similar in fundamentals (Ptychodactis and Dactylanthus), should be thought of together as forming one family. The debatable ground here is as to where the family fits into the general scheme. Carlgren includes it in his Protantheae with the Gonactiniidae. That the Ptychodactidae must be kept apart from the ordinary Actinians is pretty clear; also that they must come next to the Gonactiniids in a list. But apart from this general location, they seem to have very little to do with the Gonactiniids, and should be marked off from these by being placed in a group of their own and of higher rank than a family.

Of primitive characters they share with Gonactiniids the following: absence of basilar muscles although there is a base; similarity of structure between tentacles and body-wall—spirocysts and ectodermal muscle in both; sphincter little or none; mesenterial musculature weak, hardly forming retractors. They have no ciliated tracts on the filaments. On the other hand they have diverged from the Gonactiniids as regards size—they can get quite large—and have attained not only pairs of perfect mesenteries but often a good many of them. Ptychodactis has become very broad and has almost lost its actinopharynx (a unique case), and has numerous tentacles

and mesenteries. Dactylanthus has a good actinopharynx but has tentaculiform outgrowths of the body, curious actinopharyngeal pouches, and a fusion of the lower ends of the mesenteries into a columella-like network. Further, both genera are unique in two ways: firstly, the upper extremities of the filaments of the imperfect mesenteries are modified into curious structures like bisected funnels, the analogy of which

TEXT-FIG. 3.

One-half of a specimen of Paradiscosoma. Note the cup-shaped form, mouth on a cone at the bottom of the cup, tentacles reduced to knobs lining the cup. Mesogloea, &c., black. The base was injured, and is not fully shown. The tentacles have narrow 'stems' running through the thick mesogloea of the disc.

among other forms it would be difficult to suggest; and, secondly, the gonads and filaments are confined to different parts of each mesentery, the free border of the latter (or what corresponds to it in Dactylanthus) being occupied by filament above and gonad below, quite an unusual state of affairs.

From this one would judge that the Ptychodactids are a collection of curiosities which have diverged along a little

line of their own. Since they are in some ways primitive we may place them next to the Gonactiniids for convenience; but because of their peculiarities they should be kept sufficiently apart from those to represent a quite distinct evolutionary line. The exact rank of the group Ptychodacteae which I propose for their reception will be better discussed in other sections (see pp. 540, 552, 554–6, &c.).

§ D. The Corallimorphidae and Discosomidae.

There has been a growing feeling among those who have worked at anemones that there is a good deal of inter-relation between them and the corals, and that we can no longer insist on a separation of them based on presence or absence of a skeleton alone. This feeling has been best expressed by Duerden (**120**) in a study of the Madreporarian relationships of certain Stichodactylines. Perhaps in this connexion too little attention has been paid to the soft parts of corals. We are undoubtedly justified in retaining two groups, Actiniaria and Madreporaria; but the justification is to be found in the sum-of-the-characters principle, and not in the presence or absence of skeleton merely. The reservation is, that if we maintain these two groups we must include in the Madreporaria some forms without skeleton. I am not familiar enough with Madrepores to generalize about them, but am relying on the details given in Duerden's paper—from which I gather that there are certain aspects of their soft parts which present a fair degree of uniformity through the group. With the Actiniaria, as hitherto limited, this is not the case; but if certain forms were removed from among them it would be so to a more reasonable extent. There are two families of forms, hitherto called anemones, which have all the characteristics of coral-polyps save a skeleton—in fact which are corals but for that one thing. If these two families be removed from the Actiniaria and placed under Madreporaria in some way, the division into anemones and corals at once becomes more intelligible, and various difficulties disappear. The families in question are the Corallimorphidae and Disco-

somidae,[1] both 'Stichodactyline'. One advantage of placing these with the corals is that they are not like the remaining true Stichodactylines, which apart from them form a harmonious group (see p. 533).

Two further points arise: (i) are there any corals with the Stichodactyline arrangement of tentacles? and (ii) to which Madreporarian families do our forms most nearly approach? With regard to the first it does not much matter, for a Stichodactyline condition of tentacles could arise as a convergence anywhere, and has done so among the Ceriantharia. As to the second it is for a coral expert to suggest, and pending further investigation the families should simply go under Madreporaria without closer allocation.

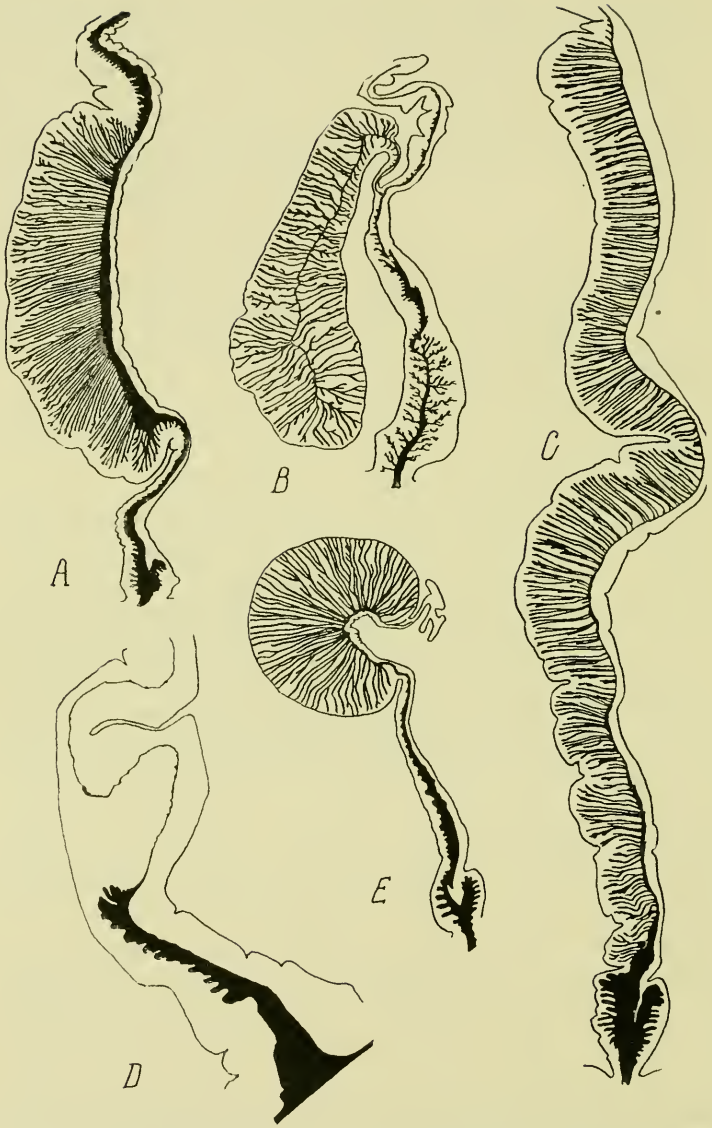
A vertical section of one of the animals in question is shown in Text-fig. 3. It is a cup-shaped form in which the tentacles have become reduced to mere knobs.

What are the points which make these forms like corals? A general statement about them might be made as follows:

They secrete no horny or limy skeleton. They may be quite solitary, or quite gregarious, sometimes living in sheets or carpets. Frequently they reproduce by fission, and often compound individuals with several mouths, or individuals connected by a basal coenosarc are found. The base is adherent. The body is without verrucae, variable in form and consistency. More than one tentacle connects with at least the older endocoels. The tentacles may be simple, or capitate (cf. Caryophyllia and others among corals), or branched; or small and wart-like, or even reduced to so little as to be invisible externally. There are no siphonoglyphes (or rarely?). The mesenterial filaments have no ciliated tracts. Sphincters are feeble or absent. Sting-cells of a size characteristic of Madreporaria, but not of Actinians in general, are usually found somewhere in the body. There are usually a good many

[1] The Discosomidae as referred to in this connexion means the family taken in Carlgren's sense, 1900, p. 58, and not in the wider sense of some authors—including only the genera Discosoma, Paradiscosoma, Actinotryx, Rhodactis, Orinia, and Ricordea.

TEXT-FIG. 4.



Transverse sections of mesenteries, to show various types of musculature. Mesogloea black, endoderm white. A, Epiactis; B, Aureliania; C, Cryptodendron; D, Actinotryx; E, Phymanthus.

perfect mesenteries, and no distinction of mesenteries into macro- and microcnemes. The longitudinal mesenterial muscle consists typically of a feeble layer, not forming the sort of sheet or retractor characteristic of anemones. There are no basilar muscles, and directives may be present or not. The ectoderm of the body-wall may or may not contain a weak muscle-layer. The mesogloea is Madreporarian rather than Actinian.

Text-fig. 4 shows the contrast between various sorts of Actinian mesenterial musculature and the sort of thing found in these 'soft corals'. In the former there may be seen dendrites or processes projecting from the general mesogloea for the support of the muscle-fibres. In the soft corals the surface of the mesogloea is typically either straight or lobed as at D, but has a weak fringe of muscle-fibres directly upon it, not elevated on processes. The sort of thing is better seen in Text-fig 5. Text-fig. 6 shows Discosomid sting-cells contrasted with typical Actinian sting-cells from acontia and acrorhagi, &c. The general difference in size between A and B ('soft corals') and the others is very marked. C is unusually large for an Actinian cell, D and E providing more average examples. A Discosomid filament, showing the absence of ciliated tracts, is to be seen in Text-fig. 17, C.

A microscopical study of a few of these forms at once suggests a difference from the anemone type running through the histology and other things. Even when anemones have weak musculature it has a different appearance. These are things which one cannot well bring out in figures without an extensive histological demonstration, but are easy to see in actual sections. The curiously feeble mesenterial musculature, the presence of very large sting-cells, the absence of ciliated tracts, the appearance of the mesogloea and cell-layers, the lack of siphonoglyphes, the tendency towards compound individuals and colonies, the weak or absent sphincters, and sometimes the strong permanent actinopharyngeal ridges and form of the tentacles, and so on, are points which, taken together, suggest Madreporaria, of some or all of which they

appear, generally speaking, to be characteristic. One or other of them may be found among anemones, but their com-

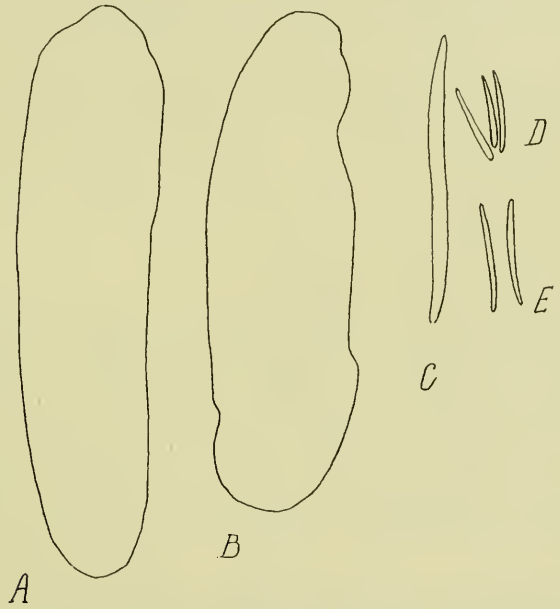
Text-fig. 5.

Transverse section of a mesentery of Paradiscosoma treated in the same way as those in Text-fig. 4. Note the heavy mesogloca (black) and absence of muscle-processes.

bination indicates coral affinities. Their distinctness from anemones in general struck me decidedly, before I thought of them as corals.

The presence of ectodermal muscle-fibres in the body-wall of Corallimorphus, &c., is doubtless a survival. Whether the weak general musculature is primitive in this case it would not be safe to say; there is much to suggest that it is a well-established thing here. Some of the other characters

TEXT-FIG. 6.



Sting-cells. All are drawn to same scale, as seen with $\frac{1}{6}$ objective and no. 3 ocular. A (Actinotryx) and B (Paradiscosoma) show the size characteristic of many 'soft coral' sting-cells. C is an unusually large Actinian cell from acontium of Artemidactis, and D (acrorhagi of Bunodactis alfordi) and E (Halcampa aspera, body-wall) show a more average Actinian size.

suggest advancement—the tentacles and their specialization of form and arrangement, the big sting-cells, numerous perfect mesenteries, and the sometimes thick and rigid bodies. The condition of mesenterial filaments they share with all corals. Taking them all in all suggestion of primitiveness here would

be much less safe than in the case of Gonactiniidae or even Ptychodactidae.

The Actiniaria as freed from extraneous skeletonless corals show general tendencies towards more complex individuality rather than towards colonial development, towards a special development of musculature in some way or another, towards different histology and on the whole more activity. They go in for expression of permutations and combinations of various characters, leading to great diversity—this diversity affecting differences among polyps, whereas it is perhaps more connected with variation of skeleton and colony-form, among corals, which may to some extent be compared with the Alcyonaria, although of course the latter much surpass them both in uniformity of the individual and diversity of the colony.

§ E.

The discussions so far have dealt with curious forms which, whatever their fate, are special cases, coming outside the main mass of anemones. Those that follow are concerned with forms the general position of which is fairly clear, i. e. they all come under the main tribe (Nynantheae in the sense taken on p. 540) of the sub-order Actiniaria, excluding Edwardsians, Zoanthids, Gonactiniids, Ptychodactids and corals whether hard or soft—or to put it another way, they are presumably the descendants of a muscular Halcampa-like stage (cf. Text-fig. 8) with ciliated tracts on its filaments. Among these forms there seem to be four main sets which can be followed, and in the following sections the exceptional sets will be considered before the majority-forms.

§ F. The Ilyanthidae.

There has been a family Ilyanthidae in use for a long time ('Actinies pivotantes'), for the more or less vermiform creatures with no adherent base. It has been subdivided somewhat arbitrarily—that it needs subdivision is not in question, but how to do it. Although, however, we are obliged

to have more than one family, it seems wise to retain the old plan to the extent of having a group to cover them, the principle of which is good. This group must be labelled by Carlgren's name Athenaria, with the Edwardsiids of course excluded. The rank of this group will be discussed in a later section, but here we may consider the general characters justifying it.

The Athenaria appear to be the representatives of those forms which, being the outcome of a muscular Halcampa-stage, have retained more similarity to their ancestor than the majority of other forms, and have kept to a more or less burrowing life. There is variation in size; the predominating shape is vermiform, the relation of length to diameter varying in different cases and different states of expansion, diameter sometimes considerable. Text-fig. 7 shows the contrast between some of these and one of the ordinary adherent anemones with short wide form. In these Athenaria the aboral end is not a definite base, but a rounded physa, which is sometimes able, however, to adhere to small objects. There is little or no sphincter. Often there are cinclides. The number of tentacles is usually small, and at most does not pass about forty. The number of mesenteries is similarly limited, and either these all have the grade of macrocnemes, or else there is a division into macro- and microcnemes—and in Peachia the state of affairs is intermediate. The mesenterial filaments have ciliated tracts.

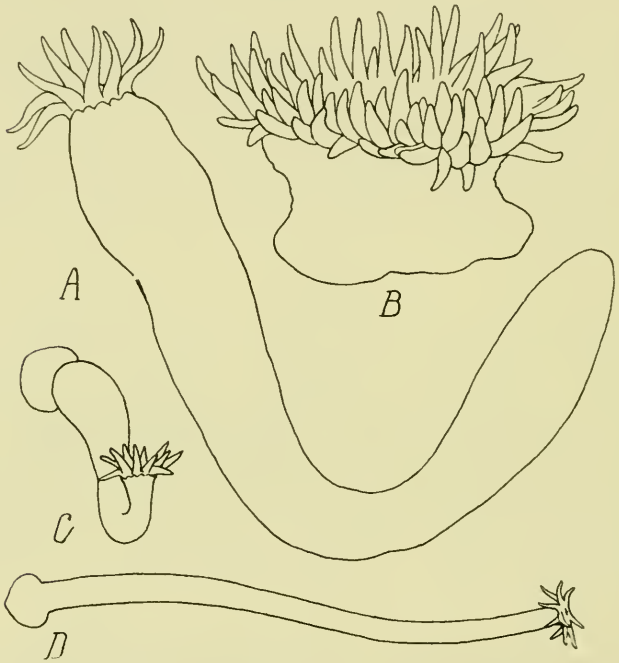
The above may be taken as a sort of definition of the burrowers or Ilyanthids. The subdivision of the group remains to be discussed.

Of course, some of the forms formerly included here have long since been removed, others more recently—the Cerianthria and Edwardsiaria. Forms with no base but with acontia are little known, but seem to fit in quite well with the Phelliidae (see Part I, p. 524), though possibly a new family may later on be needed for them. Carlgren has suggested a Halcampactidae, but it is here treated as coming under Phelliidae. Andvackiidae is not yet established. For Halcampactis see Part I, pp. 499, 509, 525.

The forms we are here concerned with are Halcampa, Halcampoides, Pentactinia, Scytophorus, Harenactis, Eloactis, Peachia, Haloclava, Ilyanthus, and Andresia.[1]

If we go into detail about all these forms we shall find that

Text-fig. 7.



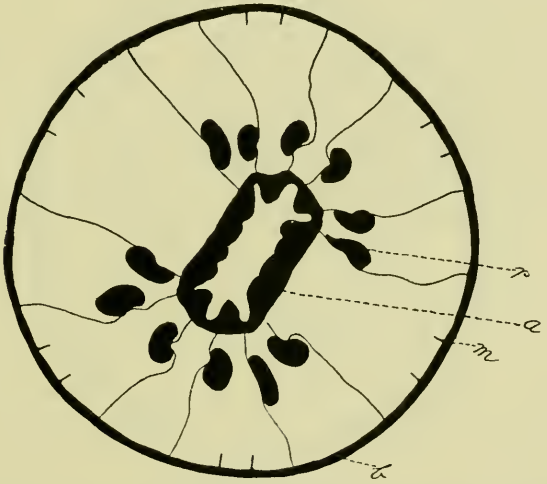
A, Peachia hastata; B, Tealia crassicornis; C and D, Halcampa chrysanthellum. To emphasize the contrast between burrowing and adherent forms. All are natural size.

almost every one could claim distinction for one reason or another; because there is diversity in rather important ways. But it would seem extravagant and hardly justifiable to give a family to each, and failing that we have to do the best we

[1] Andresia is a new name for Ilyanthus parthenopeus, which is quite unlike the more typical British I. mitchelli, and has to be separated as a distinct genus with new name. This will be formally established in Part III.

can, allowing a fair latitude of definition. It is possible to gather them into three fairly clear sets, which must be our families. It seems impossible to be content with a subdivision which has already been suggested, and based on the nature of the sphincter only—into Halcampidae, Halcampomorphidae, and Ilyanthidae. This, among other things, means that Halcampa and Halcampoides go into different families, and this seems to be straining things.

TEXT-FIG. 8.



Transverse section of Halcampa chrysanthellum, showing six pairs of macrocnemes and six pairs of microcnemes. *a*, actinopharynx; *b*, body-wall; *m*, microcneme; *r*, retractor. (After Haddon. See acknowledgement on p. 496.)

Taking first the genera Halcampa, Halcampoides, Pentactinia, and Scytophorus, we can make for these a fairly precise definition, and call them Halcampidae. They are Athenaria of more or less vermiform shape, with or without suckers or papillae or cuticle or incrustation on the body. There may be cinclides in the physa. The tentacles may be 8–12, 14, 20, or more, and their longitudinal musculature is ectodermal. The sphincter is absent, or weak endodermal, or weak mesogloeal. The mesenteries have as their

main feature six pairs of macrocnemes; but there are variations; the full six pairs may not be developed (Pentactinia and some individuals of Halcampa), or there may be an extra couple (Scytophorus). Microcnemes may be present or not.

This idea regards the genera Halcampa and Halcampoides as constituting, jointly, types of the family, and no separation of these on account of sphincter is wise. It brings in Pentactinia and Scytophorus, the one as a slightly under-developed, the other as a slightly over-developed, Halcampa-form. Indeed, these two are very like Halcampas but for mesenterial oddities slightly deviating from type. A case parallel to that of Pentactinia is that of Decaphellia, a Phelliid with subnormal number of macrocnemes. Text-fig. 8 shows a transverse section of a Halcampid for contrast with that of one of the Ilyanthidae in the strict sense as described in the next paragraph and illustrated in Text-fig. 9.[1]

If we now take the genera Ilyanthus (mitchelli), Harenactis, Eloactis, Peachia, and Haloclava, we find a rather different type of structure. The mesenteries are never fewer than ten pairs in adult animals, and vary up to about eighteen pairs. They all have virtually the grade of macrocnemes, even though there may be differentiation among them—except that in Peachia some of them are devoid of filament and gonad, but have strong retractors and are not microcnemes. For the rest they often attain fair size and may have stout bodies (capable of becoming vermiform) or very long ones. Suckers present or absent. Cinclides may occur. Tentacles simple or capitate, eight, twelve, twenty, or more, up to about forty. Little or no sphincter. There may be only one siphonoglyphe, which in Peachia is specialized into a conchula. In Peachia we have six perfect pairs of mesenteries (or rarely fewer ?) and four secondary pairs; in

[1] In this figure the gaps in the mesenteries are due to the fact that the section passes through the region of mesenterial stomata—in most regions the mesenteries would be continuous.

Eloactis and Haloclava ten pairs, all perfect; in Harenactis twelve pairs in two cycles, all perfect; in Ilyanthus the number of mesenteries varies, but is the same as the number of tentacles, and all are perfect—but there are some individual peculiarities as well.

Unless there is to be much multiplication of families the above arrangement seems the best.

TEXT-FIG. 9.

Transverse section of Eloactis mazeli. The gaps in some of the mesenteries are due to mesenterial stomata. Ten pairs of macrocnemes and no microcnemes. *a*, actinopharynx; *b*, body-wall; *r*, retractor. (After O. M. Rees. See acknowledgement on p. 496.)

There remains the case of Ilyanthus parthenopeus—or Andresia parthenopea as it must now be called. This form does not seem to fall in well with the usual idea of Ilyanthid structure, apart from its form and rounded aboral end. It has long tentacles in four regularly-graded cycles, and twenty-four pairs of mesenteries in three graded cycles. The mesenterial musculature appears to form only a weak layer, not rising into a thick (and typically circumscribed)

retractor or pad as in all other Ilyanthids. The body-margin is notched in a way suggestive of acrorhagi. In fact, but for its burrowing habit and rounded end, it would be a typical member of the Actiniidae of the less muscular sort. Whether it is an Ilyanthid which has passed the usual grade of development and moved towards that of adherent forms, or whether it is a retrograde adherent which has gone back to buried life and lost its base, we cannot tell. But in classification it ought to be separate, or probably go nearer the early Actiniids than the Ilyanthids. I have in this paper made a family Andresiidae for it, placing this among the earlier Endomyaria (see Part III).

With regard to other forms without bases excluded from the Athenaria (see Part I) these fit in better with the Mesomyaria (see pp. 541, &c.) than with the Athenaria, because of their acontia and mesogloeal sphincter, &c. In the case of some of them (Phelliidae in part) we have our finger on the transition from burrowing to adherence, and there are grades from a physa to a well-marked base ; and as these seem to be getting up to the attached stage it seems better to keep them out of the Athenaria, especially since their acontia and mesogloeal sphincter and other things show their relationship to be with the Mesomyaria. Some of the Diadumenids are also almost without base, but here it is obviously a case of retrogression or arrested development ; they are probably normally adherent forms changing under special conditions.

§ G. The Endocoelactids.

These forms start from a six-pairs-of-muscular-mesenteries or Halcampa-stage basis, with ciliated tracts on the mesenterial filaments, but work onward from this in quite an unusual way. The secondary mesenteries appear in the endocoels of the lateral primaries, and all of them have the character of directives (i.e. the retractors of each pair face away from one another). The usual plan is, of course, for the secondaries to appear in the primary exocoels, and have their retractors vis-à-vis. The contrast is indicated in Text-fig. 16, G being an Endocoelactid. Apart from this most fundamental structural

aberration, the Endocoelactids are sphincterless, and nearly always have spirocysts in the body-wall ectoderm. There is a definite base. The form of body and tentacles is variable, and may be ordinary, but the wall may be thick and heavy, the disc lobed, the tentacles often with aboral basal swellings. In fact we find here a tendency not found on the main line of Endomyaria (see p. 541, &c.) or 'endodermal-sphinctered' anemones, towards a deep-water specialization similar to that which we found earlier on in certain Paractids and Actinoscyphids, &c. (see Part I). Taking them as wholes, the Endocoelactids are a set very different from average forms, being apparently a little line of evolution to themselves; and as such they should have slightly higher rank than that of a family, forming a group Endocoelactaria equal in level to the Athenaria.

Carlgren includes Endocoelactids in his Protantheae along with Gonactiniidae and Ptychodactidae; but since they seem evidently derived from a muscular Halcampa-like ancestor with ciliated tracts, and have no ectodermal muscle in their body-walls, I cannot see the merit of that plan, or accept it. (See also pp. 541–2, 560, &c.)

There are among the Endocoelactids two rather clearly marked out groups, one of them containing Halcurias and Carlgrenia, the other Actinernus and three related genera. The two groups seem to have fairly good claims to be regarded as families, and as such they are defined later on in this paper (Part III). There is in one of the families practically a division of the mesenteries into macro- and microcnemes (macrocnemes six to ten pairs, with circumscribed retractors, gonads, and filaments; microcnemes confined to upper part of body except for four pairs of them in Carlgrenia—some of them may be perfect, but without retractor, gonad, or filament), and also there is constantly one siphonoglyphe only and no tendency to lobing of disc or tentacles. These forms, especially Carlgrenia (Text-fig. 16, G), are nearer their Halcampid ancestor than the others. In the other family we find the lobing tendency and charac-

teristically thick body-walls, two siphonoglyphes, and numerous mesenteries, the older ones at least fertile and not much marked off from the others, many being perfect and their musculature not strong. The first family is the Halcuriidae sens. strict., the second the Actinernidae.

§ H.

The next five sections will deal with the 'Sea-Anemones' in the narrowest sense (i.e. such of them as were not dealt with in Part I), the usual forms, the majority-forms, exclusive of atypicals such as Athenaria and Endocoelactaria and the pre-Halcampid groups.

§ J. The family Actiniidae.

This family, containing our commonest and most familiar anemones, has been the subject of a good deal of discussion and fluctuation. As it is usually understood at the moment, it is not much more homogeneous than the old group 'Paractidae', but contains three distinct types of mesenterial arrangement. Any discussion of it involves also the families Bolocerídae and Bunodidae, and these points will be dealt with in order.

Firstly, the Actiniidae. If we consider the aggregate of genera usually included here—Actinia, Anemonia, Condylactis, Gyrostoma, Actinioides, Condylanthus, Myonanthus, Macrodactyla, and others, we find three types of mesenterial formula, as follows :

(i) In Condylanthus the mesenteries are divided into macro- and microcnemes, the macrocnemes numbering six pairs (cf. Text-fig. 16, c).

(ii) In Myonanthus and Macrodactyla there is no division of mesenteries into macro- and microcnemes, but only six pairs are perfect (cf. Text-fig. 16, D).

(iii) In the others there is no division of mesenteries into macro- and microcnemes, but there are numerous perfect mesenteries as a rule, always more than six pairs in adults (cf. Text-fig. 16, H, and Text-fig. 10).

Without going over the old arguments again, we take it that if the ideas advocated in this paper be accepted at all, it will have been made clear in Part I that forms exhibiting these grades of mesenterial development need separation. We have, therefore, at once three families, Condylanthidae, Myonanthidae, and Actiniidae sens. strict., and these will be defined in Part III. This gives a homogeneous and intelligible Actiniidae, and has the advantage of providing two

TEXT-FIG. 10.

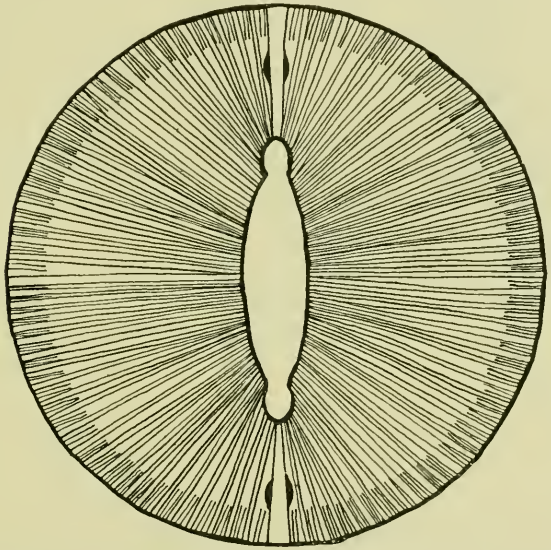


Diagram of a transverse section of Phymactis clematis, showing numerous perfect mesenteries.

families as links between the Actiniidae and Halcampidae, from near which they presumably arose. The three families might be compared with, for instance, the Diadumenidae, Metridiidae, and Sagartiidae of Part I—in which we have the same three grades of mesentery development, but acontia and cinclides and mesogloeal sphincter in all. In our new trio there is a common absence of acontia and mesogloeal sphincters and also of vesicles—as to cinclides it is hardly safe to say anything.

It has been so generally recognized that smooth-bodied and verrucose forms, and forms with and without acrorhagi cannot be separated into different families, that it seems hardly necessary to discuss this here.

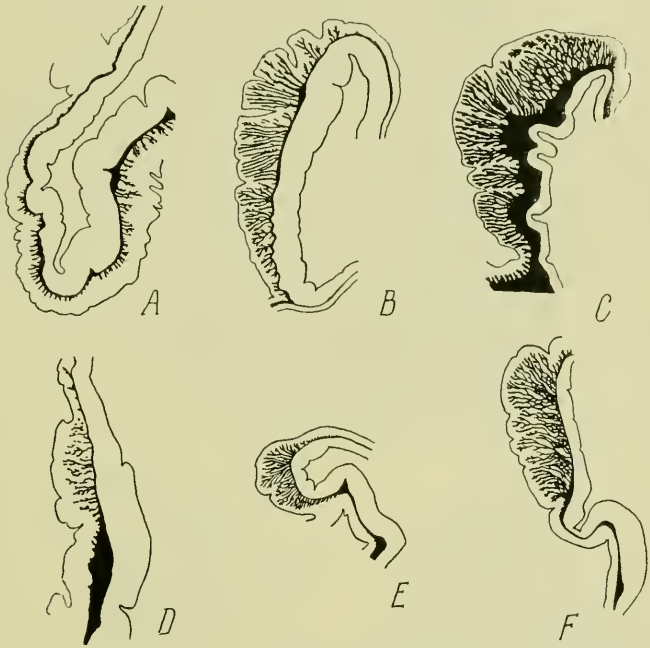
Secondly, there is the question of a separate Boloceridae. Such a family has been in use by some authors, and originally I felt a need for it (see 1918 A, p. 19), but further work has changed that feeling. It hardly seems that the deciduous tentacles are a character giving the Boloceras any right to separation, and otherwise they are exactly Actiniidae. This is especially the case since Boloceroides and Bunodeopsis have also the deciduous tentacles, and neither of them could be included in a Boloceridae in any case. One has to think of the cases as convergences. Even if Bolocera and Bunodeopsis should be further stages, along different lines, from a Boloceroides-like ancestor, this is no reason for classing the three together.

Thirdly, the Bunodidae. It seems a pity to have to attack an old-established family like this, but at the same time there seems to be no valid way whatever of separating it from the Actiniidae (in the revised sense), with which it is continuous.

Originally the Bunodidae relied for separation upon their verrucae and their strong circumscribed sphincters. The verrucal character was swept away by Epiactis and Isotealia, which are without it. We must now tackle the sphincter. In the first place the sphincter in Bunodactis (Bunodes) itself is variable, and often not a strong one. In the type-species, B. gemmacea, it may be half diffuse in some cases (I have sections of a very typical specimen showing this—see Text-fig. 11, D), and poorly developed. It is in Tealia and Epiactis (Text-fig. 12, A, B, C) that the really strong sphincters are found, and even there the size varies with species and individual. Further (this will be dealt with again under Bunodactis in Part III), there are apparently no criteria by which Bunodactis can be separated from Anthopleura and Actinioides, even generically—the three run right into each other and really form one large genus

varying as to sphincter from weak and diffuse to fairly strong and circumscribed—with too many grades to draw a line anywhere (a few are illustrated in Text-figs. 11 (D, F) and 12 (D, E)). And Actinioides is one of the genera hitherto

TEXT-FIG. 11.



Vertical sections of the sphincters of some Actiniidae, all drawn to same scale; showing various grades. A, Condylactis (here the sphincter is absent or practically so, what is shown being simply the upper part of the ordinary endodermal circular muscle). B, Actinia equina; C, Bolocera tuediae; D, Bunodactis gemmacea; E, Anemonia sulcata; F, Bunodactis alfordi. B and C are typical diffuse sphincters. D, E, and F are more of the circumscribed-diffuse grade. In all of them mesogloea is black, ectoderm and endoderm white.

classed as Actiniid. Proceeding still further towards the typical Actiniidae, if a comparative study of, for instance, Bunodactis (Anthopleura) alfordi, a Condylactis, and Anemonia sulcata be made, there is too much

similarity between them for any separation greater than generic to affect them.

In B. alfordi and A. sulcata there is a definite base, there are acrorhagi, long tentacles, lax habit of body only able to retract with great difficulty, similar habitat, weak to moderate circumscribed or circumscribed-diffuse sphincter (Text-fig. 11, E, F) (sometimes more diffuse in sulcata), numerous perfect mesenteries with fairly strong retractors, gonads on most of the older mesenteries, and the longitudinal musculature of the tentacles ectodermal. The chief difference is that B. alfordi has rows of verrucae which A. sulcata has not, and of course lesser species-differences. But obviously the relation is too close for the two to be included in different families, which has been done hitherto.

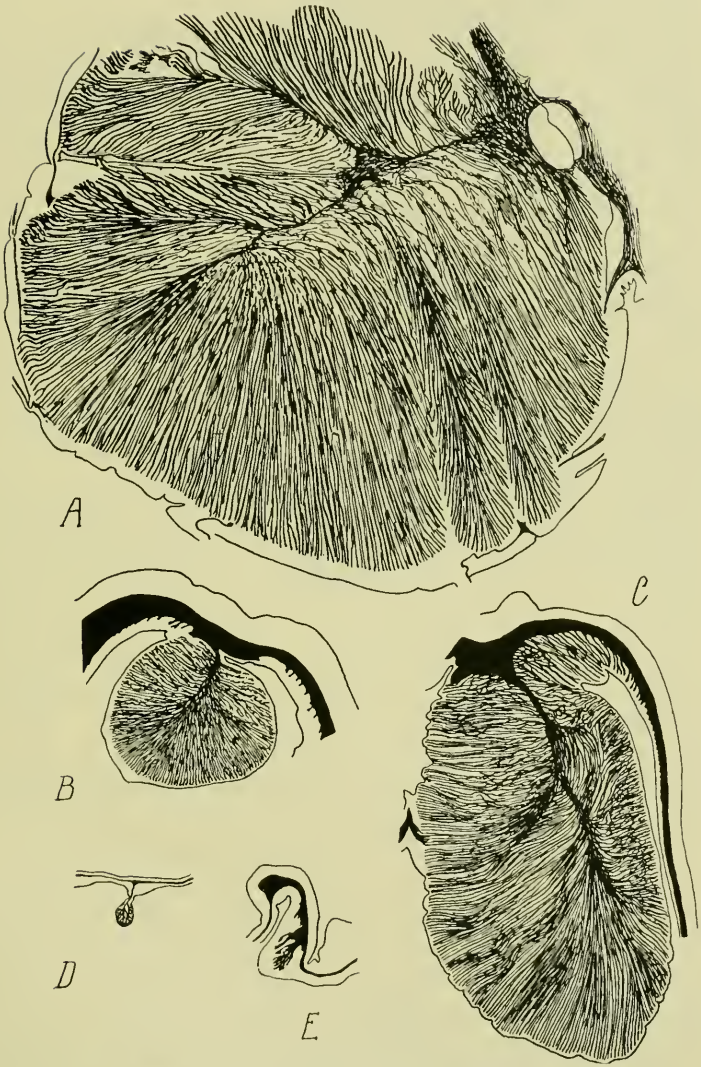
In B. alfordi and a Condylactis of which I have specimens, there is a definite base, there are verrucae, good tentacles, lax habit, numerous perfect mesenteries with fairly strong retractors, gonads on most of the older or all the mesenteries and ectodermal tentacular muscle. Here the main differences are lack of acrorhagi and a sphincter in the Condylactis. Between the points here given the similarity of the three genera should be clear. It is not always easy to distinguish them from each other if dealing with preserved material.

These things being so, where is the line to be drawn between Actiniidae and Bunodidae? Given a series of forms—such as Anemonia, Condylactis, Bunodactis (incl. Actiniodes and Anthopleura), Tealia—where is the division?

Condylactis gives us verrucae but no acrorhagi and little or no sphincter; Anemonia has the acrorhagi but no verrucae, and a weakish circumscribed or diffuse sphincter; B. alfordi has both verrucae and acrorhagi and a moderate sphincter, ± circumscribed (its relations showing other grades); and Tealia has verrucae (and rarely acrorhagi) and strong circumscribed sphincter.

The conclusion seems to be, clearly, that Bunodidae must be abandoned altogether. It should be noted that this does not impair the homogeneity of the Actiniidae, except as regards

TEXT-FIG. 12.



Further sphincters of Actiniidae for comparison with those in Text-fig. 11. All are to same scale as Text-fig. 11, and treated in the same way, but here we have various grades of circumscribed sphincter. The difference in size between A and B, for instance, is not due to a corresponding difference in size of the individuals from which they were taken. A, Epiactis sp.; B, Tealia crassicornis; C, Epiactis novozealandica; D, Bunodactis sp.; E, Bunodactis balli.

grade of sphincter ; and that, it is evident, is bound to vary within the limits of some families.

A series of sphincters is illustrated in Text-figs. 11 and 12, all of them being taken from Actiniidae in the new sense. A more evenly graded set could, I think, be made, but I have not material for it. But this one brings out the facts that I have wished to emphasize fairly well.

§ K. The Forms with Vesicles.

The anemones provided with vesicles should (see p. 500) be kept apart from those without them, but among themselves there are two kinds at least.

Taking the vesicled genera together, one can list nine clearly-distinguished ones—Alicia, Phyllodiscus, Thaumactis, Bunodeopsis, Phyllactis, Phymactis, Cradactis, Cystiactis, and Lebrunia.

There have been families in existence to cover these forms (Aliciidae, Phyllactidae, Dendromeliidae, Thaumactidae), but the definitions have been based chiefly on the form and situation of the vesicles, and this seems as unnatural as it used to be to separate Hormathia, Chitonactis, Chitonanthus, and Chondractinia on account of variation in ridges and tubercles ; and it has not been a very intelligible arrangement. So long as the vesicles are present, that is the family-character ; their form and situation are more questions of generic distinction. From this point of view the families fall to the ground. The Dendromeliidae lapses in any case ; it was formed to cover Lebrunia and Ophiodiscus. Ophiodiscus seems to be a typical Paractid (see Part I, p. 560), and in the present state of our knowledge it seems very doubtful whether, although it is a distinct enough genus, there is anything to keep Lebrunia out of the Phyllactidae. The genus Thaumactis does not seem worthy, as we know it, to have a family to itself either. The other two families (Aliciidae and Phyllactidae) must be retained, but revised in the light of mesenterial arrangement, &c.

(*a*) Alicia and Phyllodiscus are delicate creatures with vesicled scapus and naked capitulum, or with the vesicles

at or at and above the scapo-capitular junction. There is little or no sphincter, and only six pairs of mesenteries are perfect. For these the name Aliciidae should be kept, and for these only.

(*b*) The other genera are provided, usually, with numerous perfect mesenteries, have various arrangements of vesicles, may be less delicate, and have sometimes circumscribed sphincters. Some of them have mesogloeal longitudinal musculature in the tentacles. This collection has to be covered by the name Phyllactidae, and at that it had better be left for the moment. A fuller discussion of the family will fit better into Part III, where the generic definitions will be available for reference.

§ L. The Actiniidae and Vesicled Forms together.

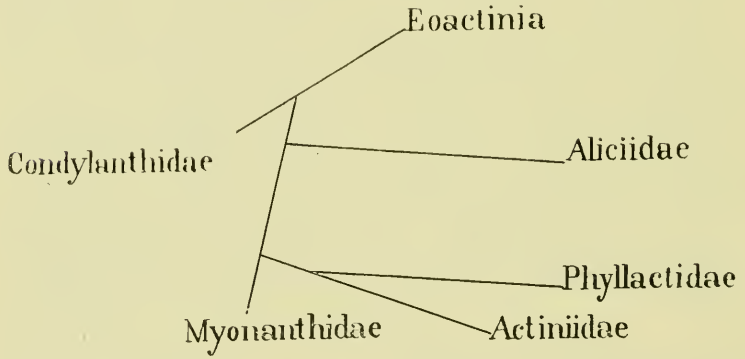
If the old Actiniidae and Bunodidae and Boloceridae (re-sorted into the new Actiniidae and Condylanthidae and Myonanthidae) and the Aliciidae and Phyllactidae be taken together, a mass of forms is presented exactly comparable to the set classified in Part I. It is worth while seeing whether they will work into a companion table like that on p. 481 in Part I. It is unfortunately necessary here to leave out cinclides, as there are not enough data about them. And of course absence of acontia and mesogloeal sphincter go right through.

	No Vesicles.	Vesicles present.
Mesenteries divided into macro- and microcnemes. Number of macrocnemes six pairs.	Condylanthidae. Condylanthus. 7	No known representatives.
Mesenteries NOT divided into macro- and microcnemes. Number of perfect mesenteries six pairs.	Myonanthidae. Myonanthus. Macrodactyla. Boloceroides. Nevadne. 7	Aliciidae. Alicia. Phyllodiscus. 8
Mesenteries NOT divided into macro- and microcnemes. Numerous perfect mesenteries, or at least more than six pairs in normal individuals.	Actiniidae. Actinia. Bolocera. Anemonia. Leipsiceras. Gyrostoma. Ixalactis. Condylactis. Pseudophellia. Bunodactis. Bolocerops is. Tealia. Glyphostylum. Epiactis. Parantheopsis. Isotealia. Dofleinia. 6	Phyllactidae. Phyllactis. Lebrunia. Phymactis. Thaumactis. Cradactis. Bunodeopsis Cystiactis. 6

In this table the number in the corner of each square is the number of characters which the members of the family in that square have in common.

Here the same characters as before go down the side of the table, but there are fewer to go above. And even without using the full number of combinations possible there is an empty square, no forms being yet described to fill it. Perhaps some will turn up, or perhaps it indicates that vesicles are structures not developed at stages of mesenterial evolution such as that represented by the Condylanthidae.

The diagram representing evolution in this group, as far as one may understand it, and for comparison with that given on p. 504 in Part I, would be as below. More will be said about it in the evolutionary section of the paper. An ancestral Eoactiniia corresponding to the Eosagartia on the other line, may be imagined—a good deal like a Halcampoides.



This diagram shows the Aliciidae and Phyllactidae as parallels, and involves the assumption that they arose independently from the main line, as some of them at any rate may probably enough have done. If they had a common origin among the pre-Actiniids, and the Phyllactids changed their mesenterial arrangement afterwards, or if some Phyllactids arose from early Actiniid forms and others from Aliciid forms, that would modify the diagram, but it is all speculation. Further details about it will be found under Phyllactidae in Part III.

§ M. The Minyadidae.

Probably unrelated forms have been placed here. There is little evidence of their relationship to each other, and there are few data altogether. It may later be found that there is no need for a Minyadidae, and that the contained forms may be allocated to different families as floating members. One form at least, Nautactis olivacea, Les., seems to be some sort of Stichodactyline. At the moment only Stichophora torpedo, Bell, can be defined, so far as I am aware, and that not fully; but for this form there seems to be justification for a family Minyadidae, even if it is not very clear, based on the definitely float-like character of the base taken with other things. At the present time it seems inopportune to say much about it, with the provision that so far there is no evidence of its ability to sustain higher than family rank, and it seems to fit in near the Actiniidae. If further details come to light—if, for instance, S. torpedo should have no ciliated tracts on its filaments—the position of the family will need reconsideration.

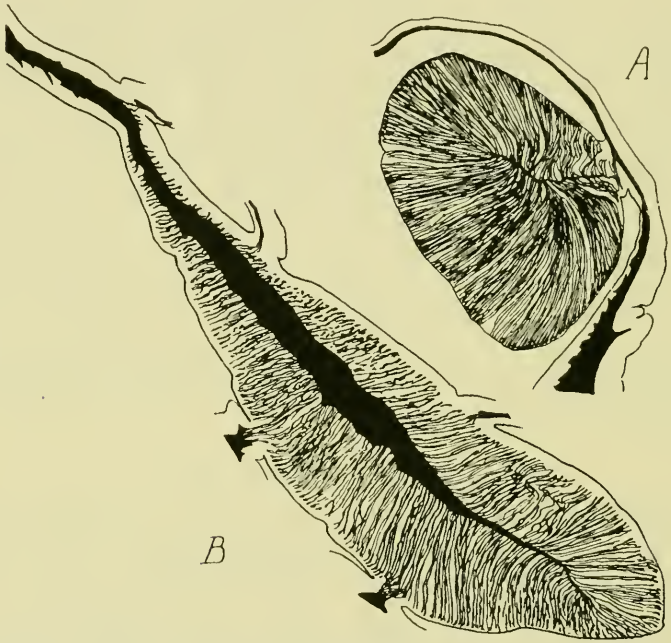
§ N. The Stichodactylines.

Here I have no suggestions to offer (save that already made about the Corallimorphidae and Discosomidae), but am prepared to accept fully the families defined by Carlgren in his 'Ostafrikanische Aktinien', 1900, and (Aurelianidae) in a smaller paper on Stichodactylines, also in 1900. These families seem to be excellently based and to represent relationships very naturally. They are the Stoichactidae, Thalassianthidae, Actinodendridae, Phymanthidae, Aurelianidae, Heteranthidae, and Homostichanthidae. They entirely supersede other arrangements, including Duerden's division of the group into Homodactylinae and Heterodactylinae; they will be defined in Part III.

There are a few points worth noting about the Stichodactylines in general, excluding always the Corallimorphidae and Discosomidae (this latter in the sense taken by Carlgren,

1900). In their main structural features they form a homogeneous group. In all of them (with rare exceptional individuals) there is more than one tentacle situated over at least some of the endocoels, and often over all the endocoels and even exocoels as well. The contrast between a Stichodactyline

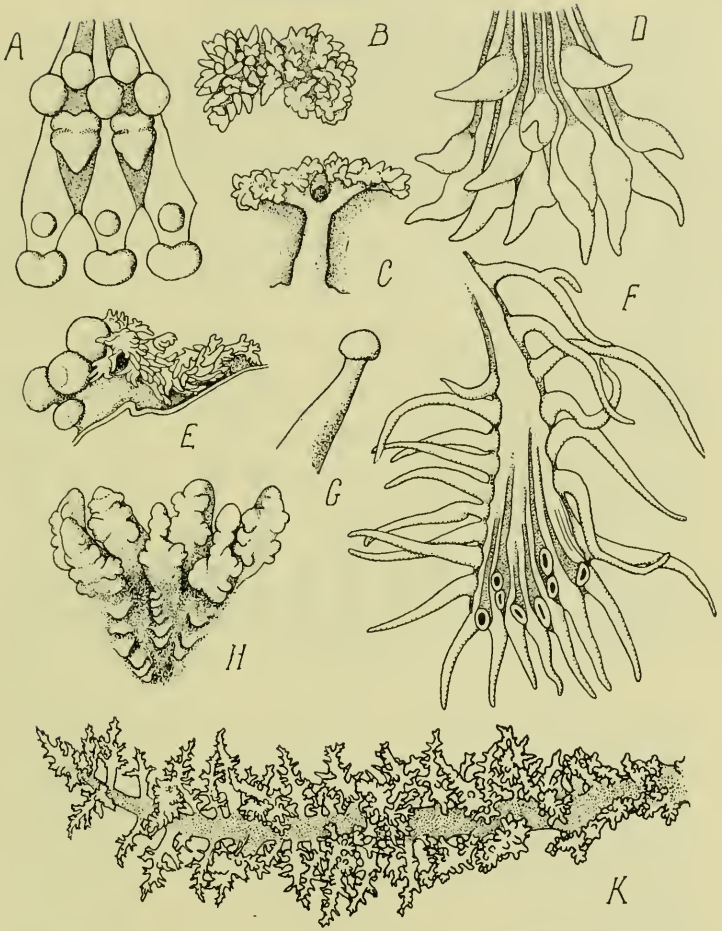
TEXT-FIG. 13.



Vertical sections of two sphincters of Stichodactylines. A, Actinoporus; B, Aureliania.

like Cryptodendron and an ordinary form (as regards tentacles) like Phyllodiscus is brought out in Text-fig. 2. A shows a vertical section of a whole specimen of the latter, and passes through one tentacle on each side of the mouth. B is a vertical section through a corner of the oral disc and body-wall of the former, and shows many short tentacles cut through in the same section—they do not all belong to the same mesenterial space, but they have not by any means one space to

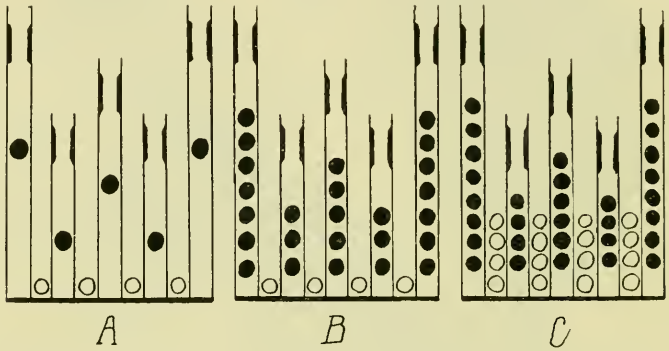
TEXT-FIG. 14.



Arrangement and form of tentacles. A, Aureliania, sector of disc, with various sorts of tentacles, and two to each exocoel and endocoel. B (surface view) and C (side view), of a dendritic tentacle of Actinotryx. In C the stem is embedded in the mesogloea of the disc. D is a sector of the disc of an Actiniine form with plain tentacles, one to each endocoel and exocoel. E, a radial group of dendritic tentacles and nematospheres from Thalassianthus. All this belongs to one endocoel. F, sector of disc of Anthcopsis for contrast with D, showing some of the tentacles in endocoelic radial rows; here some of the tentacles have been cut off for the sake of clearness. G, knobbed tentacle of Corallimorphus. H, sector of disc of Phymanthus, showing marginal pinnate tentacles in alternating cycles and small disc-tentacles in radiating rows. The pinnate character is not very clear, as the specimen was distorted. K, arm or disc-lobe of Megalactis, bearing tentacles. (After Saville-Kent. See acknowledgement on p. 496.) In A, D, F, exocoels are white, endocoels shaded.

each. The contrast is differently brought out in Text-fig. 14, D and F, which represent two sectors of the oral disc of two forms. One of these (D) has the ordinary arrangement of tentacles in alternating cycles, one to each mesenterial space; the other (F) is from an Antheopsis, and shows two of the long radial rows situated over endocoels (which are shaded) and also the arrangement of the marginal tentacles, one or two to each endocoel, one to each exocoel (exocoels are not shaded).

TEXT-FIG. 15.



Diagrams of three types of tentacular arrangement. In each diagram three cycles of mesenteries are shown, with their retractor muscles as black thickening; endocoelic tentacles black, exocoelic white. In A there are cycles of tentacles, one tentacle only to each exocoel and endocoel. In B there are radial rows on the endocoels, but only one tentacle per exocoel (e. g. Stoichactidae). In C the exocoels as well as the endocoels have more than one tentacle (e. g. Homostichanthidae).

Stichodactylines have a definite base (occasionally reduced and half like a physa). Cinclides are recorded in at least one case (see p. 501). There is a complete absence of acontia and mesogloeal sphincters, and almost complete absence of vesicles (there is one case of somewhat vesicular verrucae). The musculature is always reasonably well developed, at least in the mesenteries. There is either no approach to a division of the mesenteries into macro- and microcnemes, or if there is, there are at least twelve pairs of the macrocnemes; in the first case there are usually numerous perfect mesenteries.

Fundamental differences affect chiefly form and arrangement of tentacles and strength of musculature, and details about this will be found in Part III. Text-figs. 14 and 19 show some of the variation in tentacle-form to be found among Stichodactylines and skeletonless corals ; Text-figs. 14 and 15 show some of the modes of arrangement ; and Text-figs. 4 and 13 give details of musculature.

Taking them all in all it may be said that the Stichodactylines are the nearest analogue among anemones to the composites among plants or the birds among vertebrates. A good deal of fundamental structure is fixed, and variation more affects details or additional features. They may be looked upon as Endomyaria (see below) with, above all, tentacular specializations, often of a frilly nature.

§ O.

So far, taking this paper and Part I together, it has been sought to establish the thirty-two families listed below. It remains to discuss main subdivisions of the Anthozoa and arrangement of families within groups of higher rank.

Corallimorphidae.
Discosomidae.
Gonactiniidae.
Ptychodactidae.
Halcampidae.
Ilyanthidae.
Halcuriidae.
Actinernidae.
Condylanthidae.
Myonanthidae.
Andresiidae.
Actiniidae.
Aliciidae.
Phyllactidae.
Minyadidae.
Aurelianidae.
Stoichactidae.
Homostichanthidae.
Actinodendridae.
Heteranthidae.
Phymanthidae.
Thalassianthidae.
Diadumenidae.
Phelliidae.
Flosmarinidae.
Marsupiferidae.
Metridiidae.
Chondractiniidae.
Actinoscyphiidae.
Sagartiidae.
Choriactidae.
Paractidae.

§ P. The Groups larger than families, and the Arrangement of the families within these Groups.

At this point discussion becomes more difficult and more dependent upon individual opinion. It may be simplest to start with the class Anthozoa and work downwards. Neither nomenclature nor main subdivisions are my special concern here, but probably no one will object to one of the following alternatives, whatever names be preferred.

Bourne's division is

Sub-class 1. Octactiniaria.
Sub-class 2. Ceriantipatharia.
Sub-class 3. Zoanthactiniaria.

Or one could use

Sub-class 1. Octactiniaria.
Sub-class 2. Ceriantharia.
Sub-class 3. Antipatharia.
Sub-class 4. Zoanthactiniaria.

Either of these is a good arrangement, probably, leaving aside the vexed question of Tetracorallia—it has recently been suggested that these may have something to do with the Endocoelactid type of structure.

The next step is the subdivision of the Zoanthactiniaria. Few will object to having the Zoanthids as a separate set among them, and although Edwardsiids are sometimes included with ordinary anemones, Bourne has recently shown that they must rank as a distinct group equal to that containing the Zoanthids. So the Zoanthactiniaria may be divided into three or four, with a number of common tendencies (see p. 551).

Bourne subdivides thus :

Order 1. Zoanthinaria.
Order 2. Edwardsiaria.
Order 3. Dodecactiniaria.

His order Dodecactiniaria includes the sub-orders Actiniaria and Madreporaria. Carlgren, however, divides slightly differently.

Order 1. Zoantharia.
Order 2. Actiniaria.
Order 3. Madreporaria.

To this, now, Edwardsiaria would have to be added. In this paper Bourne's division will be used. It is when we come to the subdivision of the sub-order Actiniaria that the main divergence of opinion begins.

Carlgren divides into

Tribe 1. Protantheae.
Tribe 2. Nynantheae.

Another division in use is

Tribe 1. Actiniinae.
Tribe 2. Stichodactylinae.

In the following paragraphs I shall indicate the lines of grouping which I wish to suggest, giving an outline only. Further reasons, filling in this outline, will be found in various parts of the paper, especially under the foregoing sections dealing with sets of forms individually, and in the later evolutionary discussions.

Much has been said about Carlgren's division into Protantheae and Nynantheae, and it has been rejected by some workers, at any rate, in the sense in which Carlgren uses it. It is based mainly upon the presence or absence of ectodermal muscle and a nerve-layer in the ectoderm of body-wall and actinopharynx; and this, as has been suggested before, is probably a universal ancestral character surviving in more or less primitive forms and, otherwise, in sporadic cases. I cannot accept it as a good basis of distinction in itself, although it helps to show relationships, in some cases, when taken with other things. In this attitude I believe I am in agreement with Haddon (1898, p. 411), Duerden (1900, p. 137, and 1902), McMurrich (1904) and Bourne. At the same time I accept decidedly Carlgren's Protantheae, but in a different and much more restricted sense. I have tried to show that Carlgren's Protostichodactylines (a sub-tribe of his Protantheae) (and also the Discosomidae) are corals (see p. 510), and this restricts his Protantheae to Gonactiniidae, Ptychodactidae, and the

Endocoelactids. As mentioned on p. 523, the Endocoelactids seem to be definitely post-Halcampid and Nynanthean, and will here be treated as such. This leaves us with the Gonactiniidae and Ptychodactidae (see pp. 508, &c.); and I feel that these represent two different side-lines of evolution, not necessarily very close together even though both have some primitive features, and that in this case it is safer to give each a group, the two equal in rank. I therefore propose to limit the Protantheae to the Gonactiniidae (in the sense taken on p. 505 and exclusive of Boloceroides), and to erect a group Ptychodacteae for the Ptychodactids, equal in rank to Protantheae and Nynantheae. The Nynantheae I accept as the main tribe, provided it include Boloceroides (see p. 505) and the Endocoelactids (see p. 522), and exclude the Edwardsiids and 'soft' corals (see p. 510); and provided that not only it, but also the other tribes, be re-defined on the sum of their main characters and not on the presence or absence of ectodermal muscle in the body-wall, simply.

My suggestion for subdividing Actiniaria is therefore this one:

Tribe 1. Protantheae (including Gonactiniidae only, and not Boloceroides).

Tribe 2. Ptychodacteae (including Ptychodactidae).

Tribe 3. Nynantheae (including Boloceroides and Endocoelactids, and majority-forms, excluding Edwardsiids and skeletonless corals).

With regard to the other subdivision of Actiniaria into Actiniinae and Stichodactylinae—I used this, provisionally only, in Part I, but am letting it lapse here in favour of the above scheme. One feels that these groups have been useful as a half-way house, but that in the light of developing knowledge of the group, it is now possible to go farther. The 'Actiniine' condition is found in all Nynantheae save one section; it prevails also in Protantheae, Ptychodacteae, and most corals. 'Stichodactylinism' occurs in Ceriantharia and a few corals, and in one set of Nynantheae. There are, however, among Nynantheae, four quite distinct sets, seemingly repre-

senting four lines of evolution; and the 'Stichodactylines' form a compact group within one of these four sets. These four groups can be defined by the sums of their main characters, and clearly the Actiniine-Stichodactyline contrast must be used simply in connexion with a subordinate division of that one of the four groups in which it occurs—if it be used at all. This is only making it one degree more subordinate than Carlgren does in his scheme. It is evident that as primary subdivisions of Actiniaria the two groups are no longer adequate —they must be reduced in rank, at least, from tribes to less than sub-tribes.

Carlgren's scheme is :

Tribe NYNANTHEAE.
- Sub-tribe 1. Actiniinea.
  - *a*. Athenaria.
  - *b*. Thenaria.
- Sub-tribe 2. Stichodactylinae.

The grouping I wish to suggest, as expressive of the above-mentioned four main lines of Nynanthean evolution, is :

Tribe NYNANTHEAE.
- Sub-tribe 1. Athenaria.
- Sub-tribe 2. Endocoelactaria.
- Sub-tribe 3. Mesomyaria.
- Sub-tribe 4. Endomyaria.
  - *a*. Actiniinae.
  - *b*. Stichodactylinae.

I have put in the Actiniinae and Stichodactylinae where they must come, if used, in this scheme—as subordinate to Endomyaria.

The Athenaria of this plan is Carlgren's Athenaria without the Edwardsiids. I fully agree that it is a good group—but it represents a line of evolution within Nynantheae, all of which are derivatives of a Halcampa-like stage, and needs no subordination to anything else. Nor is there any need for a contrasting group Thenaria; the other three tribes are mostly 'Thenaria', but they represent three evolutionary lines and are best kept independent (see p. 560 et seq.).

The Endocoelactaria form a decided small line apart, and with very distinct characters (see p. 522), and it seems inevitable to give them a group to themselves. Since they seem clearly post-Halcampid, this group must come under Nynantheae, not outside it; and is distinct enough from other Nynantheae to require no further subordination.

This leaves the Mesomyaria and Endomyaria, or main mass of forms. It has been part of the purpose of this paper to show that this main mass does fall into two chief sets, following two great lines of tendency, and these two lines I propose to embody in the two sub-tribes named. The Mesomyaria contains the forms classified in Part I, the creatures with acontia and mesogloeal sphincters and so on; the Endomyaria contains those with no mesogloeal (and typically an endodermal) sphincter, no acontia, and often with vesicles, frills, &c.—for more detail of Endomyarian and Mesomyarian tendencies see pp. 560 et seq. The Endomyaria contains the whole of the old Stichodactylinae (save soft corals) and part of the Actininae, and if those names be still used it should be only as subdivisions of this group.

For most of the matter supporting the various suggestions made in this section, reference should be made to the sections on evolution and on special sets of forms, and other parts, both in this paper and in Part I.

It now remains to allocate the families listed on p. 537 to their respective groups.

1. PROTANTHEAE. Gonactiniidae.
2. PTYCHODACTEAE. Ptychodactidae.
3. NYNANTHEAE.
   - A. Athenaria. Halcampidae, Ilyanthidae.
   - B. Endocoelactaria. Halcuriidae, Actinernidae.
   - C. Mesomyaria. Diadumenidae, Phelliidae, Flosmarinidae, Marsupiferidae, Metridiidae, Chondractiniidae, Actinoscyphiidae, Sagartiidae, Choriactidae, Paractidae.
   - D. Endomyaria. Condylanthidae, Myonanthidae, Andresiidae, Actiniidae, Aliciidae, Phyllactidae, Minyadidae, Phymanthidae, Heteranthidae, Stoichactidae, Actinodendridae, Thalassianthidae, Homostichanthidae, Aurelianidae.

Discosomidae and Corallimorphidae go to Madreporaria.

§ Q. Summation of Characters.

In case it should be felt that the foregoing sections are too much of an outline and have too much connexion with evolutionary speculation, it seems advisable to point out that the conclusions have the backing of the sum-of-the-characters principle. The following lists will show that the groups suggested have a solid number of characters binding them together. Only main features are included. In connexion with some of the details given under the larger groups, Text-fig. 16 will be found useful. I will take families first, then larger groups.

FAMILIES.

GONACTINIIDAE. Genera: Gonactinia, Protanthea. Common characters, 11.

1. Definite base. 2. No basilar muscles. 3. Ectodermal muscle in body-wall and actinopharynx. 4. Spirocysts in ectoderm of body-wall (and actinopharynx ?). 5. No developed sphincter. 6. Tentacular longitudinal muscle ectodermal. 7. No true siphonoglyphes. 8. Only the eight protocnemes perfect. 9. Mesenterial musculature weak, not forming true retractors. 10. Filaments without ciliated tracts. 11. No acontia.

PTYCHODACTIDAE. Genera: Ptychodactis, Dactylanthus. Common characters, 12.

1. Definite base. 2. No basilar muscles. 3. Ectodermal muscle in body-wall and actinopharynx. 4. Spirocysts in ectoderm of body-wall (and actinopharynx ?). 5. No developed sphincter. 6. Tentacular longitudinal muscle ectodermal. 7. At least six, usually twelve or more, pairs of perfect mesenteries. 8. Weak mesenterial musculature, hardly forming retractors. 9. Filaments with no ciliated tracts. 10. Filaments of imperfect mesenteries with curious half-funnels at upper termination. 11. Mesenteries with the free edge (or its analogue) occupied by filament above, gonad below, if present. 12. No acontia.

CORALLIMORPHIDAE. Genera: Corallimorphus, Corynactis, Isocorallion. Common characters, 16.

1. No horny or limy skeleton. 2. Definite base. 3. No basilar muscles. 4. Ectodermal muscle in body-wall, at least sometimes, perhaps always. 5. Large sting-cells typically present in some part

of body. 6. No developed sphincter. 7. Tentacular longitudinal muscle ectodermal. 8. Tentacles not branched, but knobbed. 9. More than one tentacle on at least each of the strongest endocoels. 10. Not more than one tentacle per exocoel. 11. No true siphonoglyphes.[1] 12. No division of mesenteries into macro- and microcnemes. 13. Usually numerous perfect mesenteries. 14. Feeble mesenterial musculature. 15. Filaments with no ciliated tracts. 16. No acontia.

DISCOSOMIDAE. Genera: Discosoma, Paradiscosoma, Orinia, Actinotryx, Ricordea, Rhodactis. Common characters, 12.

1. No horny or limy skeleton. 2. Definite base. 3. No basilar muscles. 4. No developed sphincter. 5. Tentacular longitudinal muscle ectodermal, such as it is. 6. More than one tentacle on at least each of the stronger endocoels. 7. No true siphonoglyphes.[1] 8. No division of mesenteries into macro- and microcnemes. 9. Usually numerous perfect mesenteries. 10. Feeble mesenterial musculature, not forming true retractors. 11. Filaments without ciliated tracts. 12. No acontia.

N.B.—In this family the tentacles may be reduced or practically absent, and their form is variable; sometimes there is more than one, on exocoels as well as endocoels.

HALCAMPIDAE. Genera: Halcampa, Halcampoides, Pentactinia, Scytophorus. Common characters, 8.

1. No base (correlated with more or less vermiform shape). 2. No basilar muscles. 3. Sphincter absent or weak (if present may be mesogloeal or endodermal). 4. Tentacular longitudinal muscle ectodermal. 5. Mesenteries divided into macro- and microcnemes, or all macrocnemes. 6. Six pairs of macrocnemes the average (may be four or five to seven couples). 7. Few mesenteries and tentacles—up to forty or so. 8. No acontia.

ILYANTHIDAE. Genera: Ilyanthus (mitchelli), Peachia, Eloactis, Haloclava, Harenactis. Common characters, 8.

1. No base. 2. No basilar muscles. 3. No developed sphincter. 4. Tentacular longitudinal muscle ectodermal. 5. Mesenteries all

[1] With regard to this statement, see definition in Part III. covering Corallimorphidae and Discosomidae.

macrocnemes (in one case macrocnemes and some of an intermediate sort). 6. Never fewer than ten pairs of mesenteries, mesenteries all perfect (one exception). 7. Few mesenteries and tentacles—up to forty or so. 8. No acontia.

HALCURIIDAE. Genera: Halcurias, Carlgrenia. Common characters, 8.

1. Definite base. 2. Spirocysts in ectoderm of body-wall nearly always. 3. No sphincter. 4. Tentacular longitudinal muscle ectodermal. 5. Only one siphonoglyphe. 6. After the first six pairs, mesenteries develop as directives and in endocoels. 7. There is a fairly sharp division between the first six or ten pairs and the rest, the former being macrocnemes and the latter more or less microcnemes; retractors of macrocnemes circumscribed. 8. No acontia.

ACTINERNIDAE. Genera: Actinernus, Isactinernus, Synactinernus, Synhalcurias. Common characters, 10.

1. Definite base. 2. Spirocysts in ectoderm of body-wall. 3. No sphincter. 4. Tentacular longitudinal muscle ectodermal (or with mesogloeal tendency in part). 5. Two siphonoglyphes. 6. After the first six pairs mesenteries develop as directives and in endocoels. 7. No division of mesenteries into macro- and microcnemes. 8. Numerous perfect mesenteries. 9. Mesenterial musculature not strong. 10. No acontia.

CONDYLANTHIDAE. Genus: Condylanthus. Main characters, 7.

1. Definite base. 2. No vesicles. 3. No sphincter. 4. Tentacular longitudinal muscle ectodermal. 5. Mesenteries divided into macro- and microcnemes. 6. Macrocnemes six pairs. 7. No acontia.

MYONANTHIDAE. Genera: Myonanthus, Macrodactyla, Boloceroides, Nevadne. Common characters, 7.

1. Definite base. 2. No vesicles. 3. No mesogloeal sphincter (sphincter endodermal or absent). 4. Tentacular longitudinal muscle ectodermal. 5. Mesenteries not divided into macro- and microcnemes. 6. Perfect mesenteries six pairs. 7. No acontia.

ANDRESIIDAE. Genus: Andresia. (One species only, see p. 518.) Main characters, 7.

1. No base (correlated with very extensile body). 2. No vesicles. 3. Small circumscribed endodermal sphincter. 4. Tentacular longi-

tudinal muscle ectodermal. 5. Mesenteries not divided into macro- and microcnemes. 6. All mesenteries perfect. 7. No acontia.

It has long tentacles in graded cycles.

ACTINIIDAE. Genera: Actinia, Anemonia, Gyrostoma, Condylactis, Parantheopsis, Bunodactis, Tealia, Epiactis, Isotealia, Bolocera, Leipsiceras, Boloceropsis, Dofleinia, Glyphostylum, Pseudophellia, Ixalactis. Common characters, 6.

1. Definite base. 2. No vesicles. 3. No mesogloeal sphincter (sphincter absent or endodermal). 4. Mesenteries not divided into macro- and microcnemes. 5. Numerous perfect mesenteries—at the least more than six pairs in adults. 6. No acontia.

This is one of the few families in which the longitudinal muscle of the tentacles is sometimes mesogloeal.

ALICIIDAE. Genera: Alicia, Phyllodiscus. Common characters, 8.

1. Definite base. 2. Vesicles present. 3. Body-wall delicate, divided into scapus and capitulum, the vesicles occurring either on the scapus or at and above its junction with the capitulum. 4. No mesogloeal sphincter, no developed sphincter at all. 5. Tentacular longitudinal muscle ectodermal. 6. Mesenteries not divided into macro- and microcnemes. 7. Six pairs of perfect mesenteries. 8. No acontia.

PHYLLACTIDAE. Genera: Phyllactis, Cradactis, Phymactis, Cystiactis, Lebrunia, Bunodeopsis, Thaumactis. Common characters, 6.

1. Definite base. 2. Vesicles present. 3. No mesogloeal sphincter (sphincter endodermal or absent). 4. Mesenteries not divided into macro- and microcnemes. 5. Numerous perfect mesenteries as a rule. 6. No acontia.

Here again tentacular longitudinal muscle may be ectodermal or mesogloeal.

MINYADIDAE. Genus: Stichophora. Chief characters, 7.

1. Base a float. 2. No vesicles. 3. Sphincter endodermal. 4. One siphonoglyphe. 5. Mesenteries not divided into macro- and microcnemes. 6. Ten pairs of perfect mesenteries. 7. No acontia.

AURELIANIDAE. Genera: Aureliania, Actinoporus. Common characters, 9.

1. Definite base. 2. Sphincter strong endodermal circumscribed. 3. Tentacles have the form of small vesicles, and may be lobed. 4. More than one tentacle to each main endocoel. 5. More than one tentacle to each main exocoel. 6. One siphonoglyphe. 7. All the mesenteries, or all the older ones, perfect. 8. Mesenteries either all with the grade of macrocnemes (and with unusually strong circumscribed retractors), or else more or less divided into macro- and microcnemes. 9. No acontia.

Here the disc and tentacle radial muscle may be ectodermal or mesogloeal, and there are vesicular verrucae in one genus.

PHYMANTHIDAE. Genus: Phymanthus. Main characters, 10.

1. Definite base, sometimes reduced. 2. No vesicles. 3. No developed sphincter. 4. Disc and tentacle radial muscle ectodermal or with a mesogloeal tendency. 5. Tentacles divided into marginal and discal, the former tentaculiform and usually pinnate, the latter more usually papilliform (rarely they are absent). 6. Marginal tentacles not more than one per exo- and endocoel. 7. Discal tentacles typically in radial rows—they may occur on exocoels as well as endocoels. 8. Mesenteries not properly divided into macro- and microcnemes as a rule, though coming very near it sometimes. 9. Numerous perfect mesenteries. 10. No acontia.

ACTINODENDRIDAE. Genera: Actinodendron, Megalactis, Actinostephanus. Common characters, 10.

1. Definite base. 2. No vesicles. 3. No developed sphincter. 4. Disc and tentacle radial muscle ectodermal. 5. Disc produced into permanent arm-like lobes. 6. Numerous tentacles per endocoel. 7. Numerous tentacles per exocoel. 8. Mesenteries not divided into macro- and microcnemes. 9. Numerous perfect mesenteries. 10. No acontia.

HOMOSTICHANTHIDAE. Genus: Homostichanthus. Main characters, 11.

1. Definite base. 2. No vesicles. 3. Sphincter endodermal, not strong. 4. Oral disc not formed into arm-like permanent lobes. 5. Tentacles short and papilla-like, simple. 6. Numerous tentacles per endocoel. 7. Numerous tentacles per exocoel. 8. Tentacular longitudinal muscle ectodermal. 9. Mesenteries not divided into

macro- and microcnemes. 10. Numerous perfect mesenteries. 11. No acontia.

THALASSIANTHIDAE. Genera: Thalassianthus, Cryptodendron, Actineria. Common characters, 10.

1. Definite base. 2. No vesicles. 3. Sphincter endodermal, not very strong, may be circumscribed. 4. Disc and tentacle radial muscle ectodermal. 5. Tentacles divided into dendrites and nematospheres. 6. Not more than one dendritic tentacle per exocoel, and no nematospheres. 7. Typically more than one dendrite, and nematospheres, on endocoels. 8. Mesenteries not divided into macro- and microcnemes. 9. Numerous mesenteries perfect. 10. No acontia.

STOICHACTIDAE. Genera: Stoichactis, Radianthus, Antheopsis. Common characters, 11.

1. Definite base. 2. No vesicles. 3. Sphincter endodermal, strong or not very strong, may be circumscribed. 4. Tentacular longitudinal muscle ectodermal. 5. Oral disc not produced into permanent arm-like lobes. 6. Tentacles simple, all of one sort (but for sporadic cleft ones which are sometimes present). 7. Not more than one tentacle per exocoel. 8. More than one tentacle on at least each older endocoel, except in very rare cases; usually some or all of the endocoels have several or many. 9. Mesenteries not divided into macro- and microcnemes. 10. Numerous perfect mesenteries. 11. No acontia.

HETERANTHIDAE. Genus: Heteranthus. Chief characters, 9.

1. Definite base. 2. No vesicles. 3. Sphincter endodermal, not very strong, circumscribed. 4. Tentacles of two sorts, marginal and discal. 5. Oral disc not produced into permanent arm-like lobes. 6. Marginal tentacles short conical, disc-tentacles wart-like. 7. Mesenteries not divided into macro- and microcnemes. 8. Numerous perfect mesenteries. 9. No acontia.

The other ten families were listed and dealt with in Part I. It will be seen from an inspection of the above lists, that at the minimum each family has six common characters, and most have 7 to 11, a few even more. It must also be remembered that the lists are not exhaustive, and that most of them could be added to and even some of the characters subdivided. For instance, 'presence of ciliated tracts on the mesenterial

filaments ', ' presence of basilar muscles ', ' absence of ectodermal muscle in body-wall ', ' presence of not more than one tentacle per exo- and endocoel ', and so on, could be added where suitable ; but the addition of all these where not strictly required for present purposes would be needlessly complicating —it is mentioned only to show that the lists could be expanded rather than otherwise.

I should like to repeat here a remark made in Part I, to the effect that the arrangement suggested cannot be validly criticized on the ground that in some cases there are only one or two differences between two given families. Provided that the differences are good ones, this is all right—if families be fused up on that principle it is soon found that the whole Actiniaria will go into one or two collections, and classification breaks down altogether. The very fact that the families form enough of a series to have few differences sometimes, supports the idea that they represent relationships truly.

If sums-of-characters for groups of wider inclusion than families be now taken, the difficulty of course arises that they can be made less absolute, because in some cases there are one or two exceptions to almost everything among large series of anemones, and this is the same whatever classification be adopted. It must therefore suffice to make definitions of tendency rather than of exclusive fact, in some cases.

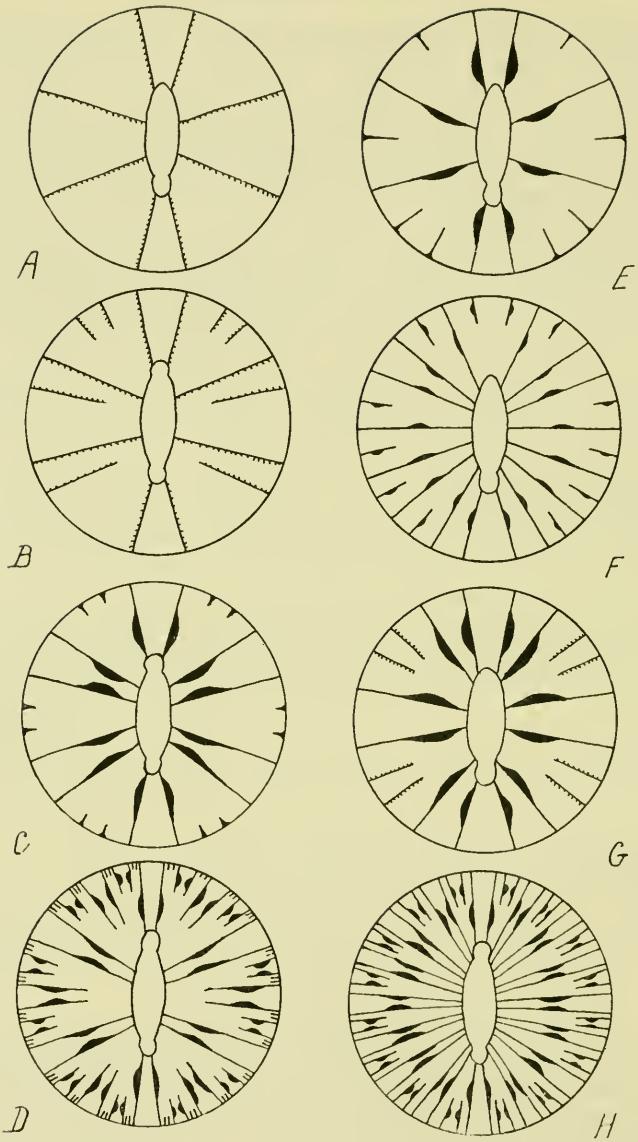
GROUPS LARGER THAN FAMILIES.

Here the wider groups will be taken first.

Class ANTHOZOA. Sub-classes included: Cerianti-patharia, Octactiniaria, Zoanthactiniaria. Common characters or tendencies, 14.

Coelenterata with (i) no medusae, (ii) ' hydroid-generation ' form, (iii) nematocysts, (iv) characteristic muscularity as compared with Hydrozoa, (v) bilateral symmetry typically, (vi) no primary cruciform symmetry like that of Scyphozoa, (vii) mesenteries, (viii) no septal funnels, (ix) no endodermal tentacles, (x) mesenterial filaments, (xi) endodermal gonads borne on the mesenteries, (xii) an actinopharynx, (xiii) no canal-system comparable to that of a Scyphozoan, (xiv) no specialized sense-organs in adults.

TEXT-FIG. 16.



Diagrams of transverse sections showing various mesenterial formulae. A, supposed ancestor of Zoanthactiniaria; B, Gonactinia; C, Halcampa; D, form with graded cycles of mesenteries but only six pairs perfect (e.g. Myonanthidae); E, an Edwardsia; F, Parazoanthus; G, Carlgrenia; H, form with graded cycles of mesenteries, and sixteen pairs perfect. Compare this with a further stage shown in Text-fig. 10.

Sub-class ZOANTHACTINIARIA. Orders included: Edwardsiaria, Zoanthinaria, Dodecactiniaria. Common characters or tendencies, 9.

1. Directive mesenteries typically present, two pairs the standard number. 2. The directive endocoels do not become subdivided in most forms, but it may occur. 3. There are always more than eight mesenteries, even if only eight strong ones, in adults. 4. The eight protocnemes do not typically get pushed out of the way in the manner typical of Ceriantharia. 5. In most cases the mesenteries form pairs, not couples. 6. There are never just eight pinnate tentacles; pinnate tentacles at all are rare, and occur in a few forms of obvious relationships. 7. There are no gular tentacles like those of Cerianthids. 8. There is no sheet of muscle in the body-wall ectoderm comparable in strength to that of Cerianthids. 9. There is no horny axis like that of Antipatharia.

Order DODECACTINIARIA. Sub-orders included: Madreporaria, Actiniaria. Common characters or tendencies, 6.

1. More than eight mesenteries, but there may be only eight perfect: but even so some imperfect ones pair with them: usually at least six pairs perfect. 2. After the first six couples, typically pairs in cycles are formed. 3. Both pairs of directives, if present, are perfect, not one pair macro- and the other micromesenteries as in Zoanthids. 4. The later mesenteries are not typically confined to two lateral regions of growth only, as in Zoanthids, though they may come in the directive endocoels. 5. Mesenteries not typically formed in unequal pairs, one perfect and macromesenterie and the other not, as in Zoanthids. 6. No canals in the body-wall save in the case of some skeleton-building forms.

Sub-order ACTINIARIA. Tribes included: Protantheae, Ptychodacteae, Nynantheae. Common characters or tendencies, 6.

1. No horny or limy skeleton. 2. No colonies. 3. Sting-cells of Madreporarian type do not occur much. 4. Tendency to muscularity greater than in Madreporaria, but not found in the most primitive forms and some others. 5. Siphonoglyphes present in the majority, but not in certain primitive and other forms. 6. Save in the earlier forms, the mesenterial filaments have ciliated tracts.

Tribe PROTANTHEAE. 1 family. See Gonactiniidae for characters.

Tribe Ptychodacteae. 1 family. See Ptychodactidae for characters.

Tribe Nynantheae. Sub-tribes included: Athenaria, Endocoelactaria, Mesomyaria, Endomyaria. Common characters or tendencies, 7.

1. Ectodermal muscle in body-wall the exception and not the rule, occurring only in sporadically-distributed cases. 2. Spirocysts in body-wall ectoderm not the rule—only of regular occurrence in Endocoelactids. 3. Siphonoglyphes present save in odd cases. 4. Mesenterial filaments with ciliated tracts. 5. Pairs of perfect mesenteries present. 6. Mesenterial musculature does not very often exhibit so low a grade of development as in the Gonactiniidae, Ptychodactidae, and many Madreporaria, weakness being usually sporadic and secondary rather than universal and inherent. 7. A fundamental number for the arrangement of parts is six, but there are a good many deviations.

Sub-tribe Athenaria. 2 families. Common characters, 9.

1. No base (correlated with more or less vermiform shape). 2. No basilar muscles. 3. No vesicles. 4. Sphincters weak or absent, though if present they may be endodermal or mesogloeal. 5. Not more than one tentacle per exo- and endocoel. 6. Tentacles and mesenteries few, up to forty or so. 7. Secondary mesenteries exocoelic. 8. Mesenteries divided into macro- and microenemes, or all macroenemes, with Peachia as an intermediate. 9. No acontia.

Sub-tribe Endocoelactaria. 2 families. Common characters, 9.

1. Definite base. 2. No genuine basilar muscles. 3. No vesicles. 4. Spirocysts nearly always in body-wall ectoderm. 5. Probably no ectodermal musele in body-wall. 6. No sphincter. 7. Secondary mesenteries endocoelic and oriented as directives. 8. Not more than one tentacle per exo- and endocoel. 9. No acontia.

Sub-tribe Mesomyaria. 10 families. Common characters or tendencies, 7.

1. Definite base with one or two exceptions. 2. Basilar muscles usually present. 3. No vesicles. 4. Acontia or a mesogloeal sphincter, or both, present. 5. Not more than one tentacle per endo- and exocoel. 6. Secondary mesenteries exocoelic. 7. No acrorhagi or tentacular complications of an Endomyarian sort—often there are basal mesogloeal swellings to the tentacles, and thick body-walls, however, and there are two cases of another sort of tentacular thickening.

Sub-tribe Endomyaria. 14 families. Common characters or tendencies, 6.

1. Definite base save in one case (it may be somewhat reduced, or may form a float). 2. Basilar muscles usually present. 3. No mesogloeal sphincters (sphincter endodermal if present). 4. No acontia. 5. Secondary mesenteries exocoelic. 6. There may be no external complications of the body or tentacles, but verrucae, acrorhagi, vesicles, and complex tentacles are characteristic of different members of the group, more than one of them sometimes occurring in the same form; but there are no tentacles with basal mesogloeal swellings.

Here there is often more than one tentacle on an endocoel, and there may be a good many on each main endo- and exocoel; or, on the other hand, there may be not more than one to each.

The above lists show that even when one is dealing with larger groups it is generally possible to base them on a fair sum of characters or at least of tendencies. It should of course be remembered that each family has not only its own special family-features, as listed, in common, but also many of the group-characters behind the family. To take a single example, the Actiniidae have in common 6 Actiniid characters + 6 Endomyarian features + 7 Nynanthean characters + 6 Actiniarian characters + 6 Dodecactiniarian characters + 9 Zoanthactiniarian + 14 Anthozoan, not to mention all their Coelenterate and Metazoan points. So that they have, back to Anthozoa, 54 common characters—the number has to be reduced of course by any characters which may occur in more than one of the lists involved, or which may be inapplicable to the particular case in point, but even then the number will be considerable.

5. Evolutionary Suggestions.

That the classification suggested here has a firm foundation in character-summation will be evident from the above lists and the definitions later on; but it allows a certain amount of latitude for alternative ideas of evolutionary history, with which it is necessarily a good deal mixed up, especially in cases of large groups, where one is almost bound to think partly

in terms of evolution. The view here taken of the evolution of the forms will now be further developed.

In Part I reasons were given for thinking of a Halcampa-like form as more primitive than such a creature as Cataldiomene (though of course more advanced than Gonactinia), and it was concluded that whatever the detail, the main direction of evolution would be in the direction Halcampa-form ——→ Cataldiomene and not the reverse, and that this would generally apply. Without going into it all again (see Part I, p. 487) it may be assumed that in dealing with such a group as the Endomyaria, some Halcampoides-like form is the end to start at, and Tealia or Phymactis, or some Stichodactyline the antithesis, for much the same sort of reason, with differences in detail. Before discussing the Endomyaria further, however, it will be well to try to get at the relationship of Endomyaria and Mesomyaria to other groups.

If it is fairly clear that both these groups originated somewhere near Halcampa, the same is still clearer of the Athenaria—i.e. the Halcampids themselves and their burrowing descendants. There is also a clear suggestion of origin from a Halcampa-like ancestor in the Endocoelactaria, and they must be thought of as Halcampa-stock diverging from the main lines. The Stichodactylina (excluding the Corallimorphidae and Discosomidae) are to be thought of as specialized Endomyaria. The first idea to establish then is that Endomyaria, Mesomyaria, Endocoelactaria, and Athenaria are the outcome along different lines of a Halcampa-stage with strong retractors and with ciliated tracts on the filaments. That is, they are ' post-Halcampid ' and form a single class, Nynantheae s.s. as defined on pp. 540 and 552, and in Part III.

Next, there are the Gonactiniidae, Ptychodactidae, and Madreporaria to be considered. The idea I hope to work out in connexion with these is that they originated in an ancestor earlier and less advanced than Halcampa (it would of course also give rise to Halcampa itself), and in fact may be called ' pre-Halcampid '.

What forms are more primitive than Halcampa? It was suggested in Part I that Gonactinia and Protanthea are survivals of something very early (see pp. 493, 496–7, &c.). The grounds are these. The 'Halcampa-stage' in evolution may be defined as a stage with six pairs of perfect mesenteries (including two pairs of directives) bearing strong retractors, gonads, and filaments with ciliated tracts; any mesenteries beyond these six pairs would be rudimentary; there would probably be little or no base, a fairly narrow body, and little or no sphincter (cf. Text-figs. 8 and 7, C, D). This is not the Halcampa-stage sometimes used in an embryological sense, but is the way in which the term is usually taken for purposes of this paper. Now the Gonactiniids have paired mesenteries, but not six pairs perfect—only the eight protocnemial couples are fully developed. The filaments have no ciliated lobes, and the mesenteries have very weak musculature, not forming retractors as in the Halcampa-stage. Moreover, the body-wall, tentacles, disc, and actinopharynx approximate to each other in structure, at least as regards ectodermal muscle, and mostly spirocysts. This gives something much nearer a possible ancestor for the groups not specified as post-Halcampid than anything else. The consideration of Anthozoa generally, suggests inevitably that mesenteries coupled before they paired, and the Gonactiniids still keep a vestige of the coupling which Halcampa has lost (see Text-fig. 16, B)—and in a case like this the generalized musculature may be taken to indicate a stage before much differentiation of tentacles from body-wall, and of good retractors, had set in.

There seems no reason to think that the Ptychodactidae or Madreporaria ever passed through a Halcampa-stage in the sense outlined above. They did not attain to much in the retractor line, and the Ptychodactids did not differentiate the parts of their ectoderm very markedly. They never have ciliated tracts on the filaments, and their whole organization and histology, especially of course in Madreporaria, suggests a difference of direction in evolution from that of the post-Halcampids.

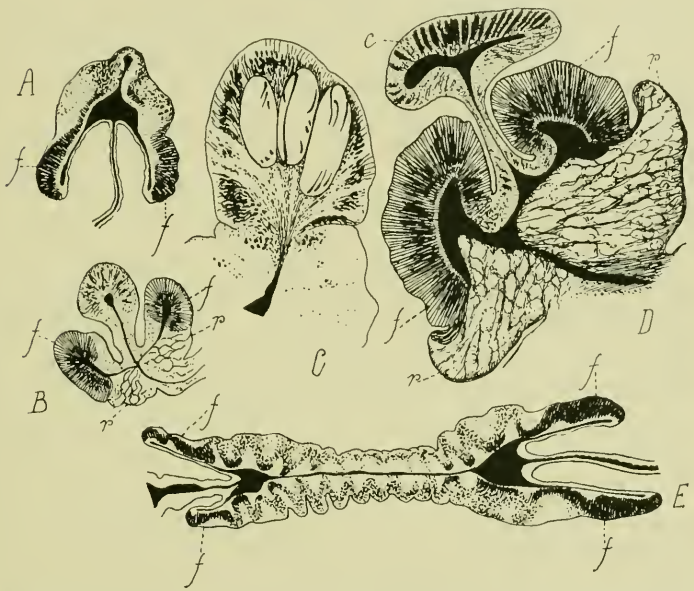
Although these forms (Gonactiniids, Ptychodactids, Madrepores) must be put down as pre-Halcampid, they have common features establishing them as distinct from Edwardsiaria and Zoanthinaria, and they form one group, Dodecactiniaria—for instance, they have typically attained pairing of mesenteries and equality of directives, and the pairs are not usually formed each of a macro- and a micromesenteric partner, nor do they usually develop in two lateral zones of increase only, after a certain point; there are no canals in the body-wall save in some of the skeleton-making Madreporaria.

So that it may be said that the Dodecactiniaria present on the one hand descendants of a Gonactinia-like form, and these are poor in muscle and lack ciliated tracts; and on the other hand descendants of a Halcampa-like form (itself, of course, the outcome of an earlier Gonactinia-like one), with the ciliated tracts stabilized and a tendency to muscularity.

Is the ancestor of the Zoanthactiniaria, the group containing the Dodecactiniaria as well as the Edwardsiaria and Zoanthinaria, simply the same sort of Gonactinia-like animal? The whole situation suggests that it must have had a good deal in common with Gonactinia—it would surely be a small form with weak muscle and generalized ectoderm and only eight perfect mesenteries (see Text-fig. 16, A); the chief point of debate is, had its filaments ciliated tracts? At first glance one would say No, the state without the tracts is more primitive; but there are other things which do not suggest that it was devoid of them. That the ancestor of all Anthozoa was without them seems certain, but that is even farther back than the one here visualized. Our Zoanthactiniarian ancestor gave rise to Edwardsians and Zoanthids as well as to Dodecactiniaria, and both the former have ciliated tracts, even if they are not quite the same as those of the Nynantheae. This suggests that either (i) the Edwardsians and Zoanthids attained them independently, or else that (ii) the Gonactiniids, Ptychodactids, and Madreporaria lost them, while Halcampa and its followers retained, stabilized, and developed them. (See Text-fig. 17 for the main types of filament here mentioned.)

The first assumption, of independent acquisition, would not be unreasonable, but at the same time it does seem likelier that the ancestor of all three had ciliated tracts, perhaps only in a slightly differentiated form; and it is a simpler way of putting things to think of some forms losing them than of

TEXT-FIG. 17.



Mesenterial filaments. A and E, a Zoanthid, with powerful ciliated tracts (*f*). E passes twice through these, as it cuts through a curved edge of mesentery. B, Edwardsia. Ciliated tracts present but less marked than in the Zoanthid; here and in D there are also reticular tracts (*r*). C, Paradiscosoma. Here there are no ciliated tracts, but three large sting-cells are shown. D, Artemidactis. Typical Actinian filament, with median cnido-glandular tract (*c*) and lateral ciliated and reticular tracts.

three groups gaining them. There seems no special reason why such an ancestral form as that under consideration should not have weak ciliated tracts, because although very distinct structures they would easily be differentiated early on, just as acontia seem to have been at the Eosagartia stage in

the history of the Mesomyaria. It provides an idea parallel to that of loss of acontia by various forms, advocated in Part I.

I do not feel that the loss of ciliated tracts by some forms can be very fully accounted for, but it is easier to explain than their independent acquisition in three cases would be. The suggestion I should like to offer in this connexion was made to me by Professor Fleure, and does seem to make it intelligible. In certain Gastropods where the gill-lamellae are not much strengthened and kept apart skeletally, there is a device for keeping open chinks between them, for the passage of water, by means of pads of cilia. It is an attractive idea that part of the function of the anemone's ciliated tracts is something of the same sort—a preservation of chinks allowing access of water between the mesenteries, for respiratory purposes and so on. In the light of this several things may be noted. Among the forms with no ciliated tracts there is little or no sphincter, which means not much tight closing-up of the body. The forms with the tracts have above all developed strong retractors or sphincter, or both (with fairly numerous exceptions), and can often spend a good deal of time tightly shut up—in which condition, of course, the pads would function very well. The marked development of the tracts in Zoanthids fits in with this idea. Among the tractless forms the only really successful ones are the skeleton-making corals, and these have got over any difficulty by keeping their mesenteries apart with septa ; and the other groups are seemingly quaint survivors, and some of them are so constituted that there is not much crowding in the coelenteron. It is not impossible that certain appearances in some of the filaments devoid of ciliated tracts represent vestiges of them ; similar appearances may be present, it is true, in forms with the tracts—but even here they might be vestiges of the weak tracts of the ancestor which were superseded by much better ones. On the other side of the question it must not be forgotten that there are analogues of the ciliated tracts in Ceriantharia, but here again the ancestor may not have been far from that of the Zoanthactiniaria.

Summarizing so far, we get the suggestion of an evolutionary course somewhat as follows :

From a small, delicate, bilateral ancestor, with eight feebly muscular mesenteries, with some degree of differentiation of ciliated tracts, and with generalized ectoderm, there arose

(i) Edwardsiaria, the mesenteries of which never paired, but some of them attained muscularity (see Text-fig. 16, E).

(ii) Zoanthinaria, the mesenteries of which paired, but which went in for various curiosities (see Text-fig. 16, F).

(iii) Dodecactiniaria, the mesenteries of which paired, and which developed along the familiar 'Hexactinian' lines.

There is just the possibility of an alternative view of the Edwardsiaria to the one adopted in this paper—namely, that they might somehow be Nynantheae in which certain mesenteries had been suppressed so that now there are only couples and not pairs. It is their histology which rather suggests Nynanthean affinities, but this idea is put forward very tentatively and further work would be required to ascertain how far it could be entertained as a possibility.

The Dodecactiniaria split on the rock of sluggishness versus muscularity.[1] The Gonactinia-like ancestors experimented a little, and gave rise to the Gonactiniidae and Ptychodactidae, perhaps trial-lines, on the one hand, and to the corals on the other ; all these losing the ciliated tracts and never getting very muscular, the majority-forms going in for strict sedentariness and skeleton-building, often colonially. In a different direction there arose from one of the Gonactinia-like ancestors a muscular Halcampa-form ; this, far from losing the ciliated tracts, developed them further, and gave rise to the individualized and typically muscular forms, which fell into four sets—Athenaria, Endocoelactaria, Mesomyaria, Endomyaria.

[1] See in this connexion Chapter VIII in Thomson and Geddes, 'Evolution'.

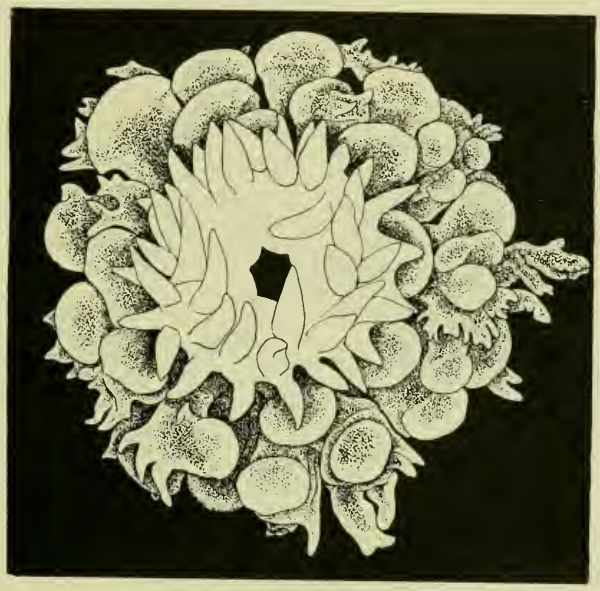
From this point evolution among the Halcampa-descendants or Nynantheae may be further considered.

About the Athenaria and Endocoelactaria little further need be said beyond what may be found in the special sections on those forms. The Athenaria are highly muscular as regards their mesenteries, this being useful in a burrowing existence. They have diverged among themselves in curious ways, and some of them present rather interesting special features, such as the immense siphonoglyphe and conchula of Peachia (presumably a development connected with drawing in a water-current when the animal is below the sand), and the knobbed tentacles of Eloactis. Harenactis has become very attenuated, with many cinclides—and indeed there are often cinclides among these forms. The Endocoelactaria are obviously divergent in another way. The earlier ones, most nearly represented by Carlgrenia, would be not far from the Halcampa-stage, but with secondary mesenteries (microcnemes at first) appearing in the lateral endocoels, and oriented like directives—this modifying the whole plan of structure. A stage further is represented by Halcurias, with ten pairs of macrocnemes instead of six, and later in the Actinernids the distinction into macro- and microcnemes has gone and numerous mesenteries are perfect, and often there are lobed discs, swollen tentacles, thick body-walls, and deep sea habitat. A sphincter never appears.

This leaves the main mass of forms, the Meso- and Endomyaria (including Stichodactylines). With regard to the justifiability of these two groups, if the work of this paper and of Part I be taken into consideration it should emerge that so far as we can know anything about these things, the Endomyaria did, as a bunch, follow a different line of tendency from the Mesomyaria, and if that is established the grouping follows. It is mainly a difference of tendency, there being, at any rate low down in the two groups, probably no essential histological difference—this might come in higher up, perhaps, in comparing such forms as Actinoscyphia and Catadiomene with Thalassianthus.

Among the Endomyaria the sphincter, if present, is endodermal. There are never any acontia. After early evolutionary stages are past, there are often vesicles, sometimes very complex ones, on the body ; verrucae and acrorhagi are frequent ; and in some cases the tentacles increase in number or become

TEXT-FIG. 18.

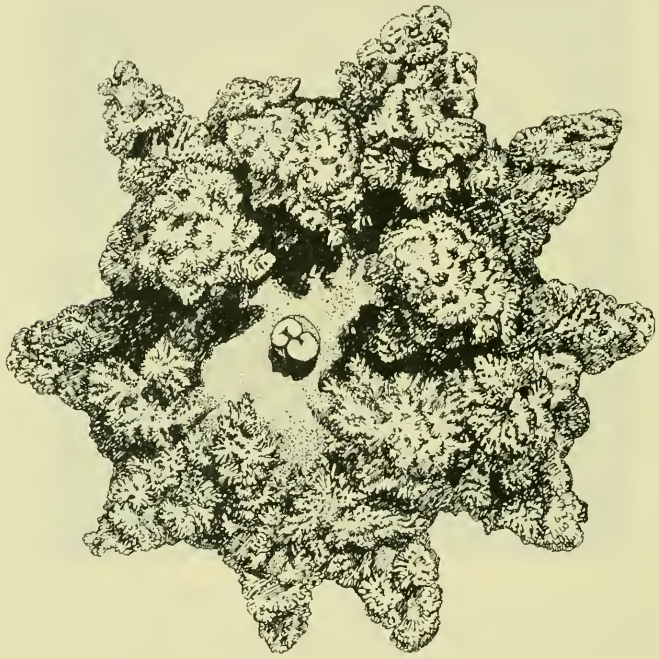


An enlarged view, from above, of a whole specimen of Phyllodiscus indicus. The tentacles are not shaded, and form the central part, and projecting beyond them is the corona or ruff of compound vesicles. An example of complexity affecting outgrowths of the body.

curiously modified in form—vesicular or branched, sometimes quite feathery in their subdivision. There is little or no tendency to thick body-walls of the sort found among Mesomyaria, and never are there basal mesogloeal swellings to the tentacles. The tentacular musculature rarely becomes mesogloeal. A definite base has been attained save in one case, and typically there are basilar muscles. The secondary

mesenteries appear in exocoels, and usually the musculature of the body-wall ectoderm is lost. The habitat of the forms with vesicles and elaborate tentacles is often tropical. Text-fig. 18 gives a good example of one of the forms with a frill of vesicles. The crown of tentacles (unshaded) is seen to be surrounded by a wider corona of compound vesicles, like a ruff,

Text-fig. 19.



Actinodendron plumosum, copied from a photograph of a living specimen by W. Saville-Kent. See acknowledgement on p. 496. An example of complexity affecting tentacles and disc.

projecting beyond it. A vertical section of the same form is shown in Text-fig. 2, A. A case in which the tentacles are dendritic and form a frill, being borne on permanent arm-like projections of the disc, is shown in Text-fig. 19, and other variations in Text-figs. 14 (tentacles) and 1 (acrorhagi).

In Mesomyaria, on the other hand, we get the sphincter,

when present, mesogloeal. Acontia are often present. Real vesicles or frilled tentacles do not occur (the tentacles are slightly complicated in one or two cases), nor do acrorhagi; there is never more than one tentacle to an endocoel (often there are more in Endomyaria, and it may be so on the exocoels also), and the tentacles often have a thick basal mesogloeal swelling aborally. Thick body-walls and knobs and crests of mesogloea are fairly frequent (see Part I, Text-figs. 24, 25, 26, 27, 31). Tentacular musculature is more often mesogloeal than in Endomyaria.

Possibly the acontia in the second group, the acrorhagi and vesicles and complex tentacles in the first, are different expressions of stinging tendencies along different lines, going with the sphincter-difference and so on, the frills especially associated with warmer seas, the curiosities of the Mesomyaria often connected with deep water. One difference is that acontia seem to have been ancestral in the Mesomyaria and to have been lost in certain cases; whereas vesicles and such things must be the attainments of certain individual sets of animals at given points.

Lastly, evolution within Endomyaria may be a little more closely thought of. For Mesomyaria see Part I.

The general direction has been decided on (see p. 554). The simplest way will be to put the route suggested by the facts as narrative, as before, and it must have been something more or less like the following:

From an Eoactinia (near to Eosagartia—see Part I) or Halcampa-like form with little or no base, no sphincter, and six pairs of macrocnemes and a few microcnemes, at first one line of evolution only started.

An adherent base was gained, at first, and an increase in the number of tentacles and microcnemes, but nothing else (cf. Text-figs. 8 and 16, c). There are survivors of this stage even now, the Condylanthidae.

Next, the distinction between macro- and microcnemes was lost, but at first only the former macrocnemes remained perfect (cf. Text-fig. 16, d). Some forms began to get an endodermal

sphincter, though not a very strong one; some developed suckers on the body-wall, and one curious animal formed special sphincters whereby it could cut off its tentacles at will—it also retained some primitive features (Boloceroides). The present-day forms which have gone no further than this are the Myonanthidae.

A large number of forms, however, did go further, and attained a larger number of perfect mesenteries (cf. Text-figs. 16, II, and 10). Often the endodermal sphincter developed and sometimes became very strong, though some forms still remained sphincterless, or with very little or a moderate sphincter. Some of the advanced ones with strong sphincters have the tentacular and discal musculature embedded in the mesogloea. Among these forms the body either remained smooth, or developed verrucae or acrorhagi or both, but never vesicles. These are the Actiniidae s.s. in the sense taken on p. 546.

To go back a little, from somewhere near the Myonanthidae arose a group of delicate forms which retained the six pairs of perfect mesenteries, but the body became divided into a scapus and capitulum, and either from the scapus or from the region where scapus and capitulum join (and sometimes above that region as well) there grew out hollow sac-like diverticula, often compound—the vesicles. Little or no sphincter was attained. These forms are the Aliciidae.

There is another set of forms with these vesicles, but with usually more numerous perfect mesenteries. They sometimes have a less delicate body, and occasionally mesogloeal tentacle-muscle. There is often a well-developed endodermal sphincter, but it may be weak or absent. Perhaps these, or some of them, arose, independently of the Aliciidae, from among the Actiniidae, or perhaps they arose from near the Aliciidae by a mesenterial change. Whichever way it was, they represent onward steps. They are the Phyllactidae—a somewhat heterogeneous group to be further discussed in Part III. A section of one of them, with many perfect mesenteries, is shown in Text-fig. 10.

A form, or perhaps several forms, which from our hitherto incomplete knowledge of them would seem to have arisen near

the Actiniidae, took to a floating life, swimming upside down. The base developed into a regular float, and certain anatomical peculiarities appeared. These are the Minyadidae.

Other advanced stages are represented by the various families of ' Stichodactylines '. These arose from some Actiniid or pre-Actiniid ancestors, and they have usually the numerous perfect mesenteries, and often endodermal sphincters, which may be quite strong. The sphincter is endodermal or absent, never mesogloeal. Among themselves they diverged into seven families, easily distinguished from one another. The differences affected the arrangement of the tentacles on the endocoels and exocoels, and their form—they might be simple, pinnate, dendritic, sessile and vesicular, feathery, modified into special stinging ' nematospheres ', and so on. The other part of the structure chiefly affected by variation was the musculature—there might be absence of sphincter in one case compensated for by strong retractors ; or very strong sphincter and retractors but poor tentacles ; and so on.

It will be seen from the above outline, and from that given earlier for Mesomyaria, that there is one thing assumed as having independently taken place in Endomyaria, Mesomyaria, and Endocoelactaria—and possibly more than once in Endomyaria : that is, that in each of these cases a start was made from the condition in which the mesenteries are divided into macro- and microcnemes, this was lost, and in the end there were graded mesenteries and numerous perfect pairs. This is, however, a convergence quite to be expected among forms making in a general way towards increase of size and diameter of the individual, and correlated multiplication of organs. Whatever arrangement be adopted, there is some convergence cropping up, but when one thinks of the vertebrate and cephalopod eye, or of the Marsupial and ordinary wolf, a convergence like that assumed here seems very simple.

In the two hypothetical ancestors of Endomyaria and Mesomyaria (Eoactinia and Eosagartia) there is no harm in assuming for them ectodermal muscle in the body-wall, and the same may probably be said for the Eoactinia-like

ancestors of Athenaria and Endocoelactaria. This would allow for the retention of the musculature, or of traces of it, here and there, with a dominating loss of it along all the lines.

6. Summary.

It is difficult to make a concise summary of a paper covering a good many inter-related discussions, but the following is an attempt to give some of the main points with reasonable brevity.

1. There is difficulty in defining specificity among Actiniaria, as in other lowly and plastic animals. Among British forms species are well enough marked on the whole, if studied alive so that colour and habit can be taken into account. When preserved, however, too little is known of possible range of specific variation in anatomy for much to be done. Foreign forms are so often known in death only that species are somewhat in chaos and there is little firm ground. Experience leads one to the view that among these low and plastic forms a species may have its peculiarities of organic constitution at an early stage of the development of their expression, such expression having affected colour-scheme and general facies of the living animal but not necessarily to any extent the internal anatomy which can be studied in preserved specimens. Much work needs doing by way of studying all forms alive, and of killing and preserving numerous individuals which belong certainly to the same species, in different ways, and studying them so as to reveal effect of reagents, age, state of contraction or distension, locality, reproductive maturity, and so on, on the anatomy. When a better knowledge of the limits of specific variation is gained (and they will be much wider in some species than in others) a revision of species might be attempted. Especially the value or otherwise of measurements of nematocysts as specific characters should be looked into.

2. Although species are in a poor way, genera and families are on the whole much easier to understand and make use of, and here there are enough data to start a methodical classification

with. Omitting for the sake of brevity any criticism of existing classifications, and regarding Actiniaria as an unclassified series, it may then be inquired what method can be applied to them to find out their inter-relations. Clearly unit characters are not much help, since they may vary independently, and may enter into combination in different genera with various sets of others. It is therefore necessary to sum up the chief features of each genus, and to see which genera have most in common with which others ; and those sharing most can be united in families. The result is a natural grouping, and one which expresses relationships of animals as wholes, and not analogies of isolated parts of their bodies. The classifications of Lamellibranch Mollusca may be referred to as an example of several overlapping schemes affecting the same group, founded on few characters, and each expressing the relationships and evolution of one set of anatomical details (be it siphons and pallial lines, hinge lines and teeth, adductor muscles, or gills), and not expressing those of Lamellibranch animals as wholes.

It is found, however, that after applying the method of summation of characters, families can be defined by half a dozen or more common features, and may form so graded a series that there are only unit-differences between some of them. On the other basis there were sometimes only single or few differential resemblances between the members of a family, accompanied by important differences. To look at it from another angle, it has been said that criticism is finding out why one likes or does not like a given book or picture. It seems fair to say that classification is finding out why a horse is more like a mule than like a wolf—we know instinctively that it is so, but if we can confirm that instinct by good reasons we have a classification. Similarly, given enough study of a group, and enough training of the relationship-instinct, it is felt that from their whole organism and make-up certain forms are more nearly related to some of their brethren than to others. This may be of very great help, but of course needs cautious exercise and confirmation. The point is that the principle of summation of chief characters gives this

confirmation in a way that an artificial system of unit-characters cannot do—it justifies and bears out the instinct. The summation principle also enables the family to be used definitely as the expression of a step in the evolution of any set of forms, and the classification represents evolution of whole anemones, not of their sphincters or tentacles only. It also provides evolutionary hints which could not otherwise come to light, and which, given a general idea of group-evolution, help to confirm and enlarge it. The general idea itself can grow from a comparison of early and advanced forms, embryology, and so on. From working through a whole group in such a way it does seem possible to get a glimpse of the rhythmical development of the life in the creatures, expressing itself in the various ways at its disposal and unfolding along various lines. It should be noted that in dealing with a group as plastic as Actiniaria, it is often necessary to define differentiation of tendency without too much insistence on hard and fast divisions without qualification or exception.

3. The classification worked out on the above lines, in this paper, is as follows. For definitions of the groups and families, and for limitation of the sense in which they are taken, reference should be made to the portions of the paper where these things are dealt with. I have accepted the arrangements of Bourne and Carlgren as regards sub-classes of Anthozoa; and that of Bourne for orders and sub-orders. The tribes, sub-tribes, families, and genera have, however, been largely revised in this paper. I have kept as near to Carlgren's tribes and sub-tribes as I felt possible, and have throughout used old names where I could; but the sense of his groups has been altered and they have been added to, and many of the families more narrowly limited, so that the old names take on a new meaning.

Class ANTHOZOA.

- Sub-class 1. Ceriantipatharia.
- Sub-class 2. Octactiniaria.
- Sub-class 3. Zoanthactiniaria.

Order A. Edwardsiaria.
Order B. Zoanthinaria.
Order C. Dodecactiniaria.
- Sub-order (i) MADREPORARIA.
- Sub-order (ii) ACTINIARIA.
  - Tribe a. Protantheae.
  - Tribe b. Ptychodacteae.
  - Tribe c. Nynantheae.
    - Sub-tribe α. Athenaria.
    - Sub-tribe β. Endocoelactaria.
    - Sub-tribe γ. Mesomyaria.
    - Sub-tribe δ. Endomyaria.

The families will be found listed under their respective group on p. 542.

4. An idea of the evolutionary history of the group has been worked out in connexion with the above classification, and may be summarized as follows.

It is possible to guess at a small plankton swimmer with eight tentacles and eight mesenteries, without much definiteness of musculature, and with bilateral symmetry, and contrasting with, not resembling, the cruciform Scyphistoma, which must have been quite an independent outcome of a Hydrozoan. This small creature would give rise to several types much like itself but with differences of detail, each of which would give rise to a main Anthozoan sub-class. Only the one which gave origin to the Zoanthactiniaria need be followed here. This stock seemingly shed out curiosities at first; some of them took to burrowing and life in cracks, and became vermiform, but did not amount to much (Edwardsiaria); others went in for colonialism and incrustation and had fair success in a coral-like way (Zoanthinaria). The main line, however, divided fairly early into two great groups, the split being upon the rock of sluggishness and colonialism and skeleton-building *versus* comparative activity, specialization of the individual, greater muscularity, and no skeleton. The two groups are of course corals (Madreporaria) and sea-anemones (Actiniaria). There are a few corals which developed no skeleton, or else lost their skeleton, and which though often simple show colonial tendencies.

They have usually been classed with the anemones, but it appears that they are almost identical in structure with coral-polyps, but unlike anemones. Their lack of skeleton cannot keep them out of Madreporaria, and the transference makes the division between the two groups, as regards soft parts, more intelligible. They are the Corallimorphidae and Discosomidae.

Returning to the sea-anemones proper, they seem first to have experimented with further curiosities, which perhaps diverged from the main stock about the same time as the corals, or a little later. These experimental forms fall into two sets, with a good deal that is primitive about them, one of them resembling as nearly as any surviving form the supposed ancestor of the whole Zoanthactiniaria. They are the Protantheae (Gonactiniidae) and Ptychodacteae. After this the main line attained a definitely muscular Halcampa-like stage with well-marked ciliated tracts on its mesenterial filaments, and from this point two main lines of divergence may be traced, and two lesser lines. Of the subsidiaries, one group (Athenaria) took to, or simply remained in, a burrowing life, and retained a good deal of simplicity ; the other (Endocoelactaria) went off in a curious direction, the reverse of that taken by most forms, as regards some details of its mesenteries, and possibly gives a clue to the origin of Tetracorallia. This group shows one tendency in common with the two main lines to be next dealt with—a general move towards increase in size of the individual, especially in diameter, and increase in the number of effective organs ; with musculature tending to change from a few strong retractors on a few mesenteries to a larger number of less specialized ones.

The two main lines both went in for development of a marginal sphincter, but otherwise their differences of tendency are marked. The Mesomyaria developed mesogloeal sphincters, and these, when they have special stinging organs, have acontia, never or hardly ever acrorhagi or frills. And although diverging among themselves, many of them tend after a time to take to deep-water life. In correlation with this they may

lose their acontia and may lose mobility, and develop stiff or thick body-walls, their metabolism slowing down and spare energy sometimes being used up in the production of knobs and crests and solid horn-like tentacular swellings. This is a tendency towards fixity of character and possibly thence towards ultimate extinction. It is interesting to note that some of the above-mentioned Endocoelactaria have reached a similar state, although along an entirely different line.

The other main line, Endomyaria, went in for endodermal sphincters if any, and their special stinging organs are never acontia, but they often have acrorhagi and other things. Some of them develop vesicular blisters and compound acrorhagi which may reach wonderful complexity of structure; in others the tentacles increase in number and sometimes they, not the outgrowths of the body, become complex, at their finest with a frill-like effect. These forms, whether it be body or tentacles that complexify, are more especially found in the warmer seas, and here the tendency to fixity of character does not seem much indicated. Along both lines various forms halted by the way, of course.

This idea of the evolution of the group may be helped out by the diagram printed below.

A more detailed outline of the history of Mesomyaria has been worked out, and will be found in Part I, p. 498, &c.; a corresponding one for Endomyaria is given in this part, p. 563, &c.

5. Apart from the above considerations, it has been the object of the paper to revise and re-define all the families and genera, sorting them out in such a way as to make them as homogeneous as possible, and to represent their relationships naturally, with the idea of getting the definitions as precise as is feasible in order to facilitate identification. It has the advantage of collecting all the definitions together, but at the same time is not meant to be an exhaustive compilation as regards species-lists and so on. Only a minimum of synonymy is included, and insufficiently known forms are left alone. The classification worked out is, admittedly, complicated rather

than simple, but that is inevitable in a large and very old group.

6. It seems fair to suggest that the principles advocated and put into practice here might with advantage be applied to other animal groups (e. g. Gastropods and Lamellibranchs). It is not for a moment implied that the classification of animals as at present understood does not group them correctly, speaking broadly and of the main groups; but that it needs revision and supplementing on the plan suggested, especially in the cases of some of the sub-groups, the classification of which sometimes seems tentative and not very clear. It appears that nearly enough data are now collected about animals to permit of entry on a new phase in the history of classification. It is becoming evident, with regard to species for instance, that some new system will shortly have to be devised which will more adequately represent their inter-relations, and allow for the idea of interlacing systems of concentric circles with the characters of the central individual in each system as those of the species, which has grown up. Some new conception will probably work itself out about classification in general also, and the revision of some groups in accordance with ideas advocated here is suggested as a small beginning along the road—a beginning which may possibly lead to further steps in the realization of the new conception. If it prove to be a blind alley, that conclusion should not take very long to emerge.

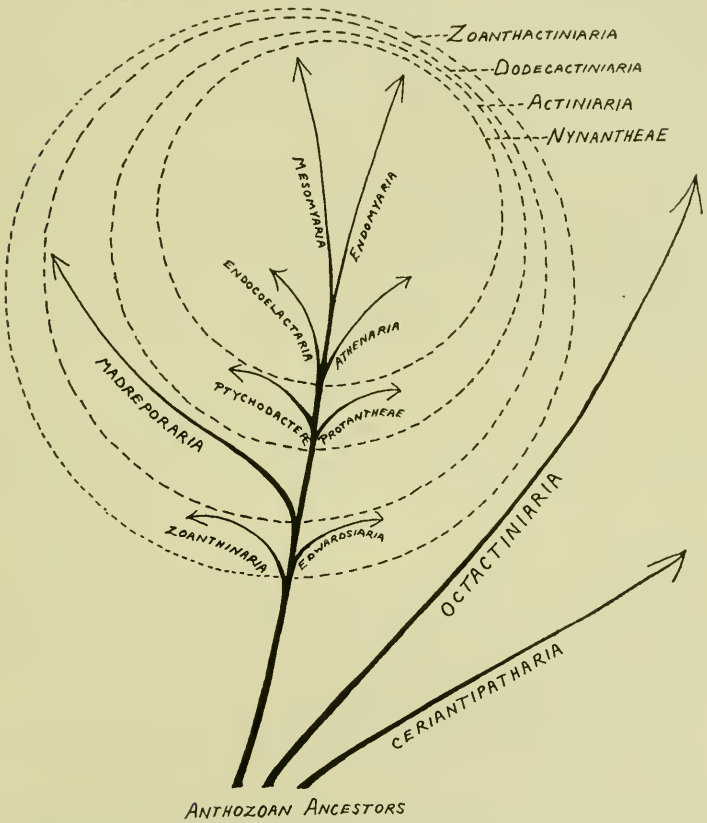
7. Short Glossary.

This is not in any way a complete glossary, but is meant for use in connexion with a few terms which more than most seem to require definition, for convenience in using the paper.

Acontia.—Slender white or coloured threads attached to the borders of the mesenteries in some families of Actiniaria, just below the mesenterial filaments. They are loaded with nematocysts, and can be protruded through the mouth, and in some cases also (accidentally) through pores (cinclides) in the body-wall, for purposes of defence or to paralyse prey. Histologically they differ from mesenterial filaments.

Acrorhagi.—Marginal outgrowths of the body-wall found in some genera of Actiniaria, and which may or may not be specialized as nematocyst-batteries. They may be simple (spherical, conical, &c.), slightly compound, or even frondose.

Text-fig. 20.



Diagrammatic representation of the classification and evolution of the Zoanthactiniaria.

Capitulum.—The bodies of some Actiniaria show a distinction into three regions: the main part of the body in such cases is termed the scapus, and may be provided with cuticle. The distal extremity, which bears the tentacles, is

termed the capitulum; it may or may not be very distinct from the scapus; usually it has no cuticle; it may be delicate and different in structure from the scapus, and introvertible into the latter. The aboral end of the body if rounded and able to become bladder-like is called a physa. Some adherent forms possess scapus and capitulum, but ordinary base instead of physa; among these the capitulum may be delicate or may be very thick-walled. There are grades between a physa and a well-marked adherent base, and some bases may temporarily become physa-like.

Ciliated Tracts (Flimmerstreifen) of mesenterial filaments. In the filaments of Zoanthinaria, Edwardsiaria, and Nynantheae, a transverse section cut at the right level will show a trifoliate outline, portions of the lateral lobes of the trefoil being composed of plain ciliated cells, these portions forming, therefore, in the whole filament, lateral ciliated tracts on either side of a median glandular or cnido-glandular tract (Nesseldrüsenstreif).

Cinclides.—Pores in an Actinian body-wall. Function perhaps connected with water-currents; in some cases they seem to provide safety-valves against rupture of the wall on sudden jerky contraction. Connexion with acontia secondary and indirect.

Conchula.—The specialized upper extremity of the siphonoglyphe in the genus Peachia. Perhaps connected with the entry or exit of a water-current when the animal is embedded in sand up to the disc.

Couple of mesenteries. See foot-note.

Endocoel. The space between two mesenteries of the same pair.[1]

[1] In this paper the word 'pair' is used of two mesenteries, both on the same side of the body, and adjacent to one another—and usually with their retractor muscles vis-à-vis. The word 'couple' is applied to two mesenteries arising at the same time and symmetrical about the long axis of the actinopharynx, but one on one side of the latter, and one on the other; their retractors facing the same way. Thus ordinary directive mesenteries are strictly couples, though usually called pairs for convenience.

Exocoel.—The space between two pairs of mesenteries.

Fosse.—Some anemones have the margin of the body raised into a distinct rim or parapet, outside the bases of the tentacles; the circular groove between this parapet and the tentacle-bases is known as a fosse.

Macrocneme.—A typical macrocneme is a well-developed mesentery which joins the actinopharynx as well as the body-wall, has a strong and usually circumscribed retractor muscle, a gonad, and a mesenterial filament. There are sometimes variations in detail from this general plan.

Metacneme.—Any mesentery formed after the earliest eight mesenteries to appear (protocnemes).

Microcneme.—Typically a narrow mesentery which does not join the actinopharynx, has little or no muscle beyond a 'parietal muscle'—no retractor therefore—no gonad, and no filament. Variations from this typical scheme are found, however.

Nematosphere.—A tentacle which has become converted into a short structure rounded at the end, or into a practically sessile sphere, and the ectoderm of at least part of which is crowded with nematocysts.

Pair of mesenteries. See foot-note on previous page.

Perfect Mesentery.—In a form where there are graded cycles of mesenteries (i.e. no division of the mesenteries into macro- and microcnemes), any mesentery which joins the actinopharynx as well as being inserted into body-wall and oral disc, is termed 'perfect'. In a form where there are macro- and microcnemes, the former are of course 'perfect' as part of their macrocnemic nature; but in some cases some of the microcnemes may join the actinopharynx though otherwise more or less rudimentary. They are then technically 'perfect' mesenteries, but are by no means macrocnemes. In the forms with graded cycles, the perfect mesenteries have filaments and retractors, but not always gonads, which in such forms may appear on the 'imperfect' mesenteries only. In such forms the older imperfect mesenteries, at least, may have retractor, gonad, and filament, so that they are not

microcnemes although less fully formed than the perfect mesenteries.

Physa.—See Capitulum.

Protocneme.—The first four bilateral couples[1] of mesenteries to be formed in a Zoanthactiniarian.

Scapus.—See Capitulum.

Sphincter.—The sphincter usually referred to in this paper is the one running round within the upper margin of the body, outside the tentacle-bases, in many anemones. It may be embedded in the mesogloea of this region (mesogloeal), or its fibres may be supported on processes of mesogloea which project into the endoderm (endodermal). It may be spread out a good deal (diffuse) or gathered up into a definite sharply marked-off cord, which at its best forms a marked projection from the body-wall into the coelenteron (circumscribed). There are various intermediate grades between diffuse and circumscribed, and various degrees of strength in sphincters.

Stichodactyline condition of tentacles. This is the term used to denote the state of affairs in which more than one tentacle communicates with at least some of the endocoels, sometimes with all endocoels, and with exocoels also.

Verrucae.—These are local, slightly differentiated sucker-like warts or slightly hollow outgrowths of the body-wall, and often they attach foreign bodies to themselves.

Vesicles.—These are truly hollow, bladder-like extensions of the coelenteron into outgrowths of the body. They may be delicate and thin-walled, simple or compound, and sometimes are well provided with nematocysts.

[1] See foot-note on p. 574

The Development of the Sea Anemone Bolocera Tuediae (Johnst.).

By

Prof. James F. Gemmill, University Coll., Dundee.[1]

With Plate 22.

Bolocera tuediae was recorded and described from deep water near Berwick by Johnston (**11**) in 1832. Gosse described it more fully in 1860 (**10**, p. 185) and the following is his summary of its characters: 'Base adherent, not much exceeding the column. Column pillar-like, the diameter and height sub-equal; surface generally very smooth, studded with warts remotely scattered. Disc smooth, circular in outline, not overlapping the column. Tentacles short, thick, constricted at foot, obtusely pointed, longitudinally furrowed, flexuous and motile, easily separated, not retractile. Mouth raised on a cone. Stomach capable of being greatly protruded.' The tentacles are, however, moderately long and slender when fully extended during life.

Carlgren (**3**, pp. 34–6) adds that the genus Bolocera is characterized by the presence of a relatively well-developed diffuse or circumscribed endodermal sphincter, that the column has no ectodermal longitudinal muscular layer, that the tentacles have a well-marked endodermal sphincter at their bases, and that probably all the mesenteries except the eight 'Edwardsia' ones are fertile. Carlgren follows McMurrich (**16**) in judging that Bolocera must be placed in a separate Family, the Boloceridae. Its nearest allies are probably among the Antheinae in which, however, the sphincter is extremely feeble if not entirely absent (see Delage, **6**, ii. 2, pp. 503–5).

In the Clyde Fauna List (Laurie, **18**, p. 367) Bolocera

[1] I am indebted to the Trustees of the Carnegie Trust for a grant towards the expenses of this investigation.

tuediae is put down as occurring at depths of from fifteen to seventy-five fathoms. My own records lower the first limit to thirty fathoms. While possessing an attaching base and capable of adhering weakly to the sides or bottom of an aquarium tank, Bolocera appears to live usually on muddy bottoms, and is almost always brought up by itself when taken with the dredge or on the long lines of fishermen. It has great stinging powers, and one has to risk a somewhat severe urticaria when handling it alive.

The sexes are separate and the gonads are at their largest in the end of February and beginning of March. Unfortunately the females very seldom spawn in captivity. The eggs are retained and undergo absorption after a time. Probably want of the natural food is a contributing reason. The males shed their sperm more freely.

Only a few eggs were obtained in 1916 and 1917, but in March 1918 large numbers were extruded by a recently-taken specimen. These after floating about in the Bolocera tank were duly fertilized, although none of the males at the time had emitted a noticeable amount of sperm.

Maturation must take place just prior to extrusion. Serial sections of full-sized ovaries show the eggs with large-sized germinal vesicles, but in similar sections of freshly-shed unfertilized eggs the nucleus is so small and inconspicuous that I could not detect it.

The eggs are spherical, 1·1 mm. in diameter, and pink or flesh coloured, i.e. of much the same tint as the animal itself. They tend to float, and when floating show no polarity as regards upper and under sides. They are surrounded by a membrane beset all round by small conical bunches of spines. The interior is crowded with small granules faintly stainable with haematoxylin, small yolk-spheres staining red with eosin, and large clear spherules unaffected by re-agents, the latter being relatively more numerous towards the centre of the egg. In certain methods of preservation (e.g. corrosive sublimate followed by graded alcohols) an inner core, about half the diameter of the egg, tends to become separated from the

outer zone. Just under the egg-membrane is a thin layer where the first-named granules are very numerous, the clear spherules absent, and the yolk-spheres few in number.

Bolocera has the largest eggs of all the Clyde Anemones I have investigated. Their diameter, 1·1 mm., compares with 0·1 mm. for Metridium dianthus, 0·3 mm. for Anthea cereus, 0·1 mm. for Sagartia, 0·25 mm. for Adamsia palliata, 0·6 mm. for Urticina coriacea (the shore Urticina), 0·7 mm. for Urticina crassicornis (the submerged Urticina). Full-grown ovarian eggs of Gonactinia prolifera and of Actinia equina measure respectively 0·07 and 0·15 mm. in diameter. The Bolocera egg-membrane and its spines resemble but are hardly so strong as those of Urticina. The egg-contents of the two are much the same. Anthea (and Actinia equina, according to Lacaze Duthiers) has spiny egg-membranes, but in Metridium, Sagartia, and Adamsia the membranes in question are smooth.

In Bolocera, as in Urticina (Appellöf, **1**), the fertilized nucleus gives rise to a number of daughter nuclei (sixteen in Urticina) before the egg-mass undergoes cleavage. In particular cases I have estimated the number as not less than twenty-four. The fertilized nucleus probably lay at a point somewhere in the deeper layer of the outer zone, about a third of the diameter of the egg inwards from the surface. The daughter nuclei, as they increase in number, spread laterally at this level from the point in question until they are more or less equally distributed all round. In the egg illustrated by fig. 1, eight nuclei were present, all of them in one hemisphere.

Slightly older eggs examined under reflected light begin to show rounded bosses or humpings which appear first at one side (no doubt the side towards which the fertilized nucleus lay), and afterwards extend all over the egg-surface. They soon become better defined and separated from one another by linear furrows. Segmentation of the egg-mass is in progress, and serial sections show that each hump is the outer end of a large more or less conical cell the apex of which is directed centrally. The whole egg increases slightly in size, and a small

central cavity filled with coagulable fluid makes its appearance. The egg-membrane is not separated off as a membrane of fertilization, but is found to follow closely every surface change of contour so long as it is recognizable. As segmentation proceeds, non-nucleated portions separate off from the inner ends of the cells, and, mixing with the blastocoelic fluid, form a central diffuse trophenchyme. At this stage one or two whole cells may share the same fate by migrating or getting pushed inwards from the surface. Their nuclei proliferate; but, soon losing control over the cell-contents which become trophenchymal, are destined to degenerate along with the other trophenchymal nuclei to be described later.

A little later the Bolocera egg shows very markedly those peculiar surface grooves and foldings which Masterman first described in the case of Cribrella (**17**, p. 8), and which have since been noted in many ova (**8**, p. 12). During this process there is a tendency, better marked in some instances than in others, for the egg to assume the form of a flattened disc the edges of which become turned upwards like those of a saucer. The surface grooves and the saucer cavity gradually fill out, so that the egg becomes almost spherical again. The saucer cavity is accordingly not the archenteron, though gastrulation, which soon supervenes, affects the part of the egg-wall which was formerly the hollow of the saucer. In the fully-formed blastula this part often remains flat while the rest of the blastula wall is spherical.

An important point to note is that as the surface folds smooth out, many single cells and groups of cells are nipped off from the recesses, and migrating inwards become included within the trophenchyme. I thought at first that these cells were going to form the endoderm of the larva. But this is not so. Their cell outlines will disappear and their nuclei degenerate.

Gastrulation.—In typical cases (see e.g. figs. 7–9) a relatively large portion of the blastula wall shows flattening and sinks gradually downwards, the margins of this portion closing in slowly to form the lip of the blastoporic opening. At the same time this lip becomes slightly

involuted giving rise to the rudiment of the stomodoeal canal.

The invaginating area soon presses against the trophenchyme, and we often find at this stage secondary flattening of the whole egg and foldings of its walls, which are probably caused by the resistance of the trophenchyme to the progress of invagination. However, in course of time, the trophenchyme finds its way through the inpushing endoderm into the cavity of the archenteron. First, the fluid and fine granules begin to get through, then the yolk-spheres, and lastly the clear spherules. The process appears to be mechanical in the sense that the trophic material passes through interstices between endoderm cells, and is not first swallowed or assimilated and then excreted into the archenteron.

As gastrulation proceeds most of the trophenchymal nuclei disintegrate, but some pass with the trophenchyme into the archenteron and are absorbed later.

It is of particular interest to note that in a few cases the end-result of gastrulation is attained by a process which may be described more accurately as unipolar immigration than as invagination. In such cases the cells over a relatively small area at one pole of the blastula begin to sink inwards through the trophenchyme, at the same time proliferating and spreading out so as almost to lose their continuity with one another. This process continues until having passed through the whole depth of the trophenchyme, they abut against the ectoderm where they soon form a continuous sheet of endoderm lining an archenteric cavity which now naturally contains all the trophenchyme. Sometimes the process is intermediate between that described above and open invagination. Similar differences occur among the eggs of different Crustacea, but not so far as I know among the eggs of the same Crustacean species. We may put down the variations in Bolocera as probably due to differences in the character of the yolk, noting that those blastulae which showed the fewest foldings and the least deformation tend also to form their endoderm by unipolar immigration.

A mesogloeal sheet only begins to form after the ectoderm and endoderm have come in contact. Accordingly it appears first at the oral end of the larva. Both layers seem to take part in its formation.

Comparison with other Anemones as regards the Stages up to the end of gastrulation.

Metridium dianthus.—Nuclear division and segmentation go together from the first; blastula with a hollow central cavity; endoderm formed by invagination (Gemmill, **9**). McMurrich, however, stated (**15**) that the endoderm is formed by delamination. Sagartia troglodytes.—As in Metridium. Adamsia palliata.—Cleavage begins after the second nuclear division; the preblastula is a wrinkled disc, becoming saucer-shaped, and then smooth and spherical or oval; the inner yolky ends of the cells separate off to form a central trophenchyme normally without nuclei; gastrulation is by invagination (Gemmill, **9**), and the trophenchyme passes through the inpushing endoderm into the archenteron. Faurot (**7**), however, stated that the endoderm is formed by delamination. Urticina crassicornis.—Development is much the same as in Bolocera. Cleavage, however, begins when there are sixteen nuclei in the egg, and the trophenchyme nuclei are sparing or absent. The crumpling and folding of the wall of the early blastula which I find to be very well marked in the eggs of Urticina have not been described by Appellöf in his otherwise excellent account of the development of this species (**1**). Actinia bermudensis.—Early stages not determined; gastrulation by invagination (Cary, **5**). Actinia equina.—Early stages not determined; endoderm formation by invagination according to Jourdan (**12**), but by immigration or delamination according to Appellöf (**1**), who states that the mouth opening is a secondary break-through.[1] Cerianthus and an Actinian allied to A. equina.—Endoderm formation by invagination (Kowalevsky, **13**).

Movements.—Cilia are acquired during the middle blastula

[1] My own observations (Millport, 1920) are entirely in favour of the open invagination method of endoderm formation in this species.

stage and show activity before the egg-membrane spines have disappeared. Blastulae and early gastrulae move irregularly, but late gastrulae and older larvae progress with the aboral end in advance, rotating at the same time in the contra-solar direction as viewed from this end. Meantime a change of specific gravity has occurred and the larvae tend to remain on or near the bottom. Elongation of the larva has also taken place in the oral-aboral axis. The shape now varies according to contraction but is usually pyriform, the aboral end being the smaller. Over this end the ectodermal cells elongate, becoming clear at their outer extremities. They are preparing a cement in view of fixation. At no stage is there present a specially elongated tuft of cilia such as is characteristic of the larvae of Metridium and Sagartia and in a less degree of Actinia equina.

Mesenteries.—The eight primary or Edwardsia mesenteries appear, first in the neighbourhood of the mouth, as folds of the endoderm, each containing a thin mesogloeal sheet continuous with the general mesogloeal layer between ectoderm and endoderm. The sulco-laterals (ventro-laterals) are the first to develop. The remainder appear practically simultaneously, but I could sometimes make out that the sulculo-laterals were a little ahead of the sulcar directives, and the latter of the sulcular directives. In the figures the mesenteries are numbered 1, 2, 3, 4, corresponding to the above sequence.

All the primary mesenteries have appeared prior to fixation, and at this stage the oral ends of the sulco-laterals are already edged by a down-growth of stomodoeal ectoderm for the mesenteric filaments, and project so far inwards as almost to meet one another. The developing muscle banners on all the mesenteries show the characteristic Edwardsia arrangement.

Fixation occurs about twenty-five days after shedding of the eggs, and is at first by cement attachment, the larvae adhering usually to the bottom but sometimes to the sides of the hatching vessel. The base, at first small and pointed, soon becomes larger and disc-like. Shortening of the larva takes place till the length of the column is less than its breadth; the oral surface flattens; the mouth opens widely and elongates

in the axis of the directive mesenteries. Then the young anemones remain quiescent except in showing the following changes.

1. Absorption of the trophenchyme within the archenteron. It is partly used up and partly absorbed into the endoderm layer, which becomes greatly thickened, as well as extended by the fuller growth of the mesenteries.

2. Down-growth of stomodoeal ectoderm to form mesenteric filaments on the sulculo-lateral mesenteries. This began prior to fixation on the sulco-laterals.

3. Formation of a new mesentery in each lateral and sulco-lateral Edwardsia space. These mesenteries can be detected near the middle of the column of the larva earlier than near the mouth or on the base. In my oldest specimens their developing muscle banners could with much difficulty be made out, each being formed on the sulcular side of its mesentery as in Urticina (1). They are thus suitably placed to form with the Edwardsia sulco- and sulculo-laterals, the primary hexactinian ulco- and sulculo-lateral mesenteric pairs on each side, the remaining pairs being of course the sulcar and sulcular directives (fig. 15).

I tried to rear the young anemones further, but so far without success, although I gave the larvae the chance of settling down on shells, stones, glass, and mud, and of living after attachment either in separate hatching vessels, or in a tank with sea-water circulation. Those which settled on mud retained a rounded base, but otherwise reached much the same stages as the attached ones. None went the length of growing out tentacles. The attached specimens were less firmly fixed, and yet crept about less freely, than the corresponding stages in Urticina, in which also, as was shown by serial sections, the mesogloeal and muscular tissues were more strongly developed.

For further comparative details and a discussion of some general problems connected with coelenterate development, reference may be made to a recent paper by the author in the 'Phil. Trans. Roy. Soc. Lond.' (9) on the development of Metridium dianthus and Adamsia palliata.

Summary and Chronology.

Egg large, floating ; maturation prior to shedding ; fertilization external ; at least twenty-four nuclei present before cleavage of egg-mass takes place (fifteen hours) ; cleavage total leaving a small central cavity ; the inner ends of the cells separate off to form a central trophenchyme (twenty-four hours) ; a greatly-folded ' preblastula ' stage during which groups of cells are included in the trophenchyme (forty-eight hours) ; the blastula becomes more or less smooth and spherical (three to three and a half days) ; gastrulation begins (four to five days) ; gastrulation complete and first mesogloea formed (six and a half to seven and a half days), the trophenchyme passing into the archenteron, and degeneration of its nuclei taking place ; blastopore narrows and virtually closes, involution of stomodaeum taking place ; larva elongates (nine to ten days) ; sulco-lateral mesenteries begin to form (fifteen days) ; aboral end shows cement gland formation, and rudiments of the other mesenteries appear (twenty days) ; fixation and shortening of the larva (twenty-five days) ; formation of four additional mesenteries (thirty-six days) ; complete absorption of trophenchyme within archenteron (thirty-six days). For cilia, movements, &c., see p. 582. At no stage is there a tuft of specially elongated aboral cilia.

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EXPLANATION OF FIGURES.

bl.c., blastocoele cavity; *end.*, endoderm; *g.*, commencement of gastrulation; *n.f.*, furrows on blastula from which trophenchyme nuclei are nipped off; *n.tr.*, these nuclei degenerating within the trophenchyme; *st.*, stomodaeum; *tr.*, trophenchyme; *tr.a.*, trophenchyme within the archenteron; 1–6, the primary hexactinian mesenteries numbered according to their order of development (see explanation in text).

Fig. 1.—Section of Bolocera egg, 10 hours after fertilization, showing five nuclei; all the nuclei are in one hemisphere of the egg.

Fig. 2.—Section of egg about 18 hours after fertilization, showing the characteristic complete segmentation (see p. 579).

Fig. 3.—Similar section about 28 hours after fertilization. Note the passage of one of the cells inwards from the surface, and commencement of trophenchyme formation.

Fig. 4.—Similar section about 36 hours after fertilization.

Fig. 5.—Similar section about 48 hours after fertilization. Note the extremely folded and crumpled surface (see p. 580).

Fig. 6.—Similar section about $3\frac{1}{2}$ days after fertilization. The folds have mostly straightened out leaving behind numerous groups of cells nipped off from their recesses and enclosed within the trophenchyme. The outlines of these cells disappear and the nuclei degenerate now or later.

Fig. 7.—Similar section about $4\frac{1}{2}$ days after fertilization. Commencement of gastrulation.

Fig. 8.—Similar section about $5\frac{1}{2}$ days after fertilization.

Fig. 9.—Similar section about 7 days after fertilization.

Fig. 10.—Similar section about 8 days after fertilization, showing (*a*) the progress of gastrulation, (*b*) the passage of the trophenchyme through the inpushing endoderm into the archenteron, and (*c*) the involution of the lips of the blastopore to form the stomodaeum.

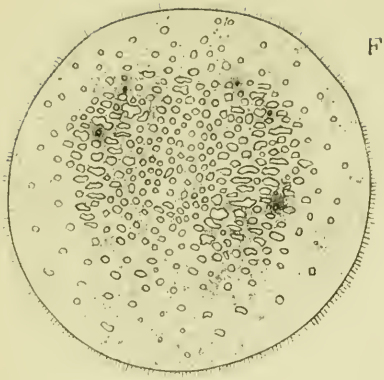
Fig. 11.—Longitudinal section of larva 12 days old. The shape is now pyriform and the cells at the aboral end are becoming elongated and glandular.

Fig. 12.—Transverse section across larva 15 days old near its oral extremity showing the two first mesenteries—the sulco-laterals.

Fig. 13.—Transverse section through larva 20 days old showing formation of all the Edwardsia mesenteries, viz. (1) the sulco-laterals; (2) the sulculo-laterals; (3) the sulcar directives, and (4) the sulcular directives. In this specimen the last named are the smallest and were no doubt the latest to appear (see p. 583). The sulco-laterals are now edged by a down-growth of epiblast for the mesenteric filaments.

Fig. 14.—Diagram of transverse section of attached specimen (25 days old) to illustrate the arrangement of the eight Edwardsia mesenteries which are numbered as in the previous figure, and on which the rudiments of muscle banners can now be made out.

Fig. 15.—Similar transverse section of attached specimen (36 days old) in which a new mesentery (numbered 5 and 6 respectively) has developed in each sulco-lateral and lateral Edwardsia space. Muscle banners are beginning to develop on their sulcular sides. The six primary hexactinian mesenteric pairs will consist of the sulcar directives, the sulcular directives, two pairs made up of two and five on either side, and two pairs made up of one and six on either side.



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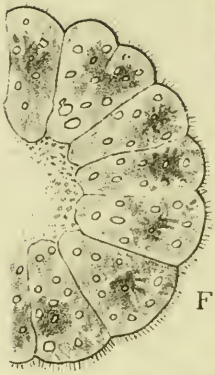

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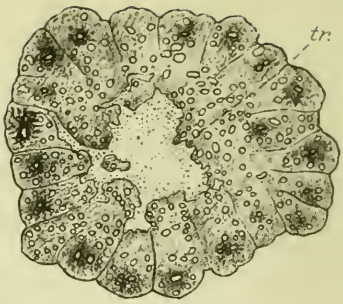

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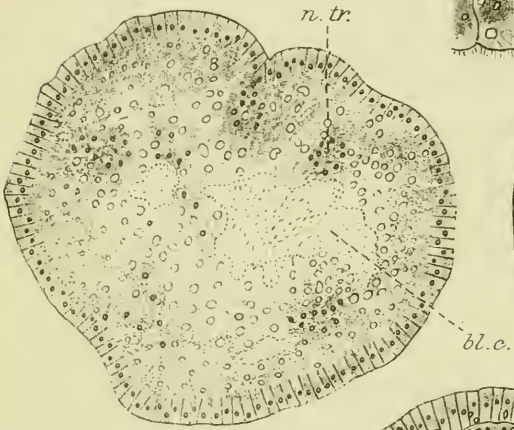

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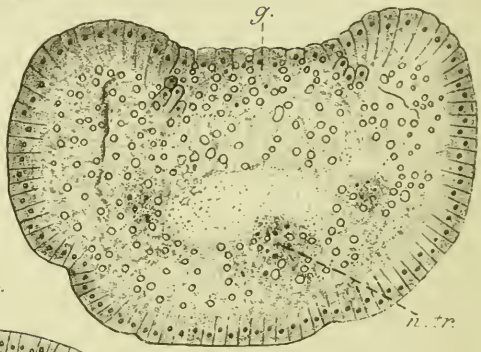

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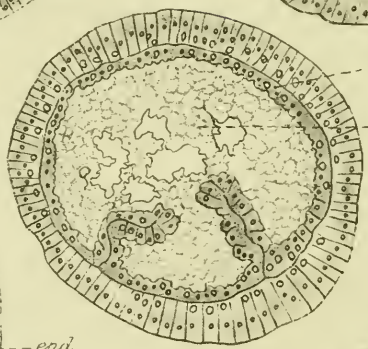

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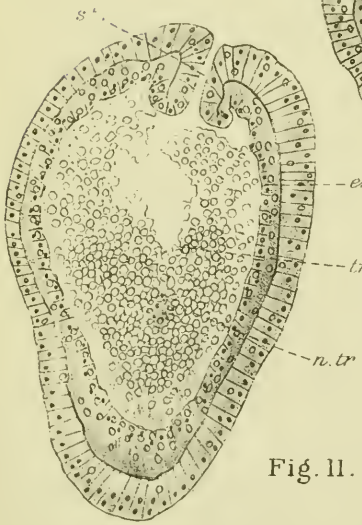

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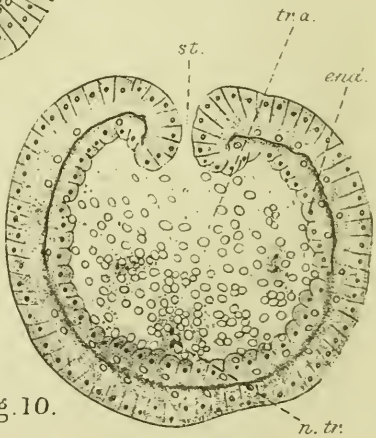

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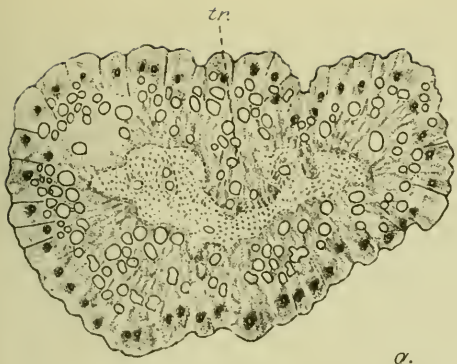

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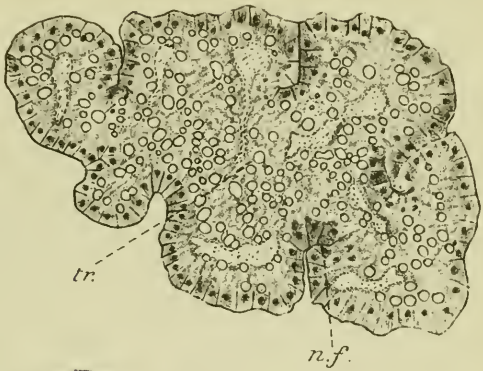

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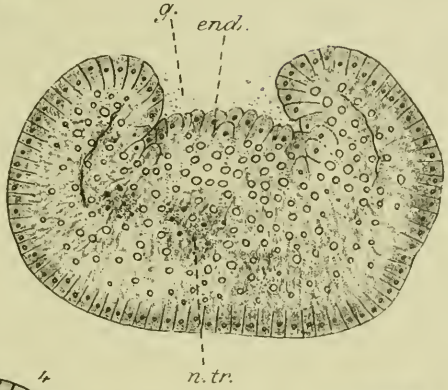

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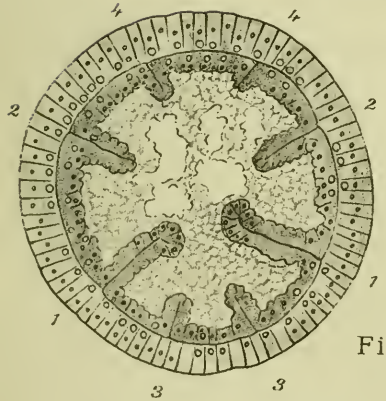

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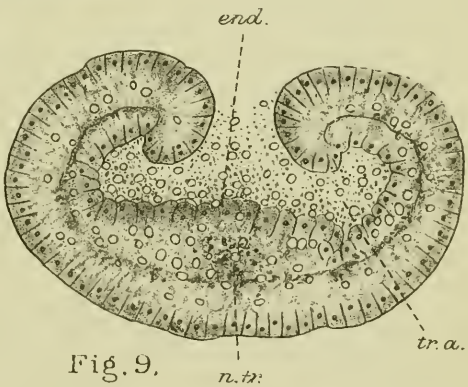

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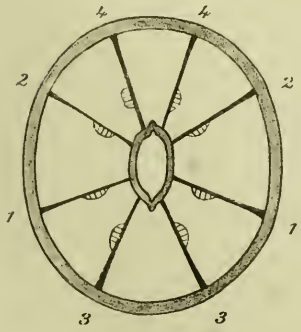

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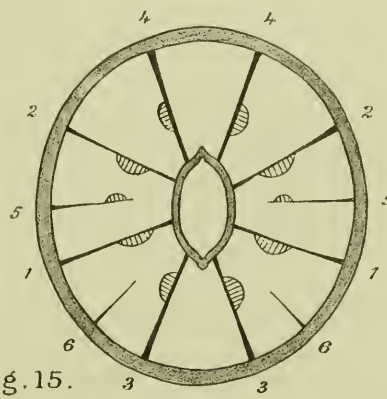

Fig. 15.

Huth London.

Observations on the Shape of the Nucleus and its Determination.

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and

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With Plates 23 and 24 and 11 Text-figures.

Contents.

1. Introductory.

That the nucleus is extraordinarily variable in shape, not only in different animal cells but also in the same cell during the different phases of its ontogeny and metabolism, is a notorious fact. In the following notes, which embody a brief description

of nuclear shape, we have also attempted to analyse, when possible, the factors responsible for this.

In the present stage of cytology the interpretation of cell-function is largely based on purely descriptive methods. Therefore such reasons as we have been able to put forward in explanation of the diversity of nuclear shape are to be regarded more as reasonable suppositions than as proven statements. We are of opinion that it is better to run the risk of assigning false causes to the phenomena which we have observed, than to explain nothing by confining ourselves to purely morphological considerations.

Only when cytology has acquired experimental methods will it be possible rigorously to determine the factors responsible for nuclear form and function.

Although the details of the structure of the nucleus—and particularly those concerning the disposition of the chromatin and the alleged 'linin' network—are controversial, observations on nuclear shape are easily verified. For not only are the appearances similar with widely different methods of fixation and staining, but they can be controlled by observations on living material. And, finally, corroborative evidence can sometimes be obtained by experimental methods such as tissue culture.

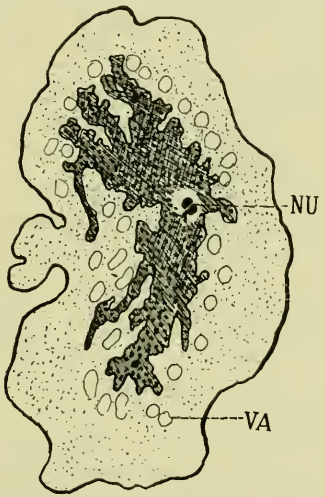
2. The Relation between Nuclear Shape and Surface Tension.

A spherical nucleus is found in hepatic and most other gland-cells, also in many nerve-cells and spermatocytes. Its shape may often be attributed to surface tension, being the result of a relatively fluid (nuclear) mass that is immiscible in the surrounding cytoplasm. Such nuclei are relatively rare in the animal body, for the spheroidal condition is not uncommonly associated with mechanical factors, e.g. furrows or canaliculi in the nuclear membrane. Such structures, which occur more often than is generally supposed, sometimes make it difficult to say whether a spherical nucleus is the result of surface tension alone or of accompanying mechanical causes.

It is a curious fact that such nuclei usually contain a single nucleolus only, and that this body tends to be in the centre of the nucleus or somewhat deviated towards that pole of it which is farthest from the centrosome. The latter seems to exercise a repellant action on the nucleus also—a fact which can be verified in many cells with large nuclei, e.g. spermatocytes.

Lobulation of the nucleus can sometimes be attributed to variations in surface tension at the interfaces of

TEXT-FIG. 1.



Phagocytic cell (amoebocyte) from a larva of Phryganea sp.—a caddis fly. Extreme polymorphism of the nucleus probably due to variations in surface tension over the nuclear membrane. NU, nucleolus; VA, vacuole. Technique: Bouin and iron haematoxylin.

nucleus and cytoplasm. A striking example of this is furnished by the large cells accompanying histolysis during metamorphosis in insects. Here, as is well known, the larval tissues are destroyed by large phagocytic cells known as Amoebocytes. Text-fig. 1 shows such an element from a larva of Phryganea sp. Here the polymorphism of the nucleus is extreme, while the nucleolus, which is single and central, does not appear to be involved in the lobulation. Of the latter, every degree

may be observed in such cells, and it seems definitely to be related to variations in surface tension caused by exchanges between nucleus and cytoplasm, as has been suggested by various authors (e.g. Prenant, **10**).

In other instances, however, the shape of the nucleus, notwithstanding its extreme lobulation, is too definite to permit of its being attributed to surface tension alone. Examples of this are the spermatogonia of some Amphibia, in which the shape of the nucleus is constant in a given species (Pl. 23, fig. 3). Here the nuclear polymorphism is apparently due to the intervention of other factors (to be considered later), and only such variations from the normal as occur during periods of intensive cell-activity—such as growth, differentiation, &c.—can be ascribed to the surface-tension changes that accompany such phenomena. Somewhat similar are the modifications which occur in many oocytes during development, as shown in Pl. 23, fig. 2. In the early stages of differentiation the nucleus in such elements is oval, containing one large central nucleolus and many smaller peripheral ones. But subsequently the nucleus becomes polymorphic, while around it is established a clear (endoplasmic) zone in the cytoplasm. Here again do we find extreme nuclear lobulation coinciding with enhanced metabolism of the cell.

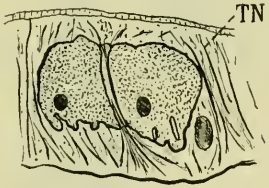
3. Mechanical Deformation of the Nucleus.

The study of our material has convinced us that nuclear shape is often due to pressure exerted on it by various cell inclusions. An obvious example of this is furnished by the thin and crescentic nucleus entirely pressed to the periphery of the fully developed adipose cell. Somewhat similar is the deformation of the nucleus in the duct-cells from the pronephros of *Triton* (see Pl. 23, fig. 4). This is due to the centre of the cell being occupied by the lumen of the duct. Again, in the early segmentation stages of ova containing much yolk, the nuclei are indented by the large, inert yolk-discs. Text-fig. 6 shows such a nucleus from a blastula of the Amphibian *Triton alpestris*. On one side there are deep indentations

between the centrosome and the nuclear membrane: these are due to other causes and will be referred to later. And, finally, similar appearances can be seen in the nuclei of the interstitial cells of the testicle of Rana esculenta, the nuclear membrane here being pitted by the lecithin globules in the cytoplasm.

Sometimes the inclusions are localized in a particular area of the cytoplasm. This may give rise to a peculiar deformation of the nucleus such as is depicted in Pl. 23, fig. 6, which illustrates a cell from the hepato-pancreas of the isopod crustacean Oniscus. Here the nucleus at the basal, i.e. attached,

TEXT-FIG. 2.



Cell from pronephros of a 3 mm. larva of Triton alpestris. Note constriction of middle of nucleus due to pressure from Tonofibrillae, TN.

pole of the cell is strikingly indented by large cytoplasmic globules of a lipoid nature. It follows from this that nuclear deformation can be produced by relatively fluid bodies.

Another instance of nuclear shape being modified by cytoplasmic structures is afforded by the intestinal epithelial cells of the same species. By appropriate staining methods (see Pl. 24, fig. 3) fine fibrils lying in the cytoplasm around the nucleus can be distinguished. They run from the basement membrane to the cuticle, apparently function as an intracellular skeleton, and may be termed Tonofibrillae after the French ' Tonofibrilles '.

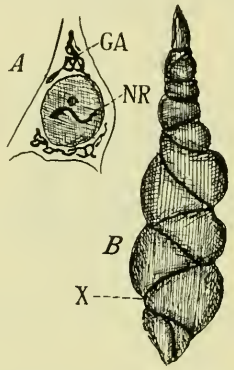
We have also observed a similar condition in cells from the excretory tubules of larvae of Triton as shown in Text-fig. 2.

In those muscles which are characterized by cross-striation

of their fibres, i.e. ordinary striated and cardiac muscle, the influence of cytoplasmic structures on nuclear shape is very marked. Thus, in striated muscle there is obvious flattening of the nuclei against the sarcolemma due to pressure from the areas of Cohnheim (i.e. groups of fibrils) of which the fibre is composed.

Often, however, other causes intervene, chief amongst which is the influence of the Membrane of Krause ('Strie Z' of the French and 'Zwischenscheibe' of the German authors).

TEXT-FIG. 3.



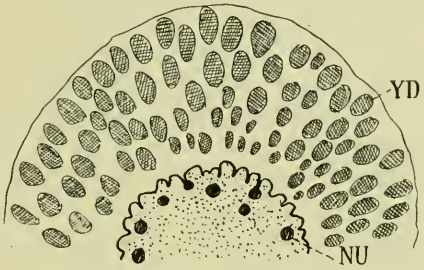
A. After Cajal, showing intranuclear rodlet, NR, in pyramidal cell from cerebral cortex of rabbit. Technique: Cajal method for Golgi apparatus, GA. B. After Retzius, depicting peri-nuclear structure (X) in spermatozoon of the Gasteropod Cypraea.

This structure is segmentally disposed along the muscle fibril and appears in the middle of the dark bands as a clear and narrow line. It is best studied in the large fibres of insects (see Text-fig. 10), where it can be seen to constrict the nucleus at regular intervals by its projection out of the fibrils into the surrounding sarcoplasm.

A similar appearance of the nuclei can be seen in human cardiac and other vertebrate muscle. This is shown in Pl. 23, fig. 7. But here we have not been able to follow the membrane of Krause as far as the nuclear membrane. Nevertheless, the nuclei bear definite constrictions corresponding to the membranes of Krause of adjacent fibrils, while the curious blunt-

ended nuclei—so characteristic of human heart muscle—can only be explained by assuming the presence of these membranes lying invisible in the sarcoplasm at each end of the nucleus.

Text-fig. 4.



Oocyte of Esox lucius—a pike. Note the pouches in nuclear membrane usually in relation with the nucleoli. NU, nucleolus; YD, yolk-discs. Technique: Bouin and iron haematoxylin.

4. Nuclear Shape and the Centrosome.

We deliberately confine ourselves to the consideration of the centrosome and nucleus in the resting cell, as the question of the spindle fibres, amphiaster and chromosome formation is beyond the scope of these observations. In the resting cell the centrosome often lies very close to the nuclear membrane and opposite an indentation in it. And since this body often does not touch the nucleus, one must surmise that the depression is due not to mechanical causes but to repulsion between nuclear membrane and centrosome. When an amphiaster is present, its influence upon the nucleus is still more marked, as is shown in Text-fig. 6, which depicts a blastomere from an egg of Triton. It will be seen that here nuclear shape is due partly to pressure from the yolk-discs (as already pointed out), partly to invaginations in the nuclear membrane in the vicinity of the centrosome. The astral rays in fact deeply indent the nucleus wherever they come into contact with it—a point possibly in favour of the view that the cytoplasmic radiations around the centrosome are of a relatively solid nature.

5. The Relation between Cell Shape and Nuclear Shape.

It is notorious that the longer a cell, the longer (usually) is its nucleus. Muscle, columnar epithelium, and connective-tissue cells are familiar examples of this (see Pl. 23, figs. 1, 8, and 10; Pl. 24, fig. 2). This elongation of the nucleus is often due to mechanical causes. Thus, in epithelia it is sometimes due to mutual cell-pressure, while the long nucleus of the smooth muscle-fibre must be ascribed to pressure from the myofibrillae. Further, the nucleus shortens or lengthens as the fibre contracts or extends. Again, in preparations of amphibian intestine fixed in different degrees of distension, there are marked differences in the height of the epithelial cells and their nuclei—the two varying in length in a parallel ratio between certain limits. Exceptions, however, exist to this general rule. For instance, in the intestinal epithelial cells of the dragon-fly Libellula (see Pl. 24, fig. 5) the small oval nucleus is quite disproportionate to the elongated cell.

As claimed by Martin Heidenhain (**8**), we must surmise the existence of a force which tends to push the nucleus towards the centre of the cell. And in view of the plasticity of the nucleus there can be no doubt but that this force must influence its shape also.

It is a fact of no small significance that the nucleus never comes into contact with the cell membrane, except in a few instances due to powerful mechanical factors, e.g. pressure from bulky cytoplasmic inclusions forcing the nucleus against the cell membrane. Two possibilities suggest themselves in explanation of this:

(1) That the position of the nucleus is due to forces exerted on it by the surrounding cytoplasm, forces which might conceivably be proportional to the mass of the cytoplasm around the nucleus. Were this so, nuclear shape in a cell of greater length than breadth would

be as in Text-fig. 9, B on p. 600, which is never the case in nature.

(2) That there is mutual repulsion between cell membrane and nuclear membrane. Such a force, acting in an inverse ratio to the distances between the two membranes is indicated in Diagram C, p. 600. This supposition explains :

(*a*) Why nuclear and cell membranes practically never come into contact with one another.

(*b*) Why the nucleus tends to elongate concurrently with the cytoplasm.

(*c*) Why the nucleus is never round so long as the length of a cell is greater than its breadth, although there is often ample room in the cytoplasm for it to become spherical.

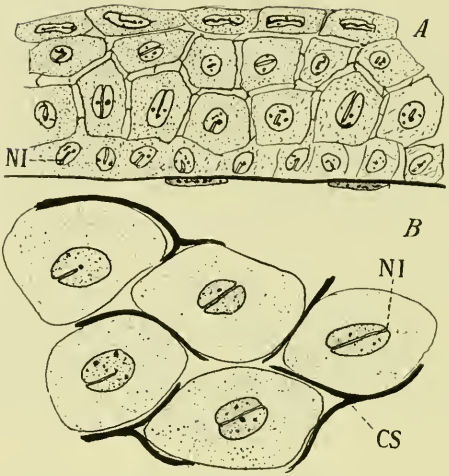
Of the nature of such a force responsible for the antagonism between cell membrane and nuclear membrane we know nothing.

6. Canaliculi in the Nuclear Membrane.

Intranuclear canaliculi are more common in spherical and oval nuclei than is usually thought. They have been described in the spermatogonia of Amphibia by Champy (**4**), and are easy to demonstrate in Rana esculenta and the Axolotl. Canaliculi in the nuclear membrane occur in many types of cell ; we have observed them in the epithelium lining the Wolffian duct in the salamander, and in pyramidal cells of the cerebral cortex in the guinea-pig. These structures are illustrated in Pl. 24, fig. 4, and in Text-fig. 8.

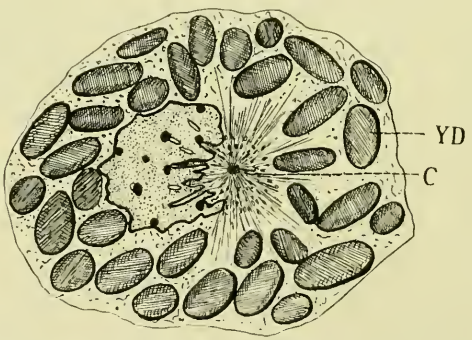
The intranuclear canaliculus is essentially a narrow invagination of the nuclear membrane. Its blind extremity, which may be bifid, often ends in the vicinity of the nucleolus. That this structure is a definite tube and not a deep furrow in the nuclear membrane, is shown in transverse sections of it. In many spermatogonia there seems to be some relation between the canaliculus and the centrosome ; at the prophase the latter comes to lie very close to the former, often exactly opposite its aperture in the nuclear membrane.

TEXT-FIG. 5.



A, Skin of sucker of Lepadogaster guannii—a 'suck-fish'. B, Supporting tissue of the same organ with intercellular stroma of cartilage. Both A and B show intranuclear canaliculi in all the cells. Probably mechanical in origin, e.g. mutual cell-pressure. CS, Intercellular cartilaginous stroma; NI, Nuclear incision.

TEXT-FIG. 6.



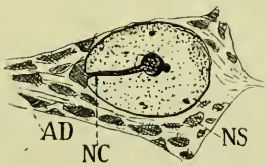
Blastomere from blastula of Triton alpestris. Note deformation on one side by yolk-discs, YD, and on the other by astral rays. C, Centrosome. Technique: Champy's fluid and iron haematoxylin.

The intranuclear canaliculus of nerve-cells (see Text-figs. 7 and 8) is sometimes demonstrated by the Cajal method for the Golgi apparatus, and has apparently been observed by Cajal himself. With standard cytological stains—such as iron haematoxylin—it appears as a single invagination of the nuclear membrane. Its aperture is often opposite the point

TEXT-FIG. 7.

Binucleated sympathetic ganglion cell from rabbit.
Intranuclear canal in one of nuclei.

TEXT-FIG. 8.



Pyramidal cell from cerebral cortex of guinea-pig.
AD, Apical dendrite; NC, Intranuclear canal; NS, Nissl substance.

of insertion of the apical dendrite in the case of pyramidal cells (see Text-fig. 8). In these elements the relation of the canaliculus to the centrosome is obscure, largely owing to the uncertainty of the existence of this structure in adult nerve-cells.

Intranuclear canaliculi are also readily observed in the cells lining the Wolffian ducts in Amphibia, while apparently similar structures can sometimes be seen in the tissues of the higher Vertebrates, though here, except in the case mentioned above, the small size of the cells renders observation difficult.

7. Folds and Incisions in the Nuclear Membrane.

Such modifications of the nucleus are common, though care is required in their observation. This is easiest after fixation in fluids which do not precipitate the nuclear contents in too coarse a manner. Fixatives such as Gatenby's Flemming without acetic (**6**) and Champy's carbol-formalin (**4**) give the best results.

Text-fig. 9.

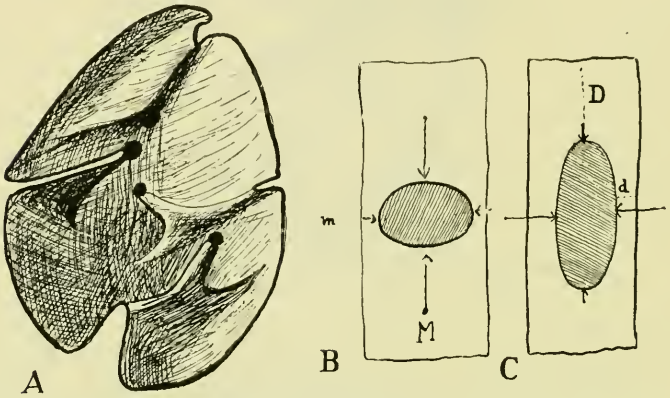


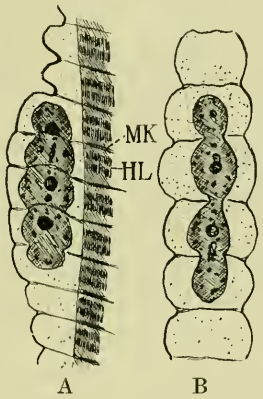
Fig. A.—Diagram showing the relation of intranuclear incisions to the nuclear membrane and nucleoli.

Figs. B and C showing that the shape of the nucleus is governed rather by its distance from the cell membrane than by any mass action of the cytoplasm. Were nuclear shape the product of repulsion between the nuclear membrane and the mass of the cytoplasm, the shape of the nucleus in an elongated cell would be as shown in B. But this is never the case. In nature the long axis of the nucleus is always in the long axis of the cell, as indicated in C. The explanation of this seems to be that the nuclear membrane is repelled by the cell membrane, and that the nearer it is to the latter, the greater the degree of repulsion.

Gastric epithelium of Amphibia, e.g. of Bombinator or Alytes, in which the cells are very large, shows clearly the folds in the nuclear membrane. In longitudinal sections of the nuclei there may be several of these structures, which may or may not traverse its entire length. They are illustrated in Pl. 23, figs. 1, 7 A, and 8. That we are dealing with folds and not with canaliculi is made clear by transverse sections of such nuclei, which are depicted in Pl. 23, figs. 7 B and C, and 8.

Folds in the nuclear membrane are found in a great variety of cells in addition to gastric epithelium in Amphibia. They occur in cardiac muscle in Man and Astacus (the crayfish), and also in the connective-tissue cells of the Testis in the latter species. In germinal epithelium they are especially common, not only in that of the Axolotl (Pl. 23, fig. 10) but also in some mammalian tissues. But in the latter it is usually difficult to make sure that the structures one can see

TEXT-FIG. 10.



Portions of muscle-fibres from nymph of Phryganaea sp.—a caddis fly. In A the membranes of Krause can be seen running across the sarcoplasm and constricting the nucleus at regular intervals. In B only the nuclear constrictions are visible, the section passing outside the zone of myofibrillae. HL, Hensen's line; MK, Membrane of Krause.

in germinal epithelium are truly intranuclear folds, though it is interesting to note that undoubted incisions exist in the pathological cysts—Cystadenomata—which are derived from this epithelium.

The nuclei of smooth muscle-fibres, after impregnation by the Cajal method for the Golgi-apparatus (Cajal, **1**; Carleton, **2**), show a peculiar spiral peri-nuclear band which has been observed by Rio Hortega (**11**). After careful differentiation, iron haematoxylin sections show that this structure is not a thickened portion of the nuclear membrane but a series of usually rather irregularly arranged spiral folds. Transverse

sections of such nuclei confirm the existence of these incisions, which we have observed in non-striated muscle from the intestine in Amphibia (see Pl. 24, fig. 2), in Mammals (muscle layers of intestine of cat), and in certain invertebrate muscle-fibres, e.g. heart of Helix as shown in Pl. 24, fig. 6.

Finally, we have noted similar folds in the nuclear membrane of developing oocytes (already described in Section 3), while a peri-nuclear reticulum—possibly comparable to that found in smooth muscle-cells—has been described by Retzius in the spermatozoa of certain Gasteropods as shown in Text-fig. 3, B.

8. The unfolding of Invaginations in the Nuclear Membrane.

It seems certain that nuclear folds and incisions expand under certain conditions, thus altering both volume and shape of the nucleus. That such a phenomenon occurs during differentiation of some cells is shown by the following example :

In Urodele Amphibia there exists a layer of lymphoid tissue surrounding the liver. Study of the lymphocytes in this layer (see Pl. 23, fig. 5) show that their nuclei, though round or oval, bear a large number of narrow incisions. The latter can be observed in various degrees of ' deployment ' in these cells, and there is no doubt that polymorphonuclear leucocytes can be formed in this manner from lymphocytes in some Amphibia —a point in favour of the ' Unicist ' theory of blood-formation. The persistence of some of the nuclear folds gives rise to the lobulation characteristic of the polymorphonuclear leucocyte.

Mutual cell-pressure may apparently in certain cases inhibit expansion of the nuclear membrane. We have observed an instance attributable to this in cells from the epidermal and sub-epidermal tissues of the sucker of the fish Lepadogaster guannii. This is illustrated in Text-fig. 5, A and B.

9. Intranuclear Rodlets, etc.

Intranuclear rodlets and allied structures, which are only found in highly specialized cells such as spermatids or certain red blood corpuscles, are responsible for the shape of the

nucleus in such elements. The peculiar shape of the head of the spermatozoon is doubtless an adaptation enabling it rapidly to move in fluids and to penetrate the ovum. In some instances, which have been described by Champy (**4**), the changes in the shape of the nucleus during the stages termed 'Spermateleosis' by Gatenby (**7**) are due to the influence of a special intranuclear apparatus.

TEXT-FIG. 11.

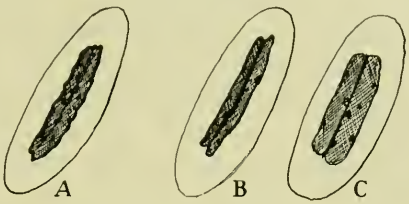


Fig. A.—Normal red blood corpuscle of bird with intranuclear rodlet faintly indicated.

Figs. B and C.—Avian red cells after four days' culture (pigeon's red cells in chicken plasma). The nuclei have become swollen and the chromatin reduced in amount; consequently the intranuclear rodlet is clearly visible.

The latter is best studied in Amphibia such as Bombinator, the Salamander, and the Axolotl. In these it can be seen within the spermatid as a thin and usually refringent rod, lying in the long axis of the nucleus. It appears to be developed from the centrosomes, originating from either the posterior or the anterior of these structures. Or sometimes it may be developed from both simultaneously. When the intranuclear rodlet does not extend the whole length of the nucleus, its free extremity, which may be bifid, is sometimes in relation with the nucleolus. All this is indicated in Pl. 24, fig. 1, which depicts spermatid nuclei from Bombinator. That this structure is not a fold in the nuclear membrane is seen in the figures of transverse sections of these nuclei. But it often co-exists with intranuclear canaliculi, from which, however, it can be further distinguished by its greater refringency.

It is well known that the red blood corpuscles of birds have

oval nuclei of a very definite aspect. These nuclei are remarkably stable, for they often retain their shape after the rest of the corpuscle has been haemolysed. Now, observation of the normal Avian red cell reveals little beyond a rather dark central portion and, often, a small invagination at both poles of the nuclear membrane. The general appearance is such (see Text-fig. 11) as to suggest the presence of some supporting structure within the nucleus, though the density of the chromatin makes its observation difficult. But when a bird's red blood corpuscles are aseptically cultivated in their own plasma, the nucleus slowly swells up before actual death of the cell occurs. As the nucleus becomes spherical, the chromatin becomes condensed into a single nucleolus, and an axial rodlet can frequently be seen under such conditions.

10. The Relation between Nucleoli and Nuclear Shape.

The nucleolus remains one of the most enigmatical of the cell components, in spite of the attention devoted to it by many biologists, and by Montgomery (**9**) and Vigier (**12**) in particular. The nucleolus is of interest in that it often shows amoeboid movements and undergoes independent fission during the life of the cell. In these observations the term 'nucleolus' is used in its widest sense, as signifying any condensation of nuclear material within the nucleus. Consequently, the word as employed in this paper applies to both karyosomes (or chromatin nucleoli) and plasmosomes (i.e. condensations of the oxyphil substance called plastin). Not only do both chromatin and plastin often occur within the same nucleolus, but karyosomes or plasmosomes sometimes contain one or more granules of unknown composition, which have been shown by the aid of special impregnation methods to divide by fission during mitosis (Carleton, **3**).

Clearly the nucleolus is often a complex structure of doubtful significance, and it is impossible at present to dogmatize on the relation of this element to nuclear shape. At the most, certain deceptive appearances may be cleared up.

Nuclear polymorphism is often—though by no means always—associated with the presence of multiple nucleoli as shown in Pl. 23, figs. 3 and 5. In elongated nuclei the nucleoli usually lie parallel to the main axis of the nucleus as depicted in Pl. 24, figs. 2 and 6, and in Text-fig. 10.

But it is in developing oocytes that the relation between nucleoli and nucleus is particularly deceptive. In the earlier stages of development the nucleoli come to lie at the periphery of the nucleus, and when invaginations subsequently appear in the nuclear membrane, they do so opposite the nucleoli. Pl. 23, fig. 2, and Text-fig. 4 illustrate this, and they suggest the possibility of nuclear incisions being formed under the influence of the nucleoli. On the other hand, it must be observed that in the case of some nuclei, the indentations in which are obviously due to certain of the mechanical causes already considered, the nucleoli are yet often in relation to the blind ends of the pouches in the nuclear membrane. In muscle, too, infolded portions of the latter often come into contact with the nucleoli, though here again nuclear incisions are primarily mechanical in origin. And finally, there are cells the nuclei of which contain nucleoli and yet have a nuclear membrane of regular contour, as shown in Pl. 23, fig. 11.

The main outcome of all this is that the relations so often seen between nucleoli and nuclear invaginations are usually secondary, and that the position of the nucleoli in such instances is rather an effect than a cause.

11. Cell Division and Nuclear Differentiation.

It is not without significance that mitoses are extremely rare—if not altogether absent—in cells the nuclei of which contain well-developed canaliculi or incisions. Such, at any rate, is the case with the following tissues in adult mammals :

Non-striated muscle.

The various segments of the urinary tubule in the kidney.

The epithelium lining the vesicles of the thyroid gland.

Nerve-cells.

Our observations suggest that while highly developed nuclear

canaliculi or incisions seem to be incompatible with mitosis, direct division may occur in cells—other than those enumerated above—which contain such structures. Thus, amitosis has been observed in nuclei of the cells of the Wolffian ducts and germinal epithelium and Sertoli cells ; also possibly in the gastric mucosa of some animals.

The behaviour of smooth muscle when cultured in plasma confirms this idea. It has been shown (Champy, **5**) that the nuclei of this tissue, when removed from the inhibitory influences of the organism, multiply actively. At first they do so amitotically, and only when the typical structure of these nuclei has disappeared by a progressive ' de-differentiation ' do they multiply by mitosis. Cultures of ovarian germinal epithelium behave in a similar manner. Again, the fundus glands of the human uterine mucosa have nuclei without incisions, while the cervical glands possess them. The former divide mitotically, the latter amitotically. And further, even in Adenomata (i.e. benign tumours) derived from the cervical glands does direct division persist. Only when such growths become carcinomatous do mitoses appear.

We would here point out that incisions or lobulations of nuclei have only too often been mistaken as evidence of direct division. In our experience such appearances are only of value when an actual increase of the number of nuclei can be established.

In conclusion, then, there is evidence that well-developed intranuclear canaliculi and incisions are incompatible with mitosis, a fact which possibly explains the tendency towards direct division in certain cells with specialized nuclei.

12. Summary.

Variations in the shape of the nucleus have been described in different animal cells. In addition, the following factors have been shown to be responsible for nuclear shape :

(1) Surface tension : when this is equal over the surface of the nuclear membrane, the nucleus tends towards the spherical condition. When surface tension varies over the interface

between nucleus and cytoplasm, nuclear polymorphism may result.

(2) Mechanical deformation of the nucleus is common and may be due to various causes, chief amongst which are: (*a*) Pressure from cytoplasmic inclusions, e.g. fat, lecithin, and yolk; (*b*) Tonofibrillae; (*c*) in striated muscle, the influence of the Membranes of Krause which constrict the nucleus along its length—and limit its ends—by their prolongation from the myofibrillae into the sarcoplasm.

(3) The centrosome, which has been shown (in the resting cell) often to repel that part of the nuclear membrane which is nearest to it.

(4) The relation between cell shape and nuclear shape has been briefly discussed. It has been noted that the nucleus never comes into contact with the cell membrane, except in the rarest instances due to the intervention of mechanical factors. Evidence has been brought forward in favour of our view that there is a mutual repulsion between cell membrane and nuclear membrane.

(5) Canaliculi and incisions in the nuclear membrane have been described in various cells.

(6) The unfolding of such incisions during development and differentiation of some such cells has been described.

(7) Intranuclear rodlets and their importance in the maintenance or the modifying of nuclear shape have been discussed.

(8) Mitotic division and a certain degree of nuclear differentiation have been shown often to be incompatible—thereby accounting for amitosis in certain highly specialized nuclei.

(9) The need for care in distinguishing between nuclear incisions and genuine amitotic division of nuclei has been emphasized.

June 1921.

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14. EXPLANATION OF PLATES 23 AND 24.

Illustrating Champy and Carleton's paper on ' Observations on the Shape of the Nucleus and its Determination '.

High-power figures drawn at various magnifications.

Arrows point towards the distal (i. e. unattached) ends of the cells.

Lettering.

BC., bile canaliculus; *BM.*, basement membrane; *C.*, centrosome; *C POST.*, posterior centrosome; *H CAN.*, Holmgren canaliculi; *LG.*, lipoid granules; *MI.*, mitochondria; *MK.*, membrane of Krause; *NC.*, nuclear canal; *NI.*, nuclear incision; *NR.*, nuclear rodlet; *NU.*, nucleolus; *PNF.*, peri-nuclear fold; *SB.*, striated border; *TN.*, tonofibrillae; *TS.*, transverse section; *X.*, invagination of nuclear membrane.

PLATE 23.

Fig. 1.—Showing nuclear incisions in a connective-tissue cell from the Testis of Astacus.

Fig. 2.—Oocytes of the fish Silurus sp., showing how the nucleus becomes polymorphic at a later stage of development.

Fig. 3.—Spermatogonium of Bombinator igneus, illustrating that the relation of nucleoli to nuclear folds is not constant. Here the nucleus has many incisions and yet the nucleoli bear but little relation to them.

Fig. 4.—Tubule cell from a nephridium of Aulostomum—a leech. Folds in nuclear membrane orientated in relation to flattening out of nucleus.

Fig. 5.—Two leucocytes from the lymphoid layer of the liver of the Axolotl. In A the nucleus is oval and its membrane highly pleated. B shows a polymorphonuclear white cell derived from A by the partial unfolding of the nuclear incisions. Technique: carbol-formalin and ferric Brazilin.

Fig. 6.—Cell from the hepato-pancreas of Oniscus (an Isopod Crustacean) showing deformation of the nucleus by large lipoidal granules in the cytoplasm. Technique: Benda fixation and iron haematoxylin.

Fig. 7.—Human cardiac muscle cells. A is a longitudinal section showing (i) the pleating of the nuclear membrane, each incision corresponding to a membrane of Krause, and (ii) the square ends of the nucleus. B illustrates the arrangement of the nuclear incisions in transverse section at a higher magnification. At the blind end of each incision there is usually a nucleolus. C is a longitudinal and somewhat oblique section of the nucleus, showing the relation of its shape to the fibrils. Technique: carbol-formalin and iron haematoxylin.

Fig. 8.—Cells from gastric epithelium of the Axolotl. The nuclear membrane shows deep longitudinal incisions. *TS.*=a transverse section of the nucleus, the relation of the nuclear incisions to the nuclear membrane being clearly shown.

Fig. 9.—Nucleus of cardiac muscle of Astacus, showing relation between nuclear incisions and nucleoli.

Fig. 10.—Longitudinal nuclear folds in germinal epithelium cell of Axolotl.

Fig. 11.—Spermatocyte of Lithobius forficatus—a Myriapod. An example of a nuclear membrane of regular contour in spite of multiple nucleoli.

PLATE 24.

Fig. 1.—Spermatid nuclei of Bombinator—a toad. Showing the fully formed axial rodlet in A. B and C are different stages in its

formation. D, E, F, and G show its appearance in transverse section. Technique: Bouin and iron haematoxylin.

Fig. 2.—Nuclei of smooth muscle from the intestine of the Axolotl. A and B are longitudinal sections of nuclei, while C is transverse. All show the spiral circular incisions in the nuclear membrane. Technique: carbol-formalin and iron haematoxylin.

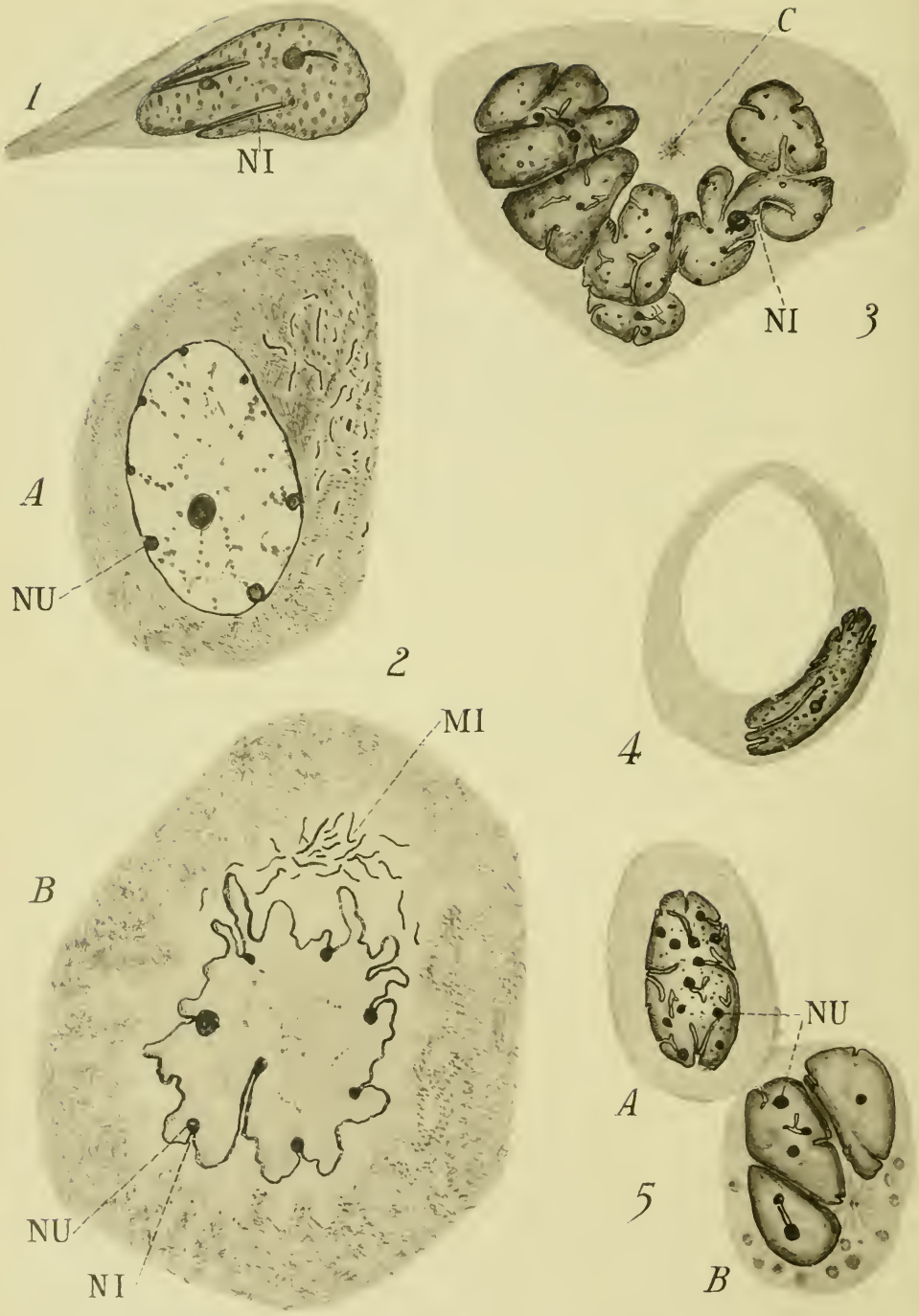
Fig. 3.—Cell from intestine of Oniscus. Note deformation of nucleus by 'Tonofibrillae'.

Fig. 4.—Cell from Wolffian duct of Salamander showing the intranuclear canaliculus and centrosomes opposite its aperture.

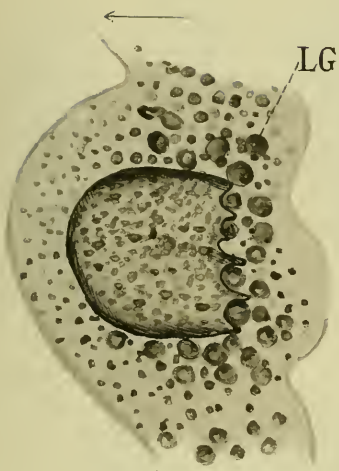
Fig. 5.—Intestinal epithelial cell from Libellula sp.—a dragon fly. Note that here the length of nucleus is not proportional to that of the cell.

Fig. 6.—Nuclei in longitudinal and transverse section from heart of Helix pomatia (snail). Incisions in nuclear membrane. Technique: Flemming and iron haematoxylin.

Fig. 7.—Hepatic cells of Salamander. At x the nucleolus is in contact with the nuclear membrane, which is slightly invaginated at this point. Technique: Bouin and iron haematoxylin.



Champy & Carleton



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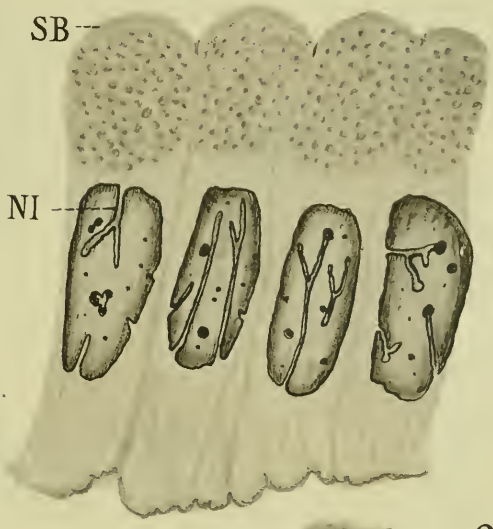

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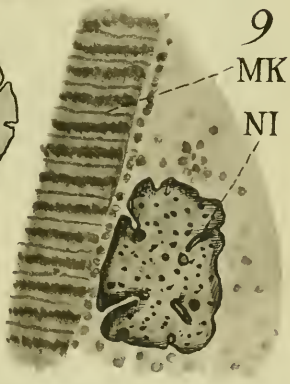

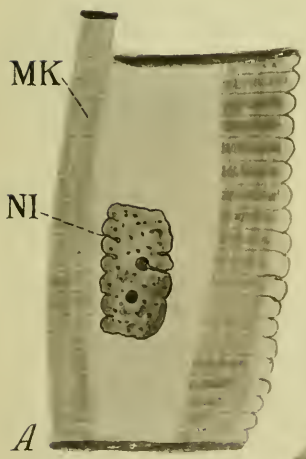

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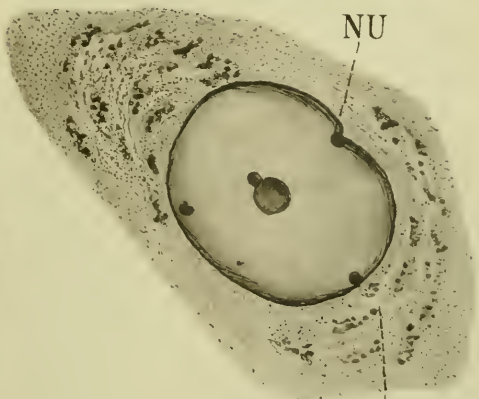

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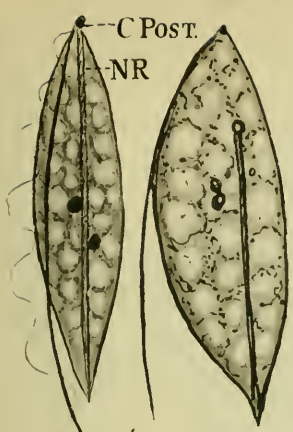

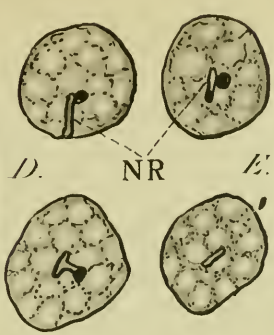

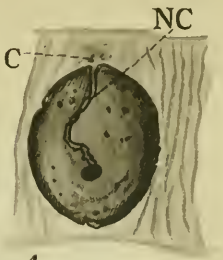

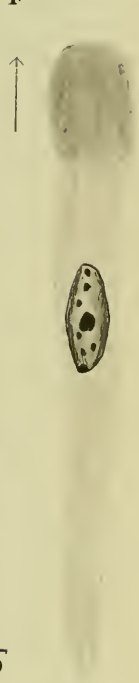

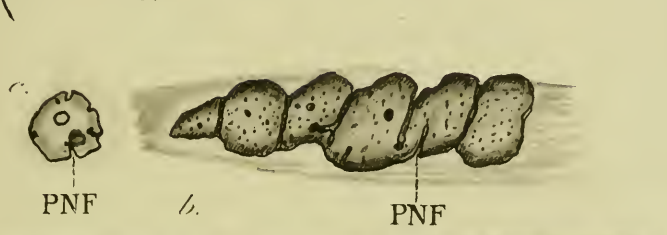

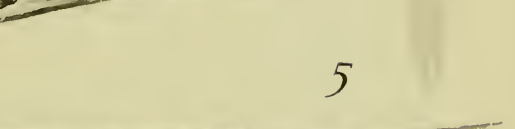

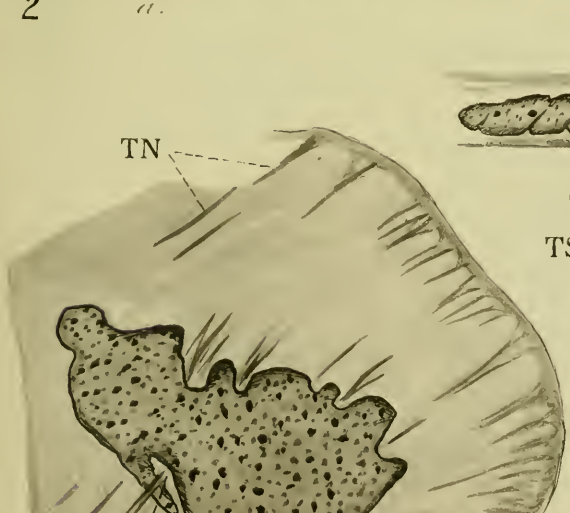

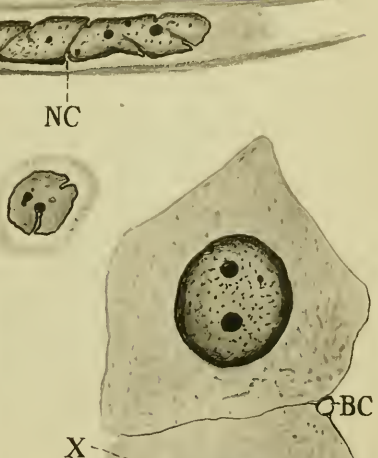

Champy & Carleton

On the calcium carbonate and the calcospherites in the Malpighian tubes and the fat body of Dipterous larvae and the ecdysial elimination of these products of excretion.

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(From the Quick Laboratory, University of Cambridge.)

With 5 Text-figures.

Contents.

1. The Presence of Calcium Carbonate in the Malpighian Tubes.

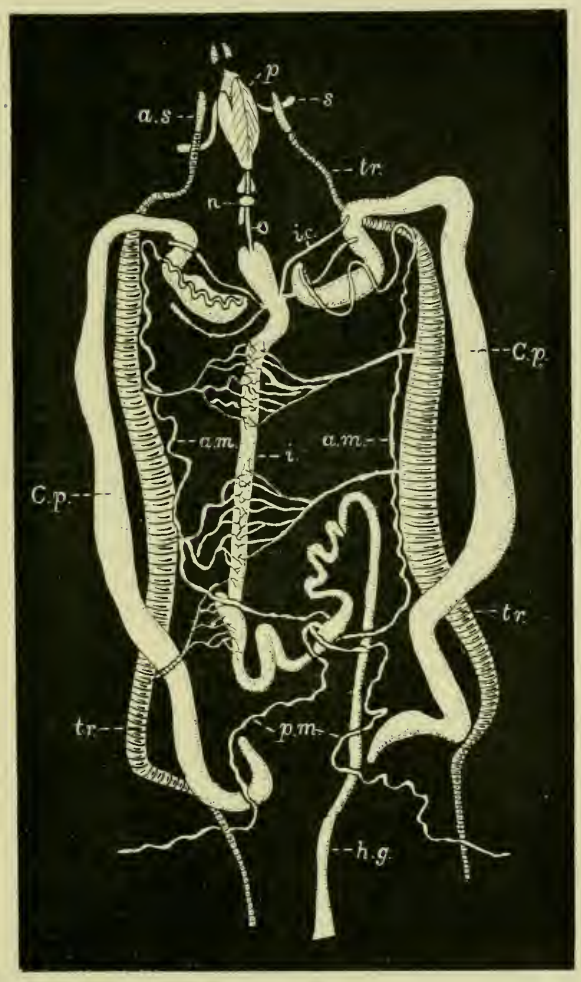
Lyonet (**11**, 1832) was the first to notice in the larva of Ptychoptera two milky-white vessels running throughout the length of the body. Similar vessels have been discovered in the larva of Eristalis by Batelli (**1**, 1879), who has rightly described them as saccate dilatations of the anterior pair of Malpighian tubes filled with calcium carbonate. Quite

independently Valery Mayet (**13,** 1896) has shown that in Cerambyx larvae, of the six Malpighian tubes, four are larger and are filled with calcium carbonate. The excretion of this product, which was described by Valery Mayet as a new function of the Malpighian tubes, was denied by Künckel d'Herculais (**10,** 1896), who at a meeting of the Entomological Society of Paris made an observation that Valery Mayet probably misunderstood the anatomy of the larva, and that the organs containing calcium carbonate were not Malpighian tubes but the intestinal caeca. Later, Valery Mayet (**14,** 1896) succeeded in demonstrating that the tubes in question were actually the Malpighian tubes; Künckel d'Herculais then suggested that the calcium carbonate of Cerambyx larvae is probably formed in other special glandular cells, and that the Malpighian tubes were eliminating only the excess of this product. P. Marchall (**12,** 1896), who took part in this discussion, observed that the excretion of $CaCO_3$ by the Malpighian tubes has nothing surprising in it; he thought, however, that the excretory function in insects is not localized in one particular organ: uric acid, for instance, can be found not only in the Malpighian tubes but in the intestine and the fat body.

Calcium carbonate has been found also by Vaney (**19,** 1900; **20,** 1902) in the anterior pair of the Malpighian tubes of the Stratiomys larva, and by Pantel (**16,** 1898) in the parasitic larvae of Tachinidae and in the larvae of Ptychoptera (**17,** 1914). In the latter, two of the five Malpighian tubes are transformed into large sacs filled with calcium carbonate.

I myself have found the excreted calcium carbonate in the Malpighian tubes of many Dipterous larvae: Eristalis tenax, L., Myiatropa florea, L., Mallota eristaloides, Lw., Merodon equestris, F., Syritta pipiens, L., Eumerus strigatus, Fln., Ptychoptera contaminata, L., several species of Stratiomyidae belonging to the genera Stratiomys, Sargus, and Odontomyia, and among the Trypetidae in Anastrepha striata, Schiner. In all of these larvae the carbonate-containing Malpighian tubes differ from the rest by being

TEXT-FIG. I.



Myiatropa florea, dissection of a full-grown larva. *a.m.*, anterior pair of Malpighian tubes; *a.s.*, anterior spiracles; *c.p.*, calcareous or terminal portion of the Malpighian tubes; *h.g.*, hind-gut; *i.*, mid-gut; *i.c.*, intestinal caeca; *n.*, central nervous system; *o.*, oesophagus; *p.*, pharynx; *p.m.*, posterior pair of Malpighian tubes; *s.*, salivary glands; *tr.*, tracheal trunks.

more developed and of a milky colour. In the larva of Ptychoptera contaminata and of a few Eristalids, these tubes, at least in their terminal portions, are exceptionally well developed and can be easily seen by transparency with the naked eye. Text-fig. 1, which represents a complete dissection of the larva of Myiatropa florea, L., shows to what extent the calcareous portion of the Malpighian tube can be developed in a full-grown larva. In this example the posterior pair of Malpighian tubes (*p.m.*) is composed of two short branches of normal structure ; the anterior pair (*a.m.*), on the contrary, is very long, its two branches in their proximal portion are of normal structure and diameter and extend to the anterior portion of the body, where they suddenly pass into two enormous sacs (*c.p.*) with milky contents, which run backwards and reach posteriorly the anal segment. These two sacs are even thicker than the intestine of the larva ; they are very fragile, and the slightest puncture causes their milky contents to flood out. The milky fluid is composed of a thick suspension of very small calcareous granules which are almost completely soluble in dilute acid, only a small central particle, probably of an organic nature, remaining.

2. Calcospherites in the Fat Body.

In all of the above-mentioned larvae the calcium carbonate of the Malpighian tubes appears in the form of crowded small granules suspended in the fluid which fills the lumen of these tubes. There are, however, other larvae which contain the calcium carbonate in form of calcospherites. The latter are enclosed either in the anterior pair of the Malpighian tubes or in special cells connected with the fat body.

The term calcospherite we owe to Harting (**7**, 1873), who was the first to prepare, artificially, calcareous corpuscles composed of two substances, mineral and organic. He obtained these bodies by precipitating calcium carbonate ($CaCl_2 + K_2CO_3 = CaCo_3 + 2KCl$) in a liquid containing organic matter (albumen, for instance). The calcareous corpuscles thus obtained were elongated or spherical, highly refractive,

composed of numerous concentric layers surrounding a central or excentric granulated body and bearing some resemblance to starch grains. When the calcospherites are dissolved in dilute acetic acid there remains an albuminoid stroma consisting of calcoglobulin. Examined in polarized light, the calcospherites show a black cross. The calcospherites, or Harting's corpuscles, have been well described by Nathusius (**15,** 1890), who found them in numerous animals and plants, and by Pettit (**18,** 1897) in cases of pathological ossification in mammals.[1]

In insects the calcospherites were discovered simultaneously by Henneguy (**8,** 1897) and Giard (unpublished observations quoted by Henneguy). Henneguy found them in the larvae of Phytomyza chrysanthemi, Kowarz. According to this author each calcospherite of this larva is enclosed in a special hypertrophied cell of the fat body. The fat of these cells disappears completely, and all that remains of the cell is reduced to a thin protoplasmic layer and a small degenerated nucleus. The calcospherites still appear in the pupa, but they are absent in the adult flies, and Henneguy thought that the imagines which he examined were probably obtained from the 'normal' larvae, i.e. 'larvae devoid of calcospherites'. Giard has observed similar calcospherites in the larvae of Phytomyza lateralis, Fall., which attacks the inflorescence of Matricaria inodora.

Personally I have found the calcospherites in the fat body of many Phytomyzine and Agromyzine larvae (Text-fig. 2). In all the species where the calcospherites are present they are to be found in every individual larva throughout its life. In this my observations differ from those of Henneguy and Giard, who considered the presence of calcospherites as abnormal and probably only seasonal. The cells which contain the calcospherites are always connected with the fat body, although they never contain droplets of fat. As a rule they lie in alveolar spaces formed among the fat cells (Text-fig. 3).

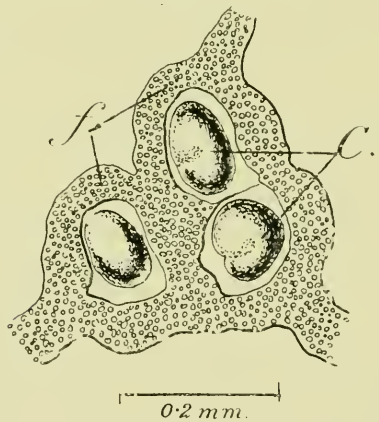
[1] To these two papers the reader is referred for numerous observations and references concerning this subject.

The calcospherites are already present in very young larvae, but not in the embryo or in those just hatched ; they seem to

TEXT-FIG. 2.

Agromyza sp., full-grown larva, slightly compressed, showing by transparency 120 calcospherites disseminated throughout the body.

TEXT-FIG. 3.



Agromyza larva, a portion of the fat body, *f.*, showing the calcospherites, *c.*

appear only after a short period of feeding. The existence of calcospherites in larvae belonging to the families Phytomyzinae and Agromyzinae seems to be so general that this character

assumes a taxonomic importance and helps one to recognize these larvae and to differentiate them from the phytophagous larvae belonging to other families like Anthomyidae and Trypetidae, the fat body of which is devoid of calcospherite cells.

3. Calcospherites in the Malpighian Tubes.

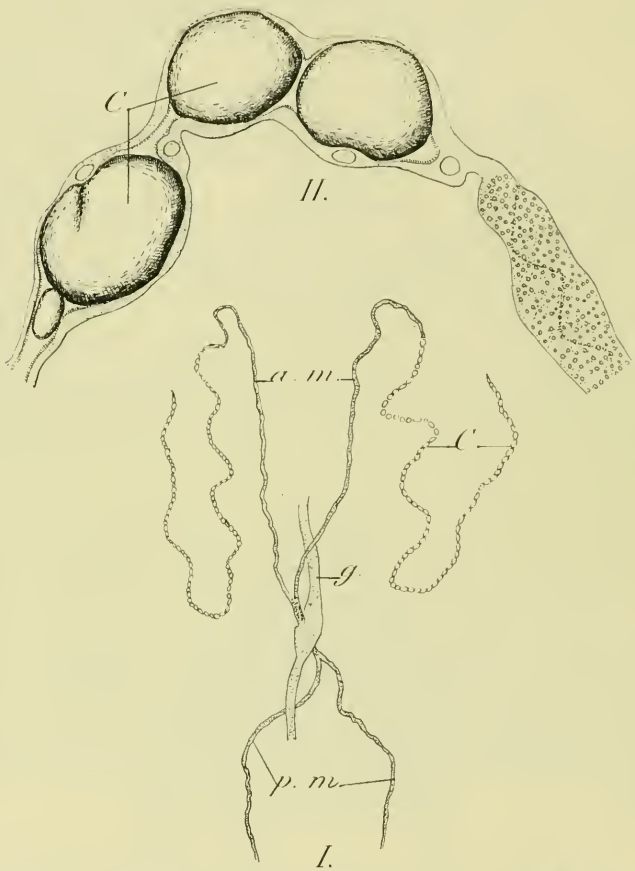
The only case of the existence of calcospherites in the Malpighian tubes is that of the larva of Acidia heraclei, the celery-fly larva. On examining a living larva of Acidia gently compressed between the slide and coverglass, I have noticed that its body contains a number of large calcospherites similar to those of Agromyzine larva. I thought at first that the calcospherites of Acidia larvae were also formed in special cells connected with the fat body. The dissection of these larvae revealed that such was not the case; all the calcospherites were lying free in the lumen of the Malpighian tubes and especially in their terminal portions (Text-fig. 4, I and II, *c*.). The calcospherites of various sizes, from 8 μ to 140 μ in diameter, distend these tubes, which have the appearance of being composed of highly refractive beads. The calcospherites when small are very often double, i. e. with two or more central granules (Text-fig. 5, *b*, *c*, and *d*). The occurrence of the calcospherites in the Malpighian tubes (Acidia heraclei) and in the fat body (Agromyzinae) of the phytophagous Dipterous larvae demonstrates once more the similarity in the excretory function of these two larval organs.

4. Ecdysial Elimination of Calcium Carbonate during Metamorphosis.

All the foregoing shows that the larvae of a great number of Diptera contain in their Malpighian tubes, or in the cells connected with the fat body, a large quantity of calcium carbonate stored in the form of minute granules or large calcospherites.

A question now arises: What becomes of the stored calcium carbonate during the ultimate stages of the life of the insect?

TEXT-FIG. 4.

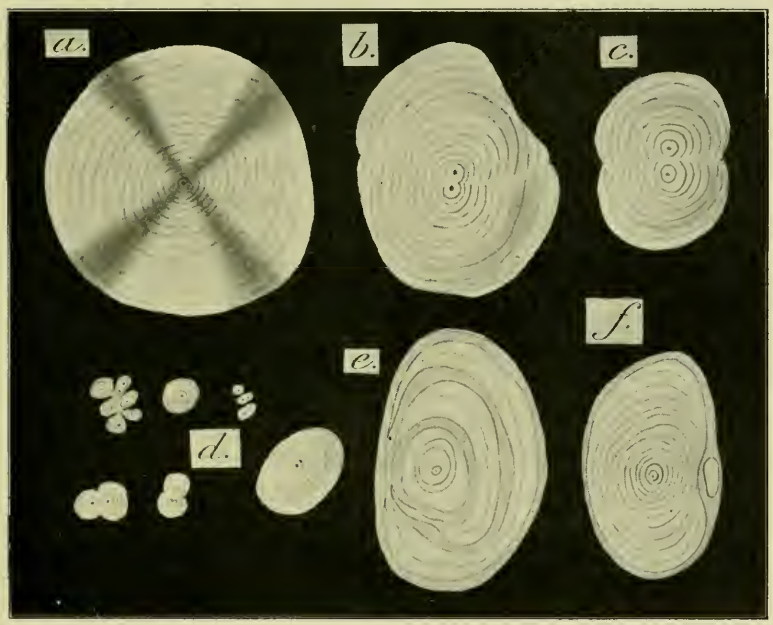


Acidia heraclei. I. *a.m.*, anterior pair of the Malpighian tubes; *c.*, terminal portion filled with the calcospherites; *g.*, gut; *p.m.*, posterior pair of Malpighian tubes. II. portion of the anterior pair of Malpighian tubes showing the calcospherites free in the lumen of the tube.

According to Pantel (**17**, 1914) the calcium carbonate of the Ptychoptera larva disappears before the metamorphosis takes place. He considered that it does not dissolve

in the body of the larva, but passes from the Malpighian tubes into the hind-gut, whence it is expelled from the body just before the larva begins to pupate. He admits, however, that he never actually saw the process of expulsion of this product

TEXT-FIG. 5.



Calcospherites of Acidia heraclei, *a* to *e*. *a*, a calcospherite examined by polarized light, showing the black cross; *b* and *c*, double calcospherites; *d*, very small simple, double or multiple calcospherites; *e*, calcospherite in diluted acetic acid showing collapsing stroma; *f*, intracellular calcospherite of Agromyza larva.

of excretion. In several cases he found the calcareous substance retained in the pupae of Ptychoptera.

Henneguy (**8**, 1897) found that the calcospherites of Agromyza larvae persist in the pupae, but he did not find them in the adult flies. He considered that the existence of calcospherites was not general, and was very probably abnormal,

assuming that the adult flies which he examined were derived from normal larvae devoid of calcospherites. Personally, I have found that in all Diptera, the larvae of which contain stored calcium carbonate, this substance disappears during the pupal phase, and the adult flies are completely devoid of this product of excretion. I must say, however, that the disappearance of the calcium carbonate seems to be a more complicated process than that suggested by Pantel (17, 1914). In the case of the Ptychoptera larva, where calcium carbonate, in form of a thick suspension of small granules, is enclosed in the distended portion of the Malpighian tubes, it is possible that the milky contents of these tubes are emptied into the hind-gut and are thus expelled from the body. It is, however, difficult or even impossible to suppose that the large calcospherites can follow the same channel in the larva of Acidia heraclei, for the Malpighian tubes of this larva, as in other Dipterous larvae, are completely devoid of peristaltic movement.

In the case of the Acidia larva and the larvae of Agromyzinae, which attack Cirsium lanceolatum, I was able to follow, step by step, the disappearance of the calcospherites during the metamorphosis of these insects. Each of these larvae, as is the case in all the Cyclorhaphous Diptera, transforms into a pupa which remains enclosed in the puparium formed by the contracted and hardened last larval cuticle. During the first day of the metamorphosis the calcospherites of these larvae can be easily seen either by transparency or by dissection. When the pupa is completely formed and separated from the last larval cuticle or puparium it loses its calcospherites, which are gradually dissolved. At the same time the puparium becomes very brittle and presents a white opaque ' fossilized ' appearance. After the emergence of the adults the empty puparia become so fragile that it is difficult to detach them from the plant, for they pulverize under the slightest pressure. On treating such an empty puparium with dilute hydrochloric acid a very active effervescence takes place, with the evolution of carbon dioxide, and all that

remains is reduced to a thin transparent larval cuticle. All this shows that the puparia of Acidia heraclei and of Agromyza are composed of a thin larval cuticle, the internal surface of which is lined and strengthened with a layer of calcium carbonate. As to the Dipterous larvae, like Myiatropa florea, Eristalis tenax, L., Syritta pipiens, L., and others, the Malpighian tubes of which contain calcium carbonate in form of a granular suspension, I could not follow with the necessary precision the course of their calcareous excretion. It is possible that a certain portion is mechanically expelled from the body before the metamorphosis takes place. On the other hand, it is certain that a good part of the stored calcium carbonate remains in the pupa and undergoes a similar process of dissolution which we have seen to occur in the Acidia and Agromyza larva. In fact, the empty puparia of these flies are also internally lined with calcium carbonate and effervesce when immersed in dilute acid. It is evident then that in Dipterous larvae the calcium carbonate (in the form of small granules or calcospherites stored in the Malpighian tubes or in the cells connected with the fat body) remains wholly or partly within the body of the larva until the latter pupates. During the first day of the metamorphosis, when the last ecdysis takes place, this product of excretion ($CaCO_3$) dissolves gradually in the perivisceral fluid of the insect. It then passes through the newly formed cuticle of the pupa into the ecdysial fluid which fills the space between the pupal and the last larval cuticle. Finally, when the ecdysial fluid is absorbed, the calcium carbonate remains as a deposit upon the internal surface of the puparium.

This mode of elimination of an excretory product from the body of an insect, being connected with the process of moulting, may well be named ecdysial elimination.

5. Hypotheses as to the Origin and Function of Calcium Carbonate in the Larval Body.

According to Valery Mayet (**13**, 1896) the calcium carbonate stored in the Malpighian tubes of Cerambyx larvae forms

a real reserve substance which has an important function during the metamorphosis. Just before the last moult the larvae, which form galleries in the wood of pine-trees, disgorge the calcium carbonate, with which they cover the walls of the galleries, thus protecting the pupae from the sap of the tree and preventing the invasion of the galleries by fungi. The opercula which close the galleries are also formed from calcium carbonate of the same origin.

Vaney (**19,** 1900 ; **20,** 1902) also speaks of calcium carbonate stored in the Malpighian tubes of Stratiomys larvae as a reserve substance.

According to Henneguy (**8**, 1897) the calcospherites of Agromyza larvae, which he wrongly supposed to be only seasonal, are probably attributable to the special conditions of feeding of these larvae during the autumn.

Pantel (**17**, 1914) considers the calcium carbonate as an ordinary product of excretion, which is probably due to an excess of calcareous substances present in the food of the larvae. The formation of calcium carbonate in the larva of Eristalis, Ptychoptera, and Stratiomys, which live in putrefying organic substances, and in the parasitic Dipterous larvae, reminds one somewhat of the calcareous excretion observed in several other organisms. Calcospherites are known, for instance, to exist in the parenchyma of Cestodes and in the excretory tubules of Trematodes, and, according to Burian (**4,** 1912, pp. 401–5), these calcospherites are derived from the neutralization of carbon dioxide. He explains thus how the parasitic worms, which live in a medium which already has a high CO_2 content, get rid of the CO_2 derived from their respiration.

According to Combault (**5,** 1909), the crystals of calcium carbonate, which fill the calciferous or Morren glands of Oligochaetes, are also products of the neutralization of the carbon dioxide which passes from the blood circulating in the lamellae of these organs.

The neutralization of the CO_2 of respiration and the formation of calcium carbonate was also shown by Bohn (**2**, 1898)

to exist in several Crustacea, e.g. Gonoplax rhomboides and others. According to this author, several Crustacea, Collapa or Ebalia, for instance, which live upon the Red algae, in a medium rich in ammoniacal alkali, do not eliminate the CO_2 derived from their respiration: they retain it to neutralize the ammonia which reaches their blood and tissues.

We know, on the other hand, that in insects the respiratory function differs markedly from that of other groups of animals. In insects oxygen is supplied to the tissues by means of a highly developed ramified system of tubules—the tracheae, while the carbon dioxide is given up by the same tissues to the perivisceral fluid and thence eliminated through the whole surface of the body.

It is possible that a part of this CO_2 is neutralized in the blood or perivisceral fluid; but at present this is purely hypothetical and needs verification by proper experimental inquiry. It indicates, however, that it would be of great interest to determine correctly the respiratory quotient of an insect larva which, like Eristalis, Ptychoptera, and others living in putrefying media, contains within its body a large quantity of calcium carbonate.

6. Conclusions.

1. The larvae of a great number of Diptera, parasitic, phytophagous, or living in putrefying substances, contain in their bodies a large quantity of stored calcium carbonate.

2. The latter is present in the Malpighian tubes or in special cells connected with the fat body.

3. Calcium carbonate is stored either in form of a thick suspension of small granules (in the Malpighian tubes) or in the form of calcospherites (in the Malpighian tubes or the fat body).

4. Calcium carbonate remains wholly or partly in the body of the larva when the latter passes into the pupal stage, but disappears by the time the adult stage is reached.

5. During the first days of metamorphosis the calcium

carbonate dissolves in the perivisceral fluid (haemolymph or blood of insects) and then passes through the newly formed pupal cuticle into the ecdysial fluid. When the latter is absorbed, the calcium carbonate remains as a deposit upon the internal surface of the puparium.

6. This mode of elimination of calcium carbonate from the body of an insect may be termed ecdysial elimination.

7. The excretion of calcium carbonate in Dipterous larvae is comparable with calcareous excretion as observed in other organisms like Cestodes, Trematodes, Oligochaetes, and Crustacea, where this product of excretion is supposed to be derived from the neutralization of the carbon dioxide of respiration. This explanation has not yet been proved experimentally.

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The Early Development of the summer egg of a Cladoceran (Simocephalus vetulus).

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With Plate 25 and 1 Text-figure.

Contents.

1. Introduction.

This paper deals chiefly with the method of germ-layer formation in the parthenogenetically-produced summer eggs of Simocephalus vetulus. A considerable amount of work has been done on the development of the eggs of various Cladocera, and a complete summary of this work is to be found in Vollmer's paper (**14**) on the development of winter eggs of Cladocera. In the same year that this paper appeared Külm (**8**) described very fully the development of the summer egg of Polyphemus pediculus, and here again is given a résumé of the work that has been done on Cladoceran development. This paper also reviews, in this connexion,

all the work done on determinate development in the Crustacea. Since then no further work has appeared on this subject, so that it would be mere repetition if that work were to be again summarized here.

Vollmer (**14**), in his summary, states [1] ' that these results point to the fact that we must differentiate between two categories of eggs which possess different modes of development in relation to their yolk-content ; eggs poor in yolk show a determinate development with practically total segmentation, eggs rich in yolk show an indeterminate superficial type of development '. Referring to his own work, he suggests that the developmental processes that he describes demonstrate an intermediate form between these two methods and make the transition less abrupt. From the work recorded in this paper it would seem that the development of the summer eggs of *S. vetulus* shows, perhaps more markedly, an intermediate stage between the determinate and indeterminate methods of development of Cladoceran eggs.

This work was carried out in Professor MacBride's laboratory at the Imperial College of Science, and I must thank Professor MacBride for valuable suggestions and for kindly reading the manuscript.

2. Method.

In all cases the embryos were dissected out of the brood-pouch into the smallest amount of water possible before being fixed. Fixing the whole Daphnid with the embryos still in the brood-pouch gave unsatisfactory results.

It was found necessary to employ different fixatives for the various stages of development. For the early stages no reliable method was found. Carnoy's fluid (Ac. Alc. Chloroform) gave good results, but the difficulty experienced was the unreliability of the fixative. The egg is surrounded by a tough membrane and it is this that causes the trouble. It is never possible to say whether it will burst or not under the action of the fixing agent. In the segmenting egg, if the membrane

[1] My translation.

bursts, the fixation is not good, while after the blastula stage the reverse is the case. If the membrane bursts, it produces a certain amount of distortion, and with all fixatives except Carnoy this was so bad as to make the material useless. With Carnoy a variable amount of swelling was produced, but with the other fixatives the whole egg usually burst. If the membrane remains intact, the egg becomes very difficult to embed owing to the embedding material not penetrating the membrane, and so it was found extremely difficult to obtain sections of the segmenting egg. One method used for the earliest stages was to employ hot water as the fixative. The eggs were dissected out of the brood-pouch and flooded with boiling water. After thirty seconds they were transferred to 70 per cent. alcohol. This gave fairly good results, but with later stages the nuclei were not well preserved and so the method was not of much use. Fixing in bichromate-formol and subsequent treatment with 5 per cent. formalin gave good results, but here again it was unreliable and gave results no better than those obtained with Carnoy's fluid. Gilson's mixture (Subl. Ac. Alc. Chloroform) gave very good fixation when it succeeded in fixing the embryo without producing excessive distortion.

For later stages Carnoy was again used, but better results were obtained with hot Flemming. The embryos were placed in Flemming's strong solution at 56° C. for ten minutes and then washed out in water. Strong picro-sulphuric gave fair fixation.

The embryos were stained with alcoholic eosin before clearing in clove oil and embedding in clove-oil ' celloidine '. This made them more conspicuous and hence easier to manipulate. After hardening the celloidine they were embedded in paraffin at 56° C.

Sections were cut 6 μ and 7 μ thick and stained on the slide. The best stain was Ehrlich's haematoxylin. Iron haematoxylin was used after Flemming fixation. Haemalum, picro-indigo-carmine and thionin were among other stains used which proved satisfactory.

3. Egg-laying.

On several occasions the actual laying of the egg into the brood-pouch was observed. Each egg is laid separately as a continuous stream of foam. The foam appears to consist of more or less opaque drops—probably yolk-spheres and transparent colourless globules—presumably oil in a continuous mass of protoplasm. Immediately after laying, the egg is of an irregularly elongated shape tapering at the end nearest to the opening of the oviduct. In a few minutes it has rounded itself off and become regularly shaped and almost spherical. The oil-drops now commence to coalesce to form one large oil-globule. About two hours after laying this large oil-drop is most distinct. It is excentrically placed in the egg and at this time has a diameter very slightly greater than half that of the egg.

As stated above, the only fixative that was found satisfactory for the earliest stages of the egg was Carnoy's fluid. In sections of eggs fixed in this liquid it was possible to recognize, according to Gatenby's diagnosis (**5**), the following structures: (1) one large oil-globule excentrically placed and surrounded by a few much smaller globules—these appeared as sections of empty vacuoles; (2) a mass of protoplasm placed almost centrally and on the edge of the large oil-globule; (3) a large number of yolk-discs staining very faintly with thionin and pervading the remainder of the egg; (4) a less number of smaller bodies scattered among the yolk-discs and staining deeply with thionin—presumably the remains of mitochondria or Golgi bodies.

Lebedinski (**9**) describes a similar arrangement of materials in the egg of Daphnia similis, but does not mention the mitochondria.

An egg-membrane is clearly distinguishable soon after the egg has been laid, and it would appear very probable, from the fact that the egg is laid as a fluid mass which subsequently rounds itself off, that this egg-membrane is produced by the egg itself after this rounding-off has taken place. It is not

a vitelline membrane if this term is restricted, as McMurrich (**10**) maintains, to a membrane which is connected with the process of fertilization, but must be termed a primary egg-membrane or 'Dotterhaut' as defined by Korschelt and Heider (**7**).

4. Cleavage.

Cleavage is completely superficial. At first the separate blastomeres remain deep in the egg as apparently amoeboid masses of protoplasm. After five hours they begin to appear on the surface, and soon after, each blastomere becomes separated from its neighbours by furrows extending a short distance into the yolk. Eight hours after the egg has been laid cleavage is complete and results in a uniform blastoderm enclosing the yolk-mass. No yolk-cells were found in the interior of the blastula. In Daphnia similis Lebedinski (**9**) found that certain blastomeres remained behind in the centre of the egg while the remainder migrated towards its surface to form the blastoderm, and that the former blastomeres functioned in absorbing the fat or yolk-drops. Vollmer (**14**) in the winter eggs of Cladocera describes the formation of a blastula with greatly reduced blastocoele by total cleavage, and states that cells are budded off from the blastomeres into the interior of the egg which function as yolk-cells. In Leptodora hyalina Samter (**12**) found that yolk-cells were budded off from the blastoderm at the same time that the endoderm plate commenced to immigrate into the egg. Agar (**1**) in Holopedium gibberum states that 'fairly late stages show occasional very flat nuclei on the separate yolk-masses, as figured by Samassa (**11**). Doubtless each yolk-mass is contained in a single cell. The origin of these yolk-cells has not been observed, but it may be safely assumed that they arise in the same way as that described by Samassa, i.e. by budding off from the mesendoderm'. Similarly, in S. vetulus embryos in which the endoderm has already separated from the mesoderm often contain yolk-masses against which flattened cells are seen to be lying. Their origin

cannot be stated with certainty, but it is thought very probable that they arise from cells originally lying round the genital rudiment which pass inwards on the inside of the blastoderm, as will be described below.

5. Formation of the Germ-layers.

The first sign of differentiation of the blastoderm is the appearance of a group of cells—more vacuolated than the rest—on one side of the embryo which subsequently proves to be the ventral side. These cells contain a large amount of yolk, and in their earliest stages their nuclei are very obscure. They will be called collectively the ' Ventral Mass ' (fig. 1).

When cleavage is complete each blastomere consists of an inner yolky part and an outer non-yolky part. In their very earliest stages the cells of the ventral mass are completely pervaded by yolk and so are conspicuous by not showing the outer non-yolky zone. Soon a few of these cells pass inwards, so that the ventral mass becomes a small heap of vacuolated yolky cells on one side of the embryo, but as yet shows no further sign of differentiation.

The cells of the ventral mass on one side, which is seen later to be the anterior side, now proliferate and form a mass of yolky cells whose protoplasm stains comparatively deeply (fig. 2). The compactness of these cells and the distinct manner in which they are marked off from each other indicate that their protoplasm has a greater surface-tension than that of the cells of the remainder of the ventral mass. The nuclei of these cells, which are now becoming distinct, are large compared with those of the blastoderm cells—approximately twice as large. Their nucleoli are distinct and stain deeply.

Behind these cells, that is at the posterior part of the ventral mass, are a few cells which still form a single layer. They are very much vacuolated and contain a large amount of yolk. Their protoplasm does not stain at all deeply and the cells are not at all compact. At first their nuclei are not distinct, as with the remainder of the cells of the ventral mass, but soon these become quite clear and show very marked characteristics.

They are several times as large as those of the blastoderm cells, as will be seen from fig. 3. The chromatin in them is either very scattered or very scanty. Each nucleus contains several nucleoli which stain to varying degrees, but none stain at all deeply. These cells are the primordium of the gonads.

Commencing at the earliest stages when the nuclei of the cells of the ventral mass are still obscure, cells can be seen round the posterior periphery which are passing inwards and dorsally up the inside of the blastoderm. These cells are apparently formed by proliferation of the blastoderm cells round the edges of the ventral mass and then migrate inwards at its periphery. They are mesoderm cells and will be spoken of as the Ectomesoderm. Later their nuclei become more distinct and are seen to be larger than those of the blastoderm and to contain distinct deeply staining nucleoli.

Soon after the genital rudiment becomes distinct there appear on the dorsal side of the embryo the primordia of the nervous system—the 'Scheitelplatten'. These consist of two groups of tall columnar cells symmetrically placed about the median plane, in which the nuclei are large and oval, approximately twice as long as the nuclei of the neighbouring blastoderm cells. The nucleoli are deeply staining and very conspicuous, and there is a marked absence of chromatin in the remainder of the nucleus. They agree with those described by other workers on Cladocera, and their further development will not be treated here.

A very conspicuous change is now brought about in the embryo by the invagination of the genital rudiment. An early indication of this inward migration can be seen in fig. 3, where the surrounding cells are seen to be pushing their way over the primordial germ-cells. The primitive germ-cells sink into the egg, a variable but sometimes considerable distance. The pit caused by this sinking in has been seen to stretch a third of the way across the embryo. The lips of this pit are formed of the ectomesodermal cells which are continually pushing their way under the edge of the genital rudiment to lie on the inside of the blastoderm (fig. 4), and as the

invagination proceeds these lips gradually approach one another (figs. 7 and 8) and thereby tend to enclose a space which sometimes persists for a short time as a small cavity (fig. 5). The lips ultimately fuse (fig. 6), so that the primordium of the gonads comes to lie completely internally. With the closure of this invagination the passage of the ectomesodermal cells into the interior stops in this region.

At the time of invagination the number of cells constituting the genital rudiment is about ten, but there seems to be no constant number. Cell divisions among these cells were found but rarely. Vollmer (**14**) states: 'Teilungsfiguren habe ich aber niemals in der Gonadenanlage nachweisen können', but in S. vetulus the number of cells in the genital rudiment most certainly increases by cell division from about four at its earliest apparent differentiation to about ten at its invagination.

While these changes have been taking place at the posterior end of the ventral mass the formation of the 'mesendoderm' has commenced at the anterior end. The original compact yolky cells at this end apparently separate into two parts—an inner mass of cells which spread themselves as mesodermal cells over the anterior part of the blastoderm conspicuously in the region of the 'Scheitelplatten', and an outer region which remains as part of the blastoderm. In the centre of this region, that is about midway between the genital rudiment and the level of the 'Scheitelplatten', the mesendoderm makes its first appearance as a group of tall, compact, comparatively non-yolky cells in the blastoderm (fig. 7). The nuclei of these mesendoderm cells show at first no difference from those of the blastoderm cells, but later, as the mesendoderm mass grows, the nuclei are seen to be nearly double as large as the blastoderm nuclei, with conspicuous nucleoli. This enlargement can be seen to take place as the cells pass inwards. The mass enlarges and its posterior end pushes its way backward in the median plane (fig. 8). The area of origin, which may be termed the blastozone, is marked a little later by a depression from which later grows the stomodaeum.

In its backward growth the mesendoderm comes up against the primordium of the gonads. There is no strict relation between the times of mesendoderm formation and of the invagination of the genital rudiment—sometimes the latter is completely internal before the mesendoderm begins to grow posteriorly. The mesendoderm pushes its way underneath the genital rudiment between this and the blastoderm, which may now be called ectoderm, so that the genital rudiment comes to lie on the dorsal side of the mesendoderm (fig. 9).

During the formation of the mesendoderm, mesoderm cells are formed at the periphery of the blastozone, most conspicuously at the anterior and lateral borders, the posterior border being obscured by the backwardly-growing mesendoderm. The mesoderm at this stage is grouped, in the posterior portion of the embryo, ventro-laterally, while in the anterior part it extends dorsally, covering the 'Scheitelplatten'.

When the mesendoderm has finished its backward growth it is a very clearly defined mass, and is sharply separated from the lateral mesoderm, as can be seen in fig. 11. It now begins to flatten out, and its lateral borders cease to be sharply cut off from the neighbouring mesoderm. Ultimately the whole of the mesoderm and mesendoderm form one flat plate of cells lining the inside of the ventral ectoderm. While this fusion is taking place the nuclei of the mesoderm and mesendoderm cells become smaller, so as to be indistinguishable from those of the ectoderm. From this plate of cells in the median plane a solid rod of cells separates off, which is the endoderm (fig. 10). At this stage the rudiments of the second antennae are already showing. Much later, when the large stomodaeum and the smaller proctodaeum have grown in from the ectoderm, this solid rod acquires a lumen.

At the time of separation of the endoderm the genital rudiment still exists in the ventral part of the embryo lying on the gut, as a mass of yolky cells with very large nuclei showing the same characteristics as the original primordial germ-cells of the ventral mass.

6. Discussion.

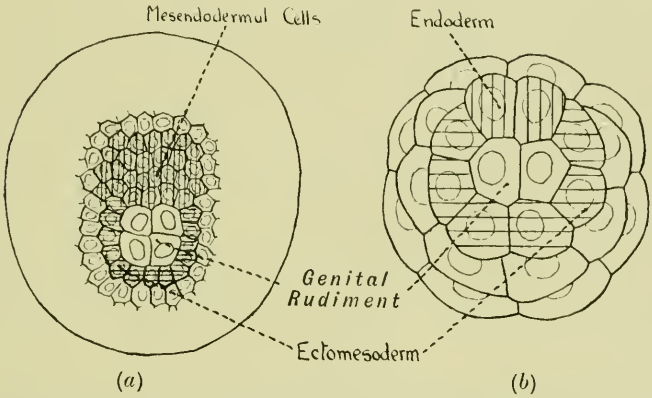
Kühn has shown in his paper dealing with the development of the summer eggs of Polyphemus (**8**) that the early development of Moina as described by Grobben (**6**) is very similar, in fact almost identical, to that of Polyphemus, and Samassa's description (**11**) of a totally indeterminate method of development of the eggs of Moina receives no support from his work. These are the only two Cladocera in which a determinate type of development has as yet been described.

Vollmer in his work on the resting eggs of the Cladocera (**14**) states that when the blastoderm consists of about two hundred cells a migration inwards takes place of about eight to ten of these from the future ventral side of the embryo. These multiply and form the genital rudiment. A similar proliferation of cells from a ventral blastozone later forms the 'untere Blatt', and from this is subsequently separated a solid rod of cells which forms the gut. Because of the early separation of the genital rudiment Vollmer states that this method of development is intermediate between the determinate development of Polyphemus and Moina and the indeterminate type of development as described by Agar (**1**) in Holopedium and Lebedinski (**9**) in Daphnia.

A comparison of the genital rudiment as described by Vollmer for Daphnia with that of Simocephalus described in this paper shows certain differences. Firstly, the mode by which it passes into the interior of the embryo is different in the two cases. In Daphnia this is brought about by a few cells that wander from the blastoderm into the interior of the egg, presumably by the action of an inwardly directed cytotaxis. In Simocephalus, on the other hand, the group of cells forming the genital rudiment passes into the interior by an invagination and only becomes internal when the edges of the pit caused by this invagination have grown together and fused. Here again the invagination may be brought about by a similar force. However, the extent of the invagination varies considerably, sometimes the pit is very

shallow, while at other times, as stated above, it has been seen to stretch one-third of the way across the egg. This fact, when it is also remembered that the surrounding cells are actively proliferating and producing cells which push their way inwards between the edge of the genital rudiment and the blastoderm, suggests that the invagination may be brought about by the ectomesoderm cells pushing the genital rudiment

TEXT-FIG. 1.



(*a*) Diagram of the ventral view of embryo of Simocephalus vetulus, showing the ventral mass before the formation of the mesendoderm.
(*b*) Ventral view of embryo of Polyphemus pediculus in thirty-two-cell stage (from Kühn).

in front of them as they themselves pass into the embryo. A second difference lies in the fact that in Daphnia the primordial germ-cells when they have passed into the interior lose their yolk. Vollmer states (**14**): 'auch in den Blastodermzellen schreitet die Dotterresorption fort, wenn auch nicht in demselben Grade wie in der Gonadenanlage'. In S. vetulus the cells of the genital rudiment always consist of large yolky cells which retain their yolk all through the development. Their protoplasm also stains very faintly, not as in Daphnia, where Vollmer states that these cells show an increased affinity for stains. However, from the position of origin of the

genital rudiment in the two forms, and from its relation to the mesendoderm and ultimate fate, it would seem that the differences are of small significance and that the two structures described as genital rudiment are really homologous.

A comparison of the mode of development of S. vetulus with that of Polyphemus as described by Kühn reveals some very close analogies. Text-fig. 1 (*a*) shows a diagram of the ventral mass of S. vetulus before the formation of the mesendoderm. In the posterior region are the large primordial germ-cells bordered laterally and posteriorly by ectomesodermal cells. In front is the group of yolky cells which are mesendodermal. The inner layers of this latter cell-mass spread out over the anterior part of the blastoderm as mesodermal cells, and from the outer layer is developed the very definite mesendoderm. While this is growing backwards mesoderm cells are still being proliferated inwards at the anterior and lateral edges of this group and possibly at the posterior edge. The fact that these latter cells originate by proliferation of cells at the edge of this mesendodermal group, together with the fact that they form mesoderm distinct from the mesoderm included in the backwardly growing mesendoderm, suggests that possibly they are a separate source of mesoderm, that they are ectomesodermal cells—a continuation forwards of the ectomesodermal cells which are formed at the periphery of the genital rudiment. If this were so, an analogy might be drawn with the development of Cyclops as described by Urbanowicz (**13**), where he states that larval mesenchyme arises from cells surrounding the primitive endoderm cell while the secondary mesoderm arises from the gut. The more recent work of Fuchs (**4**) on Cyclops has, however, failed to confirm the findings of Urbanowicz, and has, on the contrary, demonstrated an extraordinary resemblance between the development of Cyclops on the one hand and Polyphemus and Moina on the other, in neither of which is there any larval mesenchyme as distinct from secondary mesoderm. But in S. vetulus when the mesendoderm is growing backwards, although its hinder end is very sharply separated

from the laterally lying mesoderm (fig. 11), at the anterior end no such clearness exists and at the blastozone the mesendoderm merges into the plate of mesoderm lining the anterior part of the embryo. But both this anterior mesoderm and the mesendoderm clearly arise from a sharply defined group of cells at the blastozone, and it is suggested that there is no distinction between the mesoderm of the mesendoderm and the other mesoderm formed in this anterior region. If this is so, a very complete analogy can be found with Polyphemus. Text-fig. 1 (*b*) shows a view of the vegetative pole of a Polyphemus embryo in the thirty-two-cell stage. Two central primordial germ-cells forming the genital rudiment are placed posteriorly to two cells which give rise to the whole of the endoderm. Laterally and posteriorly to the genital rudiment are six cells which give rise to both ectoderm and mesoderm. Each of these six cells divides into two cells, one of which becomes an ectoderm cell and the other gives rise to mesoderm cells. In the comparison of these two figures it is seen in the two cases that the germ-cells are completely segregated in the genital rudiment as two cells in Polyphemus and as a group of about four cells in S. vetulus. Forming a crescent posteriorly round this primordium in both cases are mesectodermal cells, but anteriorly in Polyphemus are two endoderm cells, while in S. vetulus are a group of mesendoderm cells. The chief difference between the two forms is thus that the endoderm is segregated very late in S. vetulus, while it separates very early in Polyphemus—in the sixteen-cell stage. Similarly the mesoderm is segregated later than the endoderm, but still very early in Polyphemus compared with S. vetulus where the separation of mesoderm is only complete with the separation of the endoderm.

In Moina and Polyphemus Weismann (**15**) has proved that the parents nourish the young in their brood-pouch, and it is probably due to this fact that the yolk in the eggs of these two forms has diminished so considerably, and in correlation with this disappearance of yolk is the appearance of the teloblastic type of development. In S. vetulus

and also in Daphnia Agar (**2**) has shown that while the embryo is in the brood-pouch it does not receive nourishment from its parent. And yet S. vetulus shows a type of development which differs considerably from that of Daphnia in that there is a very early segregation of the genital rudiment but shows such obvious similarities to the development of Moina and Polyphemus.

The fact that has been pointed out by Fuchs (**4**), that among groups so far apart as the Copepoda and the Cladocera, in forms where there has been loss of yolk owing to the development of other modes of nutrition of the embryo, there is such an extraordinary similarity in the cell lineages, suggests firstly that the arrangement of the 'Anlagen' in the eggs of these forms is a very archaic character, and secondly that in cell lineage there is a representation of the arrangement of the 'Anlagen' in the yolky eggs that do not show a teloblastic mode of development. This view is upheld by the similarity between the cell lineages of the Cladoceran eggs that contain little yolk and of the egg of the Cirripede Lepas, where although there is abundant yolk, yet there is determinate cleavage (Bigelow, **3**). In the development of S. vetulus there is further support of this view in that in this apparently indeterminate method of development the earliest arrangement of the germ-layer 'Anlagen' shows such a close resemblance to the arrangement of the teloblasts in the non-yolky eggs of the Cladocera.

7. Summary.

1. Each egg is laid as a yolky mass of a foam and later forms a primary egg-membrane.

2. Cleavage is completely superficial and apparently indeterminate.

3. The first differentiation of the blastoderm is the appearance of a group of vacuolated yolky cells on the ventral side of the embryo which are called the ventral mass.

4. This subsequently differentiates into a few large cells with very large nuclei which form the genital rudiment, surrounded laterally and posteriorly by ectomesodermal cells,

and anteriorly to this a mesendodermal mass of cells from which arises the mesendoderm.

5. The genital rudiment surrounded laterally and posteriorly by inwardly growing ectomesodermal cells invaginates and becomes internal by the lips of the invagination growing together and fusing.

6. The mesendoderm grows backwards as a solid mass of cells, which later spreads out flat and becomes indistinguishable from the laterally-lying mesoderm, and from this layer the endoderm separates as a solid rod in the median plane.

South Kensington,
June 1921.

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EXPLANATION OF PLATE 25.

List of Abbreviations.

bl, blastozone; *ect*, ectoderm; *em*, ectomesoderm; *end*, endoderm; *ga*, genital rudiment; *gac*, cavity of genital rudiment; *i*, pit produced by invagination of genital rudiment; *me*, mesendoderm; *mes*, mesoderm; *mm*, mesendodermal mass; *v.m*, ventral mass; *y.c*, yolk-cells.

Figs. 1, 2, 3, 4, 8, and 9, are from material fixed in Carnoy's fluid. The remainder are from Gilson material.

Fig. 1.—Section through an embryo showing the earliest sign of differentiation of the blastoderm. The ventral mass is marked off from the rest of the blastoderm as a group of cells completely pervaded by yolk.

Fig. 2.—Median section through embryo showing differentiation of ventral mass into (1) genital rudiment, (2) anteriorly, the comparatively deeply staining mesendodermal mass, and (3) posteriorly, the ectomesoderm cells which are passing inwards. The nuclei at this stage are not at all distinct.

Fig. 3.—Slightly oblique section—almost median—of an embryo slightly older than that figured in fig. 2. Shows the same as in fig. 2, but nuclei are now distinct. The cells surrounding the genital rudiment are seen to be pushing their way over the latter.

Fig. 4.—Transverse section of the genital rudiment showing how the lips of the pit caused by its invagination are formed of inwardly migrating ectomesoderm cells.

Fig. 5.—Transverse section through the genital rudiment after it has become completely internal, showing its cavity.

Fig. 6.—Transverse section through the invaginating genital rudiment showing the fusion of the lips of the invagination pit.

Fig. 7.—Median section showing commencement of mesendoderm. The genital rudiment is not yet completely internal.

Fig. 8.—Median section showing mesendoderm growing backwards from the blastozone which is marked by a small depression.

Fig. 9.—Median section. The mesendoderm has grown backwards underneath the genital rudiment which is now completely internal.

Fig. 10.—Transverse section showing endoderm separated as a solid rod from the laterally lying mesoderm. The genital rudiment is immediately dorsal to the endoderm. Yolk-cells are seen in this figure enclosing yolk and oil-drops.

Fig. 11.—Transverse section through the posterior region of the blastozone showing mesoderm formation at the lateral borders of the blastozone.

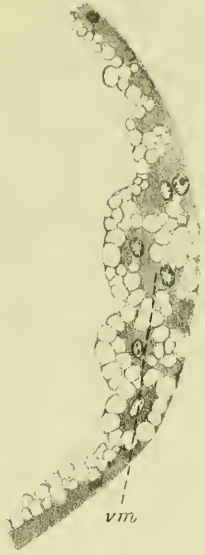

Fig. 1.

Fig. 11.

Fig. 10.

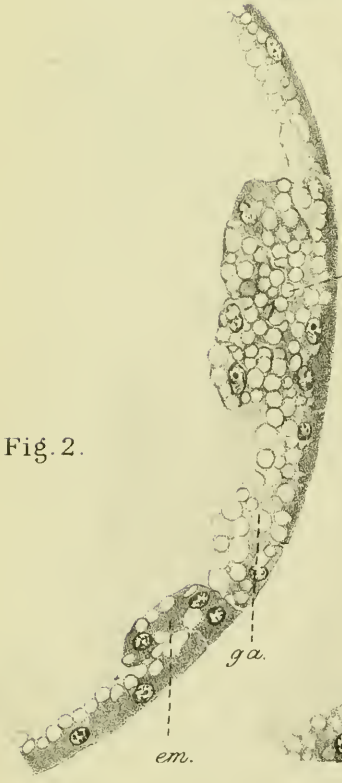

Fig. 2.

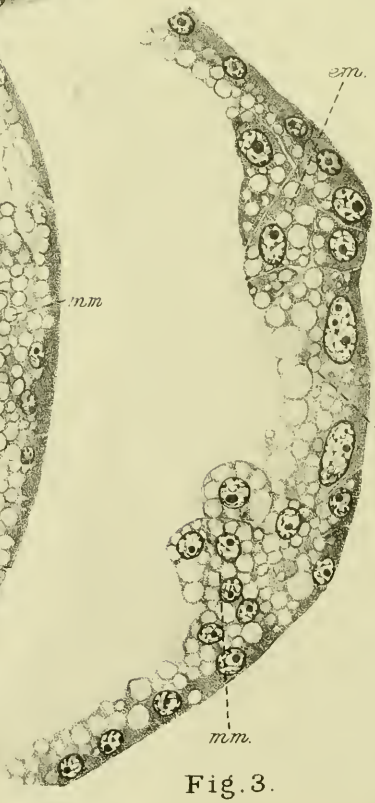

Fig. 3.

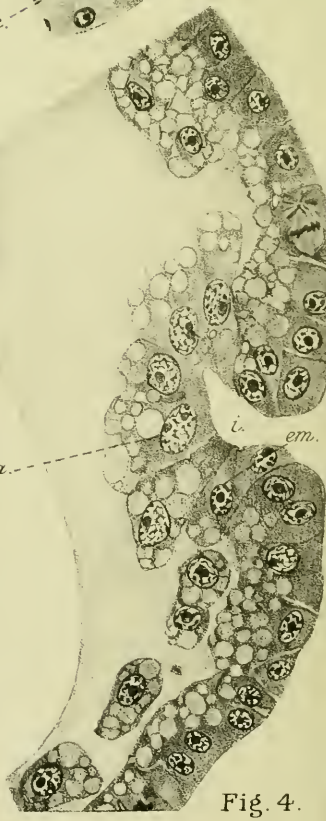

Fig. 4.

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Fig. 9.

Fig. 8.

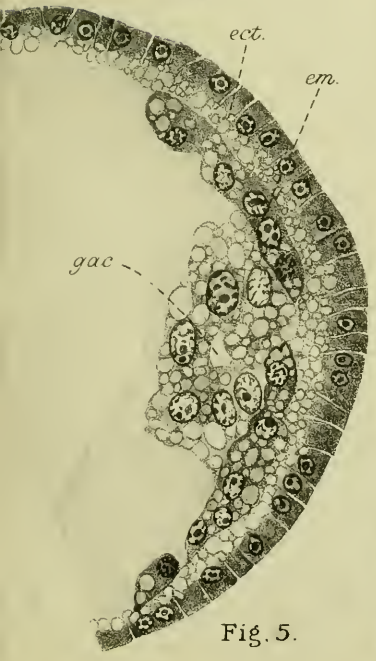

Fig. 5.

Fig. 6.

Fig. 7.

Studies in Dedifferentiation. II. Dedifferentiation and resorption in Perophora.

By

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With Plates 26–28 and 1 Text-figure.

Contents.

1. Introduction.

The observations of Driesch (1906) and E. Schultz (1907) and myself (unpublished) upon the reduction or dedifferentiation of the social Ascidian Clavellina have been mainly morphological. Accordingly I decided, while in the United States, to take up the problem from the physiological aspect. The work was carried out at Wood's Hole. Clavellina itself is not found there, but another social Ascidian, Perophora viridis, is common, and proved to be a useful form for experimental

work. As is well known, the social Ascidians reproduce asexually by means of buds given off at intervals from creeping branched stolons; but whilst in Clavellina the zooids may reach two inches, in Perophora the maximum length is only about one-quarter of an inch, and the span of life is probably limited in proportion. The branching and budding of Perophora is also much easier to follow, the stolons often growing in a straight line for a considerable distance, giving off buds at regular intervals. It is thus easy to trace a sequence from young to old individuals in Perophora, but hard in Clavellina. In Perophora it is also possible to isolate single zooids of any age by cutting the stolon midway between the neighbouring zooids on either side; and in such preparations the piece of stolon is of the same order of magnitude as the zooid, while in Clavellina the volume of the stolon is quite negligible in proportion to that of an adult, a half-grown, or even a quarter-grown zooid.

Such preparations we may call stolon-zooid systems. They are composed of two very distinct parts. The stolon is very simple: it consists of a thin external test-layer surrounding a single-layered tube of flattened ectodermal epithelium, which in its turn is divided into two by a horizontal partition composed of two very thin endodermic epithelia flattened together to form a single sheet; the space between ectoderm and endoderm contains blood, with numerous cells of several different kinds. At either end of the stolon the partition stops short, so that the blood can circulate from one half-tube to the other. It is normally kept in motion by the heart-beat of the zooid, which, as in all Ascidians, undergoes a periodic reversal of direction. The cut surface of test and ectoderm soon heals over. In a healed preparation the ectoderm at either (cut) end of the stolon is more or less cuboidal, and presents the appearance of undifferentiated tissue.

The zooid, on the other hand, is of high organization, containing, as it does, heart, stomach and intestine, elaborate branchial apparatus, nervous, muscular, and excretory systems, and hermaphrodite reproductive organs. It is also highly

sensitive in the region of the two siphons. It is connected with the stolon by a narrow tube of less diameter than the stolon, separating above into two tubes; this is generally longer in proportion in older individuals. The stolon may grow in length and form buds at the proximal end or the distal end or both.

Suitable food for Perophora has not yet been discovered; but in spite of this stolon-zooid preparations may be kept alive in the laboratory for a considerable length of time.

2. Dedifferentiation.

(*a*) General.—Processes may occur in living matter whereby whole organisms or parts of them become visibly simpler. This occurs, for instance, in Clavellina when kept in unfavourable conditions, in Hydra when starved (Schultz, 1906), and in various other Coelenterates, in encysting protozoa and in other protozoa in the ordinary course of the life-cycle, without encystment (Lund, 1917), in sponges (Maas, 1910; Müller, 1911), &c. Such a process is the reverse of differentiation, and is best called dedifferentiation. It has also been termed involution and reduction. The latter word will here occasionally be used as a convenient synonym for the more accurate but clumsier term.

In Clavellina the original observations of Driesch and the later work of Schultz was carried out on half-animals, the individuals being cut in two and the half containing the branchial sac (pharynx) used for the experiments. This portion proved capable of regenerating the whole organism. Sometimes it remained intact and produced a restitution-bud in which the missing organs were formed; at other times it dedifferentiated completely to form an opaque spheroid which later redifferentiated into a normal whole individual; or it might show a combination of the two processes. Here, when dedifferentation occurred, it was as the result of the shock of the operation and of the changes produced by it.

However, Driesch also mentions in one of his papers (1906) that he had been able to secure dedifferentiation in whole individuals. In my work I used whole individuals only. With them I found that the simplest method of obtaining dedifferentiation was to leave unchanged the water in which the organisms were kept, the accumulation of toxic waste products probably initiating the process. It was also found that only young individuals underwent dedifferentiation easily, mature and half-grown zooids speedily dying.

When full dedifferentiation, whether of half or whole zooids, occurs in Clavellina, a spheroidal white mass results, in which all the organs are very much simplified, both morphologically and histologically, becoming reduced to a series of separate sacs, some simple, others compound, of roughly spherical shape with walls of embryonic-looking cuboidal cells. On being replaced in clean water the opaque mass usually grows out to form a new perfect zooid, quite normal but smaller than the original; and this alternation of differentiation and dedifferentiation may be repeated several times. It is obvious that the term dedifferentiation may be applied equally to all retrogressive changes resulting in simplification of visible structure, provided that the reduced tissues remain alive, whether or no redifferentiation from the reduced condition is possible or not. When it is possible, an added interest attaches to the whole phenomenon; but dedifferentiation is essentially similar whether subsequent redifferentiation can occur or not, just as differentiation is essentially similar in all cases whether subsequent dedifferentiation can occur or not.

In Perophora similar methods were at first adopted, the animals being kept in watch-glasses containing approximately either 5 or 7·5 c.c. of water.

Zooids that were adult or more than half-grown never achieved successful reduction. They all died after a few days, but always after a preliminary attempt at dedifferentiation. The siphons were closed, all appearance of vigour and tone was lost, the body became contracted and opaque. The appearance

was very similar to that presented by an early stage of dedifferentiation. After this, however, a brownish colour appeared in the animals, and this heralded true degenerative changes leading to death. Adult individuals are often found in nature in a similar state, and these, too, always appear to die without full dedifferentiation; in fact it would appear that natural death occurs in Perophora through this means, the conditions in old zooids being such that they cannot maintain themselves in full tone, and thus undergo incipient dedifferentiation, which, in these old zooids, is not able to complete itself, and so leads on to degeneration and death. Similar failure of old individuals to adjust themselves to changed conditions is of course well known in the case of regeneration; a discussion of the whole subject will be found in Child's book, 'Senescence and Rejuvenescence' (1915*a*).

When smaller zooids were taken, however, quite different results were obtained.

(*b*) Simple Dedifferentiation (Clavellina type).

If the stolon be cut very close to the zooid on either side, the zooid will usually dedifferentiate as in Clavellina. That is to say, the siphons contract, the zooid shrinks, becomes increasingly opaque, and eventually draws right away from the tunic. The final stages of this process were represented by opaque spheroidal masses with a diameter of one-third to one-half that of the original zooid, and often with no or extremely slight trace of siphons. The heart usually continued to beat even in this condition. Examples are shown in fig. 1. Here the shortness of the stolon is noticeable.

In most examples of this process the stolon was either very short, or underwent dedifferentiation concomitantly with the zooid, or both. In all such cases the system, with its relatively small proportion of stolon, was similar to a stolon-zooid system in Clavellina, and behaved in an essentially similar way.

In one point there was a difference. I never observed such complete reduction in Perophora as in Clavellina. Further, I was not able to obtain redifferentiation by replacing the

spheroids in clean sea-water. This, however, is probably due simply to a greater susceptibility of Perophora to laboratory conditions, in the same way as one species may develop well after artificial insemination in the laboratory, while a closely-related species cannot be got beyond early segmentation stages.

(*c*) Dedifferentiation with Resorption.

(1) Stolon Resorption.—In systems with healthy young or moderate-sized zooids which were changed to fresh sea-water daily, the interesting fact soon came to light that so long as the full tone of the zooid was maintained and its siphons continued wide open, it did not decrease in size at all, but maintained itself at the expense of the stolon. This would also occur sometimes when the zooid was in the form of a partially-differentiated bud (e.g. fig. 4, *c–f*). The bud remained of the same size and at the same stage of development for over seven days, while the stolon was almost completely resorbed.

Later it was found that in other systems in which the zooid portion was represented by similar developing buds, these might not merely maintain themselves but actually develop further into perfect zooids at the expense of the stolon, e.g. fig. 2, where in the course of three days a very great change in the relation of zooid and stolon has taken place.

It is thus clear that in certain circumstances the zooid may be physiologically dominant over the stolon, and may either develop or maintain itself at the latter's expense.

(2) Zooid Resorption.—In other cases, however, a change in the opposite direction takes place. In most systems, after the lapse of a few days without change of water (and in some even when the water is changed), the premonitory signs of dedifferentiation become visible: the siphons close, the general tone decreases, and the whole animal shrinks slightly. But the sequel is quite different. Instead of becoming more and more opaque, on account of the cells of the various organs and epithelia becoming cuboidal and so bringing about a marked decrease in the size of all the cavities

in the organism, the zooid remains transparent. At the same time, however, it decreases in size. It is, in fact, being resorbed into the stolon. Appearances indicating the occurrence of this process are also found in nature, though not commonly. Successive stages of the process are shown in figs. 4, *a–b*, 5, 6, 9, 12, and isolated stages in figs. 7, 8, 10, 11, 13–15.

After a very short time the siphons disappear entirely, and a spheroidal mass of two-thirds or one-half the zooid's original diameter is left. In this, the ovoid heart, very little diminished in size, can always be seen pulsating steadily. A steady diminution of size continues, the heart too decreasing absolutely, although becoming relatively larger. A certain degree of opacity may appear, but it is never striking.

At a certain moment the pulsation of the heart slows down and ceases. Soon after this the heart becomes invisible altogether. Traces of other organs are visible. At first they are somewhat masked by the slight opacity caused by accumulation of blood-cells in the shrunken zooid, but later, as the zooid becomes smaller and smaller, they become increasingly clear. At about the stage when the heart disappears they are seen as two or three translucent rounded bodies, some colourless, some faintly yellowish.

The shrinkage continues after the disappearance of the heart, and soon the zooid comes to appear as a minute knob, scarcely bigger than the stalk connecting it with the stolon. This stalk represents the stolon-connexion of the original zooid, and has itself decreased in size, although but slightly. At this stage a single clear refractive area, which I take to be the vestige of the stomach, is usually the only structure to be seen in the knob. Finally the knob all but disappears, and a mere trace of the clear area remains visible. Presumably the stalk itself would also eventually become resorbed into the stolon, but resorption is much retarded after the cessation of the heart's action, and becomes progressively slower and slower as the size of the zooid decreases, so that I have never actually observed this ultimate step in the resorption of fully-formed

zooids. Complete resorption of very young buds has, however, been noted. When dedifferentiation is rapid, and especially in larger zocids, the connexion between zooid and stolon may be severed, and a spheroidal mass left isolated in the old tissue. This, of course, precludes further resorption.

Two further points of interest should be mentioned. The first is that the tunic of the zooid undergoes considerable decrease in size, presumably by means of some form of resorption. This reduction, as shown in the figures, is usually irregular, but I have seen cases of reduction in buds where the test remained closely apposed and of firm outline.

The second is that the stolon, especially during the late stages of the process, performs spontaneous movements of contraction, thereby causing a rudimentary and irregular form of circulation through the system. This may be called stolon-circulation. The contraction is effected by the ectoderm cells becoming cuboidal in one place and later extending again to become flattened ' pavement '-epithelium (fig. 24). Corresponding with these circulatory movements back and forth, the now minute zooid could be seen now to contract, now to expand slightly, cells moving from it into the stolon or vice versa. A similar contractibility of the ectoderm I have also observed in the stolon of Clavellina, and in the coenosarc of Hydroids (Campanularia and Obelia).

During the resorption of the zooid the stolon usually grows in length, at least during the earlier stages (figs. 5, 6*a*). Later on the stolon often remains constant in size, or decreases slightly. It then becomes more or less opaque, owing to the accumulation in it of cells from the zooid. Such packed opaque stolons, however, may send out transparent slender new growths at one or both ends. Quite often the final length may be greater than the original length, and buds may even be formed. The process of resorption may take a considerable time. The zooid in fig. 9 took seven days in all, four days to the cessation of the heart-beat and three days more until only a stalk was left, but in other specimens it was much more rapid. For convenience the process may be divided into

stages as follows: (1) shrinkage alone, (2) siphons closed, (3) siphons withdrawn from test, (4) spheroidal form assumed, (5) cessation of heart-beat, (6) reduction to stalked knob.

It will be seen that this process is the reverse of that previously described as stolon-resorption. In both cases, however, the equilibrium of the stolon-zooid system is altered, the alteration results in the resorption of one or other of its members, and this resorption may be total.

Resorption of an organ like the stolon cannot be considered a very unusual phenomenon. It is paralleled, for instance, by the resorption of various larval organs at metamorphosis, such as the gills and tail of a frog-tadpole. Resorption of whole individual organisms, however, is much more unusual. So far as I am aware, it has only been noted at all adequately by Loeb (1900), who found it to occur in the Calyptoblast Hydroid Campanularia. I have re-investigated the phenomenon in Campanularia and also in Obelia, and can confirm the facts entirely. Something rather similar occurs in those Echinoderms where almost the whole of the larva is absorbed into the growing rudiment of the adult, but there remains an essential difference, namely, that resorption in such a case is determined as part of a normal development, whereas in Perophora and Campanularia it does not occur except as the result of circumstances which must be called abnormal. This is also true for the interesting observation made by Child (1904), who found in the chain-forming Turbellarian Stenostomum that, if a cut be made through one of the zooids, the posterior half of such a zooid is completely resorbed by the zooid behind it. Resorption of whole zooids is also recorded (see later, p. 675). The case of Perophora is more remarkable than any yet recorded, partly owing to zooids being resorbed by subordinate systems, and partly owing to the great complexity of the zooids, which is very much greater than in Hydroids or Turbellaria.

In all three cases, however—Ascidian, Flatworm, and Hydroid alike—the mechanism of resorption appears to be the same, namely, that the organs all decrease in bulk by the

actual migration of single cells out of their union in the tissues into the cavities of the body (in Hydroids into the coelenteron, in Stenostomum into the parenchyma, in Perophora into the haemocoel). In no other way can we explain the rapid decrease in size of the zooid, or the marked increase in the number of cells in the cavities. The stolon in Perophora always becomes crowded with cells during the later stages of resorption. I have seen no sign of the cells disintegrating on release, there being no increase in the number of granules, &c., in the plasma; and the process can certainly not be explained as due to the using up of cells as nutriment *in situ*.

We have thus the singular spectacle of the organs and tissues unbuilding themselves. It is as if a house were to become smaller and smaller through individual bricks leaving their places here and there in the walls and accumulating in the passages and garden, the rooms meanwhile closing the gaps in their walls and progressively diminishing in size.

During the process it appears that dedifferentiation also is going on. For one thing, the ectodermic epithelium becomes more and more cuboidal, and then also all cells that appear in the blood-stream are of a simple, irregularly-rounded type, and not visibly specialized in any way.

The long persistence of the heart as a functional organ, and its final sudden disappearance are closely paralleled in simple dedifferentiation in Clavellina.

Presumably what occurs when the stolon is resorbed into the zooid is similar, the cells of the ectodermic epithelium and of the endodermic partition also becoming dedifferentiated and migrating out of the tissues into the blood-stream. The process is merely not so remarkable here, owing to the less differentiation of the tissues involved, and the subordinate status of the stolon as an organ. To sum up, we find that in Perophora (and in Campanularia) adverse conditions lead to a form of reduction in which dedifferentiated cells migrate out of their fixed position in the tissues into the general cavity of the body, and the whole differentiated zooid finally disappears by resorption. This combination of dedifferentiation

and resorption will probably be found to occur also in other colonial organisms, the zooids of which are united by relatively undifferentiated portions.

When the stolon is resorbed in Perophora a similar process appears to be at work. It is further probable that in many other cases of resorption of subordinate organs, and of grafted tissues, a combination of dedifferentiation and resorption is also taking place, although in many higher organisms the factor of phagocytosis also enters, but probably often as a secondary phenomenon.

3. Experiments with Potassium Cyanide.

The next step was to find out something as to the factors involved in the reversal of dominance and the initiation of resorption. With this end in view some experiments with dilute solutions of KCN were made. I have to thank Professor Child for advice.

As a preliminary the effect of an n/250 solution of KCN in sea-water was tested. It was found that this affected the whole system, zooid and stolon alike. Shrinkage of all parts took place, and death-changes were in progress after twenty-four hours. A series of solutions was therefore prepared as follows: n/250, n/500, n/1,000, and so on to n/64,000, together with a control vessel. All vessels were protected as far as possible from evaporation, and the solutions changed every twenty-four hours.

The detailed results are to be found in Table I. They may be summarized as follows: Solutions of n/1,000 and higher concentration affect both stolon and zooid very adversely, and lead to death in about forty-eight hours. The ciliary action of the gills is much slowed down, and the action of the heart badly affected. Almost always the stolons become contracted and opaque. The zooids were never drained completely by resorption; they usually shrank slightly, became opaque, and then died. In one or two cases the appearances were very similar to those seen in the dedifferentiation of Clavellina. In solutions from n/2,000 to n/8,000 inclusive

there was no growth of the stolons (except a very slight growth in one case). In n/8,000 the appearance of the stolons was nearly normal, but in the two higher concentrations they were adversely affected and showed contraction. As regards the zooids, the circulation was in all subnormal. A considerable degree of draining (resorption) took place, but was never complete. Several became opaque and spheroidal without appreciable draining (Clavellina type of dedifferentiation). The zooids mostly still showed normal tone after twenty-four hours, while in higher concentrations all had begun to shrink by this time. A slight effect on the stolon was indicated by opacity and clubbing of the ends.

In solutions from n/16,000 to n/64,000 inclusive, a considerable proportion of the stolons showed new growth. In no case was the stolon adversely affected, but it always remained of normal appearance with flat cells. Of those zooids which did not die the large majority had begun to be resorbed in the typical way before forty-eight hours, and some of them became completely drained. The n/32,000 solution seemed to be the most effective in causing this draining, but this may have been an accident, although it is perfectly possible that the n/64,000 solution is less effective because too weak.

The controls, apart from a small proportion which started to drain early (an occurrence which takes place in all collections of stolon-zooid systems chosen at random, and presumably depends on the internal condition of particular zooids), remained normal, the zooids completely expanded, for forty-eight hours and most of them for seventy-two hours. Most of them showed slight new growth of the stolons, as is customary in the early stages of stolon-zooid systems, but they were not kept long enough to see whether stolon-resorption, which only occurs after several days, would supervene.

We can classify the effects broadly as follows. High concentrations kill the whole organism speedily. The next lower degree of concentration causes contraction (dedifferentiation) of both stolon and zooid. No resorption is possible in this case, whether of the zooid or of the stolon. The next

lower grades of strength adversely affect the zooid, but only affect the stolon sufficiently to inhibit its growth, not to cause its dedifferentiation. Partial resorption may take place in these circumstances.

Still lower concentrations have no appreciable effect upon the stolon, but yet adversely influence the more sensitive zooid. The stolon is thus able not only to maintain its form, but to grow. The zooid starts dedifferentiation, and this is followed by resorption, which, typically, is complete. Finally, we get dilutions beyond which no effect is produced on the zooid or the stolon, with the result that the normal dominance of the zooid is maintained, and it is the stolon which is resorbed.

We thus see that these processes occurring in nature can be experimentally controlled to a considerable degree. Other toxic agencies were not tried on Perophora ; but from what we know of the reactions of other organisms we should expect that the results of KCN treatment are non-specific, and that essentially the same phenomena would occur in other toxic solutions.

Our results of observation are therefore to be thought of as due to the following causes :

(1) In Perophora, in the absence of food, there is a competition for nutriment among the parts of the colony.

(2) In normal conditions, in the absence of food, the most active and differentiated parts (the zooids) are dominant in this competition over the less active and differentiated parts (the stolons), which are used up as nutriment by the zooids.

(3) Correlated with this difference of success in competition there is a difference of susceptibility, the more highly-organized zooids being more susceptible than the stolon to unfavourable agencies.

(4) The result of unfavourable agencies on Perophora is to cause dedifferentiation.

(5) Once dedifferentiation has started the zooid ceases to be more active than the stolon, and so ceases to be dominant in the intra-organismal struggle.

(6) In Perophora dedifferentiation may be followed by

resorption due to the migration of cells from the tissues into the blood-stream ; when the stolon is little affected, therefore, zooid-resorption, or the reverse of (2), occurs.

In the most general terms we have a system the two parts of which are in equilibrium. This equilibrium may alter in either of two opposed directions. There is differential activity of the two parts ; the one which is more active is capable of causing the reduction of the other and utilizing it as food. But differential activity is correlated with differential susceptibility, which results, in certain unfavourable conditions, in a reversal of the direction of change ; for these induce dedifferentiation of the zooid, and in this condition it is less active than the stolon.

Similar conditions, viz. (1) a balance in an organic system ; (2) differential activity of the parts of the system leading to physiological dominance of the most active part ; (3) consequent differential susceptibility of the parts leading to a possible reversal of dominance ; and (4) the resultant reversibility of the reactions of the system—play an important part in general physiology. Often they are not easy to investigate ; but in Perophora we are fortunately provided with an organism in which they appear in a striking form, and are readily accessible to study.

It should be added that in all but the weakest KCN solutions a grey tinge, not seen in dedifferentiating individuals in sea-water, was observed in the zooids during resorption.

4. Experiments on Reduction in Animals without Circulation.

At Professor Loeb's suggestion, to whom I here tender my thanks, experiments were undertaken to see whether the action of the heart in Perophora was stopped by potassium chloride, and if so whether zooids without an active circulation would show typical reduction.

The experiment was carried out as follows. A large and a small stolon-zooid system were placed together in finger-bowl

TABLE I

Experiments with KCN.

Series A. Young and medium individuals.
Series B. Very young individuals and almost complete buds.
A and B, four stolon-zooid systems in each vessel.
Dediff. = opaque, Clavellina type of reduction. Stages 1–6 refer to stages of resorption.

Strength of KCN.	*Twenty-four hours.*	*Forty-eight hours.*	*Seventy-two hours.*	*Remarks.*
Control A	Normal	Normal	3 normal, 1 draining, stage 3	New growth on all stolons.
Control B	3 normal, 1 dediff.	2 normal, 1 dediff. 1 draining, stage 4	As at 48 hrs.	Slight new growth on all stolons.
n/64,000, A	3 normal, 1 stage 4	2 dead, 2 stage 5	—	Stolons healthy, new growth on 2.
n/32,000, A	Normal	All unhealthy, 1 stage 4	—	Stolons healthy, slightly turgid, new growth on 2.
n/32,000, B	2 normal, 2 stage 3	All draining, stages 3–5	2 dead, 1 stage 5, 1 stage 6	Stolons healthy, no new growth.
n/16,000, A	3 normal, 1 dediff.	2 dead, 1 stage 5, 1 stage 3	—	Stolons healthy, new growth on 1.
n/16,000, B	2 normal, 1 stage 3, 1 stage 4	1 dead, 1 dediff., 2 stages 5–6	—	Stolons healthy, new growth on 2.
n/8,000, A	Expanded, circulation affected	1 dead, 3 dediff.	—	Stolons nearly normal, no new growth.
n/8,000, B	2 normal, 2 stage 3	1 stage 1, 1 dediff., 2 stages 4–5	1 dead, 1 dediff., 2 stage 5	Some stolons healthy, some opaque, 1 with new growth (very slight).
n/4,000, A	Expanded, circulation poor.	2 dying, 2 stage 4	—	Most stolons contracted; no new growth.
n/4,000, B	2 normal, 2 stage 3	1 dediff., 3 stages 4–5	All dead	All stolons opaque; no new growth.
n/2,000, A	Expanded, circulation very poor	1 dying, 2 dediff., 1 stage 4	—	All stolons contracted. Cilia slow.
n/2,000, B	2 subnormal, 1 stage 3, 1 stage 5	2 dying, 1 dediff., 1 stage 6	—	All stolons opaque, clubbed.
n/1,000, A	All subnormal	All dead or dying	—	All stolons early affected.
n/500, A	1 subnormal, 3 dediff.	All dead or dying	—	All stolons early affected.
n/250, A	All abnormal, dediff.	2 dead, 1 dying, 1 dediff., unhealthy.	—	All stolons early affected.

containing 50 c.c. sea-water together with a certain amount of n/2 KCl. The results are summarized in Table II.

TABLE II

+ denotes active heart-beat; (+) slow; (−) slow and intermittent; − no heart-beat. The upper sign in each compartment denotes the larger zooid, the lower the smaller.

No. of c.c. n/2 KCl added.	*Minutes.* 15	20	30	35	40	50	70	160
0 (control) . .	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+
2 . . .	+	+	+	+	+	+	+	−
	+	+	+	+	+	+	+	−
4 . . .	+	+	+	+	(+)	−		
	+	+	+	(−)	−	−		
8 . . .	+	+	+	+	not	−		
	+	+	+	(−)	noted	−		
10 . . .	+	+	+	(−)	−			
	+	+	+	(−)	−			
15 . . .	+	+	+	+	(+)	−		
	+	+	(−)	(−)	(−)	—		
20 . . .	+	(−)	−					
	+	(−)	−					
40 . . .	+	−						
	−	−						

KCl thus exercises a very marked effect upon the Ascidian heart. The stronger action of the salt on small zooids is to be noted. The organisms were left in the solutions to see what type, if any, of dedifferentiation they showed.

Those in the two highest concentrations died in under twenty-four hours without reduction; their stolons also were killed or damaged. Those to which 10 and 15 c.c. KCl had been added were scarcely affected after twenty-four hours, but were dead by forty-eight hours, having previously shrunk very considerably and become opaque.

In the solution with 8 c.c. one had died; the other had started to dedifferentiate. Both stolon and zooid were affected (fig. 20). The zooid showed a characteristic sign of KCl reduction in the cellular strands extending from the retracted siphons to the test. Also characteristic, and directly dependent on the absence of circulation, was the congestion

of the network of small blood-vessels close to the surface with the green blood-corpuscles. This gives a premature green opacity to zooids dedifferentiating in KCl. This animal was dead on the succeeding day.

In the solution with 4 c.c. one died, after only slight reduction, after three days. The other exhibited dedifferentiation of a type very similar to that just considered, but this time accompanied by a little growth in the stolon, which remained healthy and tonic. Although reduction had started, resorption never ensued, and after five days the zooid had died and was represented by a blackish spheroidal mass about half its original diameter, while the stolon was still healthy. (Fig. 19.)

In the solution with 2 c.c. matters were very similar. The stolons remained healthy, though distended with blood-cells (and possibly others) from the zooids, for over five days. The zooids withdrew their siphons from the test, shrank, and became opaque (i.e. started to dedifferentiate), but died with change of colour to brown or blackish before any marked resorption had occurred.

It will thus be evident that there are at least two factors concerned in resorption in Perophora. The first is the shrinkage of the whole organism and reversion of its cells to a cuboidal type which we may call simple dedifferentiation, the second is the migration of cells out of the tissues, which does not take place, or takes place only to a negligible degree, in the absence of the circulation.

Thus in the presence of KCl, with consequent cessation of heart-beat, the aspect of the process is altered in many particulars. High concentrations of KCl damage both zooids and stolon, and both contract. The cessation of the circulation in lower concentrations leads to a very speedy dedifferentiation of the zooid; but this never goes very far before death supervenes, and is unaccompanied by resorption.

The experiments were repeated, with variations, with forty-five more specimens; essentially similar results were obtained. Twenty of these showed dedifferentiation without resorption. In addition one showed a slight, one a moderate,

degree of resorption. Twelve formed new stolon outgrowths of fair length. The solutions used were 2 c.c. and 4 c.c. n/2 KCl in 50 c.c. sea-water.

5. Experiments with Low Temperature.

Eight vessels, each containing several individuals, were put in an ice-chest, with a temperature of 3° to 8° C.

Several points were noted when these were examined eight days later. Over half had turned brown or blackish, and were dead or dying. No cases of extreme or even considerable resorption were found. Most healthy-looking individuals had shrunk and become opaque, i.e. had dedifferentiated. The opacity was more marked than usual. Usually, however, the siphons were left open and attached to the test at a stage when at room-temperature they would have been closed and withdrawn. The heart-beat was very slow or absent, though the heart was usually visible. Sometimes the heart-beat began again soon after transference to room-temperature for examination. Very young individuals were less dedifferentiated than older ones.

The stolon seemed to be unaffected, and often remained of normal appearance even when the zooid was dead or dying; no new growth, however, was ever seen. Recovery did not occur at room-temperature.

Here again it is clear that the zooid has been much more affected than the stolon, and that the slowing or cessation of circulation has, as in KCl, prevented resorption.

In one system a new bud was produced on return to room-temperature, and grew to a normal zooid after six days.

6. Miscellaneous Notes.

(*a*) Tone of Stolon.—The turgescence of the stolon appears to depend on two quite different causes—first the physiological condition of the ectoderm cells, and secondly the pressure of the blood. Observation on a stolon which was undergoing retraction showed that the ectoderm cells were capable of great passive extension. At intervals the tip of

the stolon was dilated by the blood-pressure, the flattened ectoderm cells becoming still more flattened. The test also underwent passive dilatation.

However, even when the heart has ceased to beat, the stolon may be quite turgescent, and the ectoderm cells flattened, not cuboidal. Fullest turgescence, however, is thus only to be expected when the circulation is active and when the ectoderm cells are healthy.

It may be mentioned that the first step in dedifferentiation may be regarded usually as a diminution of tone (turgescence).

(*b*) Growing-points of Stolon.—At the tips of growing stolons the ectoderm is usually columnar (fig. 26) and the lumen generally filled with a dense mass of cells, into which the circulation does not penetrate. Sometimes, as in fig. 27, there is an increase in the number of green cells as we pass away from the tip. Often a layer of blood-cells will become attached to the walls of the stolon over a considerable distance, giving it an opaque appearance, though circulation continues internally.

(*c*) Lateral Outgrowths of Stolon.—Some lateral outgrowths, as in fig. 25, were occasionally seen. They did not represent rudimentary branches. Their meaning and origin is obscure.

(*d*) Attachment of Stolons.—The stolons will usually attach themselves to the substratum. This I have seen accomplished within three and a half hours.

(*e*) Bud-formation.—When medium-sized zooids attached to stolons of fairly large size were employed, buds were often formed from the stolon when dedifferentiation began in the zooid. Sometimes two buds or more might form. Buds may form at either or both ends of a piece of stolon. Resorption might occur at any stage in the development of the zooid from the earliest bud up to half-grown individuals.

(*f*) Penetration of Zooids by Stolon Branches.—An individual was seen in which apparently a branch of the stolon had grown up inside the test of the stolon-connexion and

encircled the zooid. The actual origin of the branch could not be traced in vivo. When old zooids die, stolon branches will frequently grow into the test previously occupied by the zooid.

(*g*) Death-changes.—Death-changes in Perophora usually involve a change of the green colour to a hard brown or black.

(*h*) Change of Position of Stolon.—When a stolon-zooid system is isolated, and new growth of the stolon with subsequent bud-formation takes place at one end, not only may the original zooid be completely resorbed, but the stolon tissue may abandon the original region and become concentrated in the region of the new bud. This 'moving-on' of the stolon is common in regeneration in Hydroids.

(*i*) Segmentation of Stolon.—In not very dilute solutions of KCl and KCN in which the stolons were affected, the stolon-tissue sometimes contracted into a series of separate ellipsoid portions giving the appearance of a necklace without a string.

7. Experiments on other Species.

(*a*) On Amaroucium.—Some experiments were also made on a form of compound Ascidian very abundant at Wood's Hole—Amaroucium pellucidum, var. constellatum. For information and advice as to this form I have to thank Professor Caswell Grave.

Twenty small pieces of Amaroucium colonies, consisting each of from two to twelve or fifteen individuals, were cut out and placed in separate dishes in a small volume of water. The experiment was started on July 11 and was terminated after twenty-nine days. Controls were kept in the circulation-tanks.

Those kept in the unchanged small volumes of water showed alterations as follows. The larger pieces remained normal longer than the smaller. The larger individuals, however, usually showed reductional changes sooner than the smaller, ceteris paribus; but they did not usually remain as healthy as the small ones during reduction. Often they

exhibited a phenomenon characteristic of Amaroucium—the protrusion of the pharynx from the test and its subsequent decay, the abdomen and post-abdomen remaining and dedifferentiating. The small individuals underwent a process obviously analogous to the dedifferentiation of Clavellina. They shrank in size and decreased in transparency. The siphons at first remained attached to the test (unlike Clavellina), but later became completely detached. The pharyngeal region, as in all other reducing Ascidians, shrank much more than the rest, and finally a stage was reached in which the two main portions of the body were still distinguishable, separated by a slight constriction ; the general shape was thus that of a constricted sausage ; the organism was completely opaque, the colour being white with patches of red. (Certain organs of the normal zooid show this same red colour.) A curious feature was the frequent formation of clear projections of the test. These were generally stalked, and spheroidal or ellipsoidal, like bubbles or bladders. Healthy-looking test-cells could be seen in them. Very frequently new buds would be formed from the dedifferentiating zooids during the process of reduction. These would attain a certain degree of organization, but would not usually reach full development unless the piece were replaced in clean and regularly-changed water. This replacement in clean water, however, did not lead to the redifferentiation of the reduced original zooids.

After seven to twenty days, when it had become evident that it was not possible to obtain the extreme stages of dedifferentiation seen in Clavellina, the surviving pieces were all placed under gauze in the circulation. When examined twenty-nine days after the inception of the experiment it was found that a few had remained in approximately the same condition in which they had been placed in the circulation. More than half, however, while the original zooids had not redifferentiated, had given rise to new zooids, usually in one or two clusters of four to six zooids each.

It thus becomes clear that Amaroucium shows yet a third type of dedifferentiation. The specialized method of forming

a large number of buds practically simultaneously by segmentation of the very long post-abdomen, with subsequent differentiation of each segment to form a whole zooid, is apparently responsible for this. After dedifferentiation of the primary zooid has proceeded a certain way, either death supervenes or else the post-abdomen, released from subordination now that the dominant region is thus adversely affected, manifests its independence by producing new individuals. Once these new individuals start to develop they become dominant. The non-recovery of the partially-dedifferentiated original zooids may be ascribed to this, or to greater susceptibility. In spite of this absence of the power to redifferentiate the process of dedifferentiation is very similar to the early stages of the same process in Clavellina. For such behaviour there is ample evidence as regards numerous forms reproducing asexually in the work of Child and his pupils (Child, 1915*b*). We may thus say that, under the conditions which prevail in the colony, or in pieces of it, in Amaroucium, complete dedifferentiation of single zooids is not possible. The colony or piece regarded as a whole, however, may be said to undergo dedifferentiation followed by redifferentiation.

Oozoites.—These had the advantage over blastozoites that they could be obtained singly. They were got by allowing larvae to metamorphose in the laboratory. They could be induced to dedifferentiate either by lack of change of water, or, after a longer period, by starvation. The process was very similar to that in the blastozoites, with the exception that the formation of buds was never observed. This latter fact is undoubtedly to be correlated with the small relative size of the post-abdomen and the small absolute size of the whole organism.

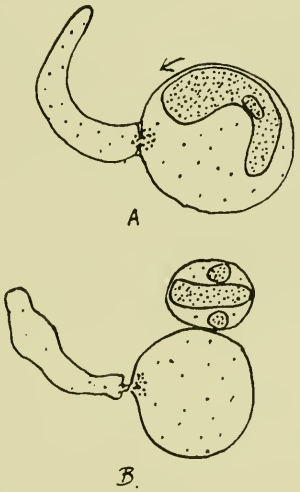
Here, too, dedifferentiation never got beyond a stage in which a sausage-shape was assumed (fig. 27, Text-fig. 1). The complete opacity and the spheroidal shape of the final stages of the process in Clavellina were not observed ; neither did I succeed in obtaining redifferentiation.

On the whole, dedifferentiation in oozoites went a little further

than in blastozoites, and appeared to be a healthier process unaccompanied by so many abnormal swellings of the test, extrusions of parts of zooids, phenomena of local decay, &c.

Treatment with Alcohol.—A few experiments were made to test the effect of a 2 per cent. solution of alcohol on the process. It appeared that under its influence, dedifferentiation, both in oozoites and blastozoites, started sooner

Text-fig. 1.



Reduction in oozites of Amaroucium.
A. Zooid in stage 3, test spherical, test of tail degenerating.
B. As A, except that the zooid shows detached cell-masses, and lies in a spherical portion of test detached from the rest.

than in the controls, but that it did not progress in a normal way. Opacity might be attained, but the loss of form, especially in the pharynx, was not as great as usual, e.g. the siphons remained visible relatively much longer after their retraction from the test than in normally-reducing specimens (fig. 28).

This appears to indicate that there are two distinct processes at work in normal dedifferentiation, the first being a mere shrinking as a result of exposure to an unfavourable environment, the second a real despecialization of the cells, resulting in loss of typical form. This latter then is due to active positive

changes in the cells, changes which are partially restrained by the action of a narcotic like alcohol.

It should perhaps be mentioned that not only oozoites which had lived some time in the circulation, but also those which had only just metamorphosed, could be induced to dedifferentiate. Larvae were allowed to fix on slides on July 28. After seven to nine days in the laboratory they showed the first signs of reduction. On the tenth day their water was changed, but without effect on the result, for on the eleventh and twelfth days all were markedly reduced. A sausage-shaped mass, sometimes showing a slight constriction between pharynx and abdomen, lay in a much-swollen, but healthy, test, which was usually attached to the substratum in the form of a flattened sphere. The remains of the test of the larval tail could be seen attached to one point of the main test. In some examples an interesting modification was observed—a small portion of test surrounding the reduced zooid became constricted off from the main portion, which, though thus empty, remained healthy (Text-fig. 1, B).

In one or two specimens, detached, rounded masses of cells were to be seen outside the limits of the reduced zooid; these were also occasionally seen in reduced blastozoites. I believe them to have been derived from the organism itself, and not to have been merely collections of cells of the test. Such collections were also seen, but never had the compact appearance of the first-mentioned masses.

Swellings of the Test.—These have been already referred to. In connexion with experiments on dissociated sponges which were proceeding at the same time, it was decided to see whether portions of test were capable of re-organization or of regeneration in sea-water, or of growth in a nutrient solution. Accordingly a number of these 'test-bladders' were snipped off and isolated. After cutting the pieces were always torn and quite flabby. Some were placed in sea-water, others in weak solutions of peptone made up either in tap- or sea-water. In all cases a marked reorganization had taken place within twenty-four hours. The wound was completely

healed, and the piece was irregularly lobed, as if swollen out from two or more centres. It would appear that there was an actual accumulation of fluid in the interior, as in the spheres produced by sponge choanocytes (Huxley, 1921*a*). Usually the test-cells were nearly absent in some regions, rather densely aggregated in others. No regeneration, however, took place, and death occurred quicker in the peptone than in the sea-water. Death took place in one to three days in peptone, two to four in water. An interesting point was that, before death, many of the test-cells always left the matrix of the test, and crawled out on to the bottom of the dish. They still preserved their characteristic shapes at first, but eventually all rounded off preparatory to dying.

(*b*) Botryllus.—This genus is unsuitable for experiment owing to the small size of its zooids and their intimate connexion. One system, however, was seen in which all the zooids had become reduced to shapeless but healthy-looking lumps; the test round them had degenerated save for a thin layer. Some form of dedifferentiation had obviously occurred.

8. Discussion.

Perophora happens to be an organism in which dedifferentiation and resorption affect the whole individual in a very striking way. In higher forms, thanks to their self-regulating mechanisms, their size, the bulk of their skeletons, and other factors, the processes do not affect the individual as a whole. None the less, similar processes play a large part in many phenomena, both normal and abnormal, throughout the animal kingdom.

In the first place it is important to realize that the 'struggle of the parts', to which Roux (1881) first drew attention, is a very real struggle; that the organism is in one aspect simply an equilibrium between a number of parts, some in a relation of simple competition, some in a relation of control of or subordination to others; and that the relative success or failure of any one part, the degree to which it is developed, depend or have depended upon its success in this struggle.

Secondly, we must realize that success in the struggle, i.e. time and degree of development, may depend largely on rate of metabolic activity. It is not for a moment suggested that this is the only factor at work, nor that it is the most important factor (in the higher organisms the relationship of the nervous system to the tissues of course masks it to a considerable extent), but that it is an important factor.

Child (1915*b*) has drawn attention to its importance for problems of regeneration and asexual reproduction; he finds that the most actively-working portion of the organism (or, in higher forms, the portion containing the higher centres of the nervous system) is not only formed first in regeneration, but exerts some sort of controlling effect upon the rest of the organization of the body. For instance, once a head is formed in the regeneration of a Planarian or an Oligochete the old organs are remodelled, some being broken down, others built up, until what exists stands in normal relation to the new head. But if, for some reason or other, a head is not formed (in Planaria it can be experimentally prevented from forming), then this remodelling does not occur. The production of a new pharynx, for instance, in a pharynxless posterior half of a Planarian, will not take place unless a head is formed at the anterior end.

However, this controlling effect of the head is only exerted up to a certain distance. Once this distance is overpast the tissues of the body are free to react in the way characteristic for them when not under any control, i.e. by the formation of a new head. In other words this control or dominance of the head or oral end (or apical bud in plants) is what regulates the important temporal and spatial relations of asexual reproduction. As is to be expected, it varies with external circumstances, and Child has performed some pretty experiments on the experimental control of dominance.

It would appear, especially from some of his recent work upon plants, that this dominance exerts an effect analogous to that of the nervous system by means of some form of conduction, and that it is not, as might at first be expected,

simply dependent on nutritional relations. Child has naturally stressed this important point (Child, 1919). However, the nutritional aspect is also important, and does as a matter of fact determine many relations of dominance and subordination of parts in organisms ; and it is to some of the implications of this aspect that I wish to draw attention.

If one reaction or associated set of reactions is proceeding faster than another, in the same system, it will occur to a correspondingly greater extent ; cf. Mellor, ' Chemical Statics and Dynamics ', p. 70 : ' In any system of parallel chemical reactions which consume the same substrate and are proceeding simultaneously in a mixture, the extent to which each reaction will occur is proportional to its velocity.' This means that if two sets of reactions are going on in an organism at an equal rate and that subsequently one of them is stimulated to a 10 per cent. increase, then the end-products of those reactions (the amounts of two different types of tissues, let us say) will, if the available food remains constant, change from the proportion 1000 : 1000 to 1048 : 952. A similar result will occur if the other reaction's intensity is correspondingly lowered. This is important in explaining many changes resulting from a change in environment acting upon the tissues which respond to the change at different rates (see Child, 1916 ; Robertson and Ray, 1920 ; Lillie and Knowlton, 1902, &c.).[1]

One of the best examples is the relation of head-size to body-size in a regenerating piece of Planaria. Apparently the temperature-coefficient of the processes of the head-region is greater than those of the body, for the relative development of head increases with temperature. It is also decreased by increase in concentration of narcotics.

There is, however, another aspect of the question which it is rather more difficult to understand. That is the fact that if in an organism two sets of reactions are going on at different

[1] This will, of course, only occur up to a certain limit. A condition of hyper-activity may be induced, as for instance by excess of thyroid-secretion, or by excess of nervous stimulation in certain forms of neurasthenia, which results in a wasting of the tissues concerned.

rates, in two different regions, then, if the food-supply is reduced, the one which, *coteris paribus*, has the higher speed will be able to maintain itself in its normal state and at its normal level.[1] This may be due to the fact that the assimilatory processes are reversible; this would imply that not merely the dissolved food-substances in the body-fluid are to be regarded as the 'substrate' from which the various reactions draw their materials, but that this substrate must be taken as including the tissues themselves. If, therefore, two reversible reactions A and B were proceeding simultaneously in two regions of an organism while the organism was starved, we should have each reaction making demands upon the end-products of the other, i.e. upon the tissues of the two regions. Four processes would therefore be involved—first and secondly, the reactions A and B proceeding in their normal direction; thirdly, B proceeding in reversed direction in response to the demands of A; and fourthly, A proceeding in reverse direction in response to the demands of B. Since A's speed is greater than B's, the end-product of A will continue to increase, while that of B progressively diminishes. We can represent such a state of affairs symbolically thus: $P \rightleftharpoons X \rightleftharpoons Q$, where P is the end-product of A, Q of B, and X the common substances utilized by both. If the rate of formation of P is greater than that of Q, the reaction will proceed until no Q remains.[2]

However that may be, we are confronted with the fact that if two reaction-systems are competing in the organism for an amount of nutriment which is not sufficient for both, then the more rapid, or the one which subserves the more highly-differentiated region, will not only get first call on the available nutriment, but will actually nourish itself at the expense of the other.

[1] This again is masked in higher animals by the fact that the nervous system, apparently owing to its controlling and co-ordinating function, has come to be the system least affected by starvation.

[2] Similar ideas are put forward by Runnström (1917) in his important paper on dedifferentiation in Echinoid larvae, to which unfortunately (owing to the war) I have only just had access.

This is particularly well seen in malignant tumours, which will continue to grow at the expense of the rest of the body, even when this is in a condition of relative starvation. In tumours derived from adipose tissue, the tumour-cells may be full of fat after all vestige of fat has disappeared from the normal tissues. The fact that regeneration will proceed actively and normally in starving Planarians exemplifies the same state of affairs.

We saw above that we should expect the reaction to proceed to a limit, all the product of the slower process being utilized by the faster. As a matter of fact this limit can often not be reached, since life is not possible when the 'subordinate' region is absent, or else its reduction brings about subsidiary changes.

It is also complicated by the supervening of dedifferentiation. That starvation can produce dedifferentiation has been shown by Schultz (1906), by Runnström (1917), &c. It therefore follows that the tissues of the less active region will usually, as a result of the starvation induced, reach a stage at which they are unable to maintain themselves, and will start to dedifferentiate. In the dedifferentiated state they will possess a still lower rate of metabolic activity, and so the resorption-process will be accentuated.

Next we meet with the fact of differential susceptibility. This is a corollary of difference in rates of reaction. The more highly-differentiated region and system, or the one with higher metabolism, will be, *ceteris paribus*, more susceptible to unfavourable conditions. If it is placed in a toxic solution, for instance, it will enter into reaction with more of it in a given time than will a slower system. There are a number of complicating factors (such as acclimatization) which enter into the problem, but, broadly speaking, we may say that a more highly-differentiated and more active system will be relatively more interfered with than a less highly-differentiated and less active system.

After a certain point of interference is reached, dedifferentiation will set in. Dedifferentiation is the primitive reaction of organisms to unfavourable circumstances. More energy is

necessary to maintain a cell in a differentiated than in a dedifferentiated condition. This is especially clear when, as in most instances, differentiation involves an increase in the surface of the cell relative to its bulk ; here the maintenance of differentiated form alone involves the expenditure of more energy. Thus when the processes of life are interfered with by unfavourable agencies, the cell is unable to continue to produce the energy necessary for the maintenance of its differentiated state, and must either die or dedifferentiate.

The main characteristics of dedifferentiation are the following :

(*a*) Cells revert, if isolated to a spheroidal, if in epithelia to a cuboidal form.

(*b*) Cytoplasmic differentiation is lost.

(*c*) Organs containing cavities revert to simple spheroidal sacs. Junctions between organs are often broken.

(*d*) Apertures usually disappear altogether.

(*e*) The whole organism diminishes in size and reverts to a spheroidal form, owing to the form-changes in its constituent cells. This has the effect of increasing the opacity and density of the organism.

Once dedifferentiation has started in any region or system the previous level of metabolic activity in that system is inevitably much reduced. Thus, if dedifferentiation occurs in a dominant and not in a subordinate system, this dominant system will lose its dominance and become subordinate. Such alteration of equilibrium by unfavourable agencies we may call differential inhibition ; it is a corollary of differential susceptibility. Differential inhibition need not, however, involve dedifferentiation, nor reversal of dominance. In a growing organism unfavourable agencies will depress the growth of the dominant or more active regions relatively more than that of the rest, and we shall, as outlined above (p. 669), get a decrease in size of the former, an increase in the latter—a decrease and an increase which will be absolute as well as relative. This is illustrated in some of Child's experiments (see later).

A chemical analogy, for which I am indebted to Mr. H. R. Raikes, of Exeter College, Oxford, may help illuminate the point. If one equivalent each of hydrochloric acid, boric acid, and ammonia are mixed, a negligible amount of boric acid will react with the ammonia owing to its small degree of dissociation. We may say that the hydrochloric acid is completely 'dominant' in the system, owing to a greater speed of reaction. If, however, the mixture is heated, the more volatile hydrochloric acid will be driven off, and the less volatile boric acid left to react with the ammonia. This we may call 'differential susceptibility' (to rise of temperature) involving 'differential inhibition' of one portion of the system, and consequent 'reversal of dominance'. If the mixture were contained in a very large closed space, cooling after heating would restore the original 'dominance' of the hydrochloric acid, giving a parallel to reversible dedifferentiation.

The emergence of the cells from the tissues in dedifferentiation is a phenomenon which deserves further study. Though probably by no means universal it is doubtless commoner than is generally assumed. It occurs not only in Perophora but also in Hydroids, in Turbellarians, and in Echinoderm larvae, and in many cases of actual poisoning, e.g. by mercury salts (Child 1917, Huxley 1921*b*) and other agencies (Gray 1920).

Once the cells start to emerge they may collect close to their place of origin, or if space and means of transport are available, be removed to regions at a distance. When the stolon portion is large in a Perophora stolon-zooid system, and the heart is beating normally, the latter is the case; it is also the case in Hydroids when the coenosarcal portion is large in comparison with the hydranth. The difference between the two possibilities appears to be similar to that between a reversible chemical reaction when the end-products are not removed, and the same when they are removed. Why, in the first case, the tissues should not simply resolve themselves into their constituent cells *in situ* is difficult to see, but the fact remains that they do not (e.g. Clavellina; Perophora with very small stolon attached, or with circulation stopped by KCl).

In ordinary organic systems, therefore, we must recognize that we may have to deal with any of the following phenomena :

(1) Physiological dominance and subordination of parts, manifesting itself first as regards conduction and control of asexual reproduction, secondly as regards nutrition.

(2) Differential susceptibility.

(3) Dedifferentiation.

(4) Differential inhibition.

(5) Resorption.

(6) Reversal of dominance.

Parallel phenomena occur in other organic systems in which parts are related in equilibrium. Thus dominance, subordination, differential susceptibility and inhibition, a form of dedifferentiation, and reversal of dominance, also occur in psychophysical systems, both in some where consciousness is involved and in some where it is not, as will be dealt with more fully later. Here the dominance may be called neurological and psychological, and the dedifferentiation is of course unaccompanied by physical dedifferentiation of nerve-tissues.

Many of the phenomena of inhibition, e. g. of buds by growing tips in plants, and within the central nervous system, obviously depend upon relations of dominance and subordination. A few examples will perhaps serve to illustrate some of these general statements.

We may start with the example already referred to, of Stenostoma (Child, 1904), since here dominance and resorption are very clearly shown. When a solitary Turbellarian is divided, regeneration of a head usually occurs from the anterior cut surface. In Stenostoma, however, which is a chain-forming organism, if a cut is made across the body of one of the central zooids, such regeneration from the anterior cut surface does not take place. Instead, the half-zooid which is attached to the anterior end of the posterior half-chain will shrink, assume a more rounded form, and eventually disappear altogether.

Not only this, but the relative age of zooids determines

dominance. In Stenostoma, fission occurs according to a regular system, so that the relative age of each head-region in a chain can be determined. If now a cut is made so that a younger zooid is left in front of an older zooid at the anterior end of a piece, this younger zooid, though morphologically complete, will be resorbed by the posterior. If completely isolated from the posterior zooid the younger one would have been capable of leading an independent and normal existence, so that the age-relation of zooids clearly determines dominance.

During the process, ' disintegration ' (presumably migration of the cells from the tissues) of the subdermal structures occurs, and the pseudocoel becomes filled with cells and granules. The posterior undestroyed zooid grows more rapidly than usual, apparently because of the excess of nutriment thus provided (although this nutriment is in the pseudocoel and not in the gut). Child did not undertake a histological examination. From his observations in vivo, however, it is clear that the cells migrate out of the tissues, as in Perophora. The most highly-differentiated organ, the pharynx, disintegrates very early. The intestine, however, does not do so until late. From the ectoderm of the resorbed portion a very gradual migration probably occurs. The portions undergoing resorption are wrinkled and collapsed.

The reversibility of the process is shown by the following observations. If an older posterior zooid has in front of it another almost as old, resorption will begin, but fission will occur before it has finished, and the two zooids will separate; after this the anterior zooid redifferentiates. The converse of this is seen when a long anterior fragment is present. In this case the beginning of regeneration occurs, but reduction finally takes the upper hand, and the whole fragment is resorbed. The rapidity of the change is noteworthy, complete resorption usually occurring in twenty to thirty hours. Provided that the brain-region of an anterior fragment is absent, resorption will occur; even when a system consists of a very long but brainless anterior fragment, and only the brain-region of the posterior zooid, resorption happens.

To sum up, whenever in Stenostoma a system is artificially produced in which a posterior brain-region is older than any brain-region anterior to itself, or has a brainless region anterior to it, resorption of such anterior regions will start; it will be completed unless fission of the system occurs during the process, which only happens when an anterior zooid is far-developed.

A brain-region is physiologically dominant over all other tissues of the same and other zooids, and over all younger brain-regions than itself. When a region comes to lie anteriorly to a physiologically dominant region, it cannct maintain itself, and is resorbed. Antagonistic to resorption is the process of regeneration (morphallaxis). Both processes often start simultaneously in a fragment; which of the two eventually gains the upper hand is determined by the age of the fragment. The systems resemble the stolon-zooid systems of Perophora, except that the different members of the system are all similar to each other except in age. Further, reversal of the effects by altering the environment has not been attempted. This would provide an interesting field for experiment.

As the facts stand, the dominance is caused entirely by the internal factor of physiological state due to (1) presence and (2) age of brain or brain-region, and resorption is produced when a part is caused to lie in an abnormal position relatively to a dominant region. As Child points out, similar resorption of parts in abnormal positions is frequently seen in grafting experiments in Hydra and Planarians. Subordinate portions in a normal position relative to a dominant region do not of course become resorbed.

Once more the essential fact is that, in certain conditions, parts of a system are unable to maintain themselves of their normal size or their normal form, and, once they start dedifferentiating, become subordinate in the system, and can be used as food for the remaining dominant part.

I suspect that investigation would show that the first change, here as in Perophora, is the loss of the normal cell-form of the

differentiated organs of the subordinate region, and that resorption follows upon this.

Numerous other cases of tissues, regions, and whole organisms being unable to maintain themselves as such in changed circumstances are known. Of these may be mentioned the degeneration of muscle-fibres when the nerves supplying them are cut. Here the ' normal environment ' apparently includes constant nervous stimulation, and in the absence of this the elaborate structure of voluntary muscles cannot be maintained in equilibrium. Similar dedifferentiation of muscle-fibres takes place in the stump of an amphibian limb which has been cut off preparatory to regeneration (Towle, 1901).

In the interesting studies of Child on differential inhibition during development we do not get the total disappearance of one part of the system, but merely a change in the proportions of the various parts. The simplest example studied was the effect of dilute poisons upon the development of the marine Polychaet worm Chaetopterus (Child, 1917).

He found that during the earliest stages of development the apical region of the egg and blastula is the most susceptible to various poisons, in certain concentrations a regular death-gradient being obtained from the animal to the vegetative pole. By the time the early trochophore larvae has been produced, however, a new development occurs ; the posterior (previously vegetative) region suddenly becomes highly susceptible, its metabolic rate being raised apparently in preparation for the active growth-processes that are about to occur in this region ; for the formation of the permanent growth-zone, from which all the body-segments of the adult worm will be produced, takes place here.

The death-gradient will now advance from the two ends of the larvae to meet in the middle region, which, with its lower metabolic activity, survives the effects of the poison longer than the rest. In the later larva the anterior region is differentiated as a head with ciliated band and apical tuft ; and posteriorly there is a well-defined growing-region, with a small posterior prolongation.

Immersions of the fertilized egg in solutions of poisons so dilute as to allow development to proceed, while yet exerting an influence on the more susceptible parts of the organism, give the following results. (Essentially similar facts were discovered for other Polychaetes (Nereis and Arenicola).)

Immersion continuously up to the late larval stage gives a form with both anterior and posterior regions smaller and less differentiated than the normal. The middle region is either almost as large, and of the same form as the normal, or else considerably distended. This latter condition implies possibly that the cells of this region have been able to develop practically normally. The anterior and posterior regions are not so active as normally, and hence are not able to make use of so much of the yolk; there is thus more for the middle region, which is capable of utilizing it, and secretes an excess of fluid. If immersed for eleven hours only, and then replaced in sea-water, the apical region is small, but the growing region as well as the middle region is nearly normal. If, on the other hand, the development is allowed to proceed in sea-water for twelve or twenty-four hours, and the larvae are then placed in the solution, the apical region, having been completed before immersion in the toxic solution, is normal and the posterior end is much affected.

In another paper, giving an account of similar experiments on Echinoderms, he makes an interesting suggestion to account for the great over-development of the skeleton often found in larvae which have grown in dilute solutions of toxic agents. The mesenchyme cells appear to be least susceptible, and thus when the other cells of the organism are inhibited, can obtain a greater quantity of food, which results in a multiplication not only of themselves but of the products of their activity, i. e. the skeleton (Child, 1916).

A recent important attempt to apply similar principles has been made by Robertson and Ray (1920, where reference to earlier papers are given).

Robertson found that mice to whose diet had been added tethelin from the anterior lobe of the pituitary, showed first

a retardation of growth in weight, then an acceleration, and finally lived about 12 per cent. longer than normal controls. Other experiments had led him to conclude that tethelin (or pituitary extract) caused increased growth in cellular tissues, a conclusion strengthened by the recent grafting experiments of Allen (1920) on tadpoles. His explanation of the facts is as follows. Tethelin causes at first an absolute increase in the growth-rate of the cellular tissues of the body; this involves, as we have seen, a relative decrease in the weight of the supporting tissues. Since these latter are the heavy tissues, this involves an absolute decrease in total weight. Eventually, however, the characteristic relation between the amounts of cellular and supporting tissues is established, but later than normal. Relative increase of the supporting tissues characterizes old age; and the onset of senility is delayed by that period by which the establishment of the cellular-supporting balance was postponed. The reason for the more rapid growth of the cellular tissues at the beginning is that the tethelin stimulates them to greater activity, and that consequently they obtain first call on the available foodstuffs.

This view-point, it will be seen, is very similar to that of Child.

A beautiful example of differential inhibition depending only on the two quantitative factors of size and distance is given in the interesting paper of Detwiler (1920; see especially pp. 149–51). Detwiler transplanted the limb-rudiments of Amblystoma autoplastically, cutting the rudiments out and transplanting them a varying number of segments posteriorly from their normal position. The experiments were undertaken at a stage when the rudiments were represented only by circular thickenings of somatopleuric mesoderm in segments 3–5. He found, as had previous workers such as Harrison, that in many cases the rudiment was not completely excised, a few of its cells being left in the normal position. When this was so, these cells usually begin to regenerate on their own account. It is of interest to note that this regeneration is

greater when the wound is not covered—a result presumably due to the greater stimulation which the unexcised limb-cells then receive (Harrison, 1915).

After a short time a small nodule of cells begins to protrude from the body in this region. If the main limb-rudiment is completely removed the nodule may grow into a perfect limb. When, however, the main limb-rudiment is transplanted less than four segments back on the same side, these nodules, after growing a longer or shorter time, begin to shrink, and eventually disappear altogether. When the limb-rudiment was only transplanted one segment back the nodules appeared after about four days, but very speedily began to decrease and had disappeared after eight days. When the limb was transplanted two segments back the nodules continued to increase till the fifth or sixth day, and had disappeared by the eleventh day: when the distance of transplantation was three segments, nodule-growth continued until the tenth or eleventh day, when the 'nodule' was almost as large as the transplanted limb; but after this, decrease set in, and all nodules eventually disappeared, although not until the eighteenth to twentieth day. Finally, when the main limb-bud was removed more than three segments from its original site, the regenerating nodules always developed into a normal appendage, so that two limbs were produced from the one original rudiment.

The cells of the limb-bud constitute an equipotential system, as Harrison has shown. It is therefore clear that the inhibiting effect exerted by the main transplanted rudiment on the cells left at the original site must be due simply to the greater size of the former. The strength of this 'dominance', however, also depends upon the distance of the two systems; and when this distance is increased beyond a certain limit, there is no longer any inhibitory effect. If we like, we may say that the reason why the cells constituting the normal limb-rudiment of Amblystoma do not usually form more than one limb is that they occupy such a small area that any one rudiment growing within that area inhibits the growth of any other.

Detwiler did not investigate the actual mechanism by which

the 'nodules' decreased in size, and leaves it open as to whether the cells composing them are actually translocated into the main limb-bud, or are simply resorbed into the body. The former view is less probable on general grounds, and the latter is supported by the facts of resorption in Perophora. The limitation of physiological dominance by distance has already been brought out by Child (1915*a*, chap. 5), but is here particularly well illustrated. The relation of dominance to simple size-difference between two portions of otherwise identical tissue has not, however, so far as I am aware, received any special attention, but is obviously of considerable theoretical importance. Further, in no other case with which I am familiar, is the importance of purely quantitative relations so well brought out. It is perfectly clear that inhibition and consequent resorption can take place at any stage of growth of the 'nodule' (regenerating limb-rudiment), and that it is not due to anything in the nodule itself, but entirely to its relations with a second developing system.

We now pass to the very different field of neurology and psychology.

In recent years the phenomenon known as mental regression has been carefully studied. Patients suffering from this return to an earlier stage of mental existence. Grown men may show the behaviour and the mental processes of boys of ten or five or even younger. A review of our knowledge of this condition is given by Nichol (1920).

When properly analysed this state of affairs would seem definitely to be due to the presence, in individuals affected by it, of two competing systems of mental organization, i. e. of two possible main channels for the flow of 'nervous energy'. (I purposely use this latter somewhat vague but non-committal term to emphasize the fact that the existence of competing systems and of some form of activity transmissible along their paths is all that we need to assume for a preliminary discussion of the problem.) In normal conditions the adult system is dominant, the main flow of nervous energy is along its paths, and the childish system or systems are dormant, existing for the

most part only as potentialities of action. Under severe stress (e. g. modern warfare, prolonged worry, &c.), the adult system becomes in some way affected. It is no longer so easy for the nervous energy to flow along its paths. Under these conditions there is more nervous energy available for the other, juvenile, system, which has remained undamaged. Finally, there will come a moment at which the balance is so altered that the adult system ceases to be dominant, and the potentiality of the juvenile system is transformed into actuality. The juvenile system now becomes dominant in its turn, and the adult system retreats into potentiality. During recovery a remarkable picture is presented: the two systems are almost equally balanced, and we get—not a blending of the effects of both—but a rapid alternation, first one and then the other, the two never co-existing. A somewhat similar state of affairs exists in Perophora; once absorption of either portion has started it proceeds rapidly. Alternation, however, is not possible, since in Perophora it is structure, and not merely possibility of function, that is being destroyed.

In the neurological cases structure is not destroyed. Further, the rapidity of change from the dominance of one system to that of the other is enormously more rapid, since this is apparently accomplished simply by the passing of a threshold-value. Once this is passed a sluice is opened, and a different neural system flooded so as to permit of function. For this sudden appearance of one or the other sub-system some psychotherapeutic writers use the expressive term 'puffing-up'. It is a well-known phenomenon of convalescence in such cases.

Such occurrences are one aspect of the general principle laid down by Hughlings Jackson, that, as the result of lesion, 'dissolution occurs first in the most highly-organized products of neural or mental activity, leaving the more lowly at liberty to express themselves freely in the resulting symptoms'. This, however, only stresses the aspect of differential inhibition, not that, of equal importance, of intra-organismal struggle.

Part of this latter aspect of the question is expressed, however, by Head (1918), who lays down as one of his general principles of neurology that 'Integration of function within

the nervous system is based on a struggle for expression between many potentially-different activities '. Integration of function, however, is not all. A number of integrated minor systems may exist, one in actuality, the rest in potentiality, in the developed human psycho-neural system as a whole ; and there is also a form of struggle between them. The particular type of mental disorder known as regression is only one special case of the results of differential susceptibility among two or more such minor systems. In other so-called neurasthenic cases the second, normally-suppressed system may not be a system of childish memories, but an imaginary ' ideal ' world of thought along whose paths consciousness flows instead of along those necessary to maintain adaptation to everyday life ; or else it may be the system of ' negative ' emotions, leading to depression and possibly to suicidal attempts. Dissociation of personality and subsequent alternation of the sub-personalities may also, though less directly, be included under the same rubric. Rivers, in a recent work (1920), has emphasized the same point of view ; he points out for psychological systems what I have drawn attention to in this paper for physical systems—that reversal of dominance in a balanced system may occur either through the action of unfavourable agencies on the dominant system (differential inhibition) or of favourable agencies on the subordinate system (differential stimulation).

In a case of regression mentioned by Dr. W. MacDougall and Dr. Hadfield in their lectures and confirmed to me in conversation by Lt.-Col. Good, of Ashhurst Hospital, a young man actually regressed to the condition of an infant.[1] He was unable to talk or walk, and could tolerate no food except milk. (By some freak of the nervous mechanism two associations and two only remained from adult life : if a cigarette were offered him he would light and smoke it ; when shown a horse or a picture of a horse, he would get astride of some object and ' tchk ' as if encouraging a horse. It turned out that he

[1] Since the above was written, I find that an account of this and similar cases has been published by MacDougall in 'Journ. Abn. Psych.' 15, 1920, p. 136.

had been a jockey.) His recovery was interesting for various reasons. The intolerance for all diets save milk he lost earlier than the other infantile symptoms. As regards purely mental symptoms his growth or redifferentiation was gradual and progressive, though with considerable rapid oscillations. It is therefore clear that the picture is not quite as simple as I have drawn it above. Each stage is really in some ways dominant to the one below, subordinate to the one above, and if there has been a considerable degree of regression, the redifferentiation must apparently be by steps (although the regression itself is a sudden instantaneous process). In the normal adult each lower stage is kept in its proper place in the hierarchy, and most of the associations and types of reactions connected with it exist *in posse* only. When it is released from the inhibitory control of the processes associated with higher stages it becomes dominant, and then these potential associations, memories, and reactions become actual and functional again. Normally, since each stage of growth represents a necessary step towards the next stage, some of the reactions of each stage are functional even in the adult, as foundations for normal adult activity; but they are altered by the dominant higher processes to a form different from that which they would have if released from control. This is parallel, though not identical, with the behaviour of dominant and subordinate regions in regeneration (see later). Regression takes place suddenly to that stage whose system has been encouraged; if the patient has dwelt upon a particular time of childhood, to the system associated with that time; if he has dwelt on mere release from control, to an infantile stage. But recovery must be by gradual building-up, as in physical development.

Individual mental development is thus an epigenetic process; and the different stages of this development are arranged in a functional hierarchy or series in which each stage is dominant to the one below, subordinate to the one above.[1]

[1] The alternation of dominance seen in dual and multiple personality (Prince, 1908, 1920) is presumably based upon essentially the same principles, the difference being that typically the two systems are very evenly balanced,

We shall now see that similar relations may exist in non-conscious neural processes, of which the lower have never been fully dominant in ontogeny (though possibly in phylogeny).

This is well shown by the observations of Head and Riddoch (1917) on the activities of ' spinal man '. They found that when the spinal cord was completely divided, the reflex activities which manifested themselves after the initial shock-period were very different from those occurring in the uninjured individual. In the normal person the activities of the spinal cord are modified by influences reaching it from pre-spinal levels. The isolated spinal cord, however, responds to stimulation predominantly by a type of ' mass-reflex ' not normally seen in man. In ' spinal man ' any form of nocuous stimulation to a hind-limb causes not merely flexion of the limb stimulated, but violent flexion of both limbs, abdominal contraction, voiding of the contents of the bladder if the contained fluid is above a certain very small volume, and sweating. Conversely, injection of the bladder with fluid induces a flexor spasm of the lower limbs, combined with sweating. (The reaction may be called an excessive and non-discriminate reaction to harmful stimuli, resembling in many ways that seen in certain lower animals, e.g. the toad, in which voiding of the bladder accompanies limb-flexion when the animal is alarmed by handling.) The same mass-reflex also appears in higher forms and in man himself when the higher centres are put out of action under the influence of an excessive degree of an emotion such as fear (differential inhibition). The mass-reflex may be looked on as a very primitive response of the organism to nocuous stimuli.

In higher forms the mass-reflex has become subordinated to the influence of other types of reaction ; among these are the postural reactions and the conscious direction of movements of escape. Head and Riddoch found that so long as any

and both adapted (though incompletely) to adult life. The emergence of the juvenile personality ' Sally ' in Morton Prince's case is especially interesting as it only occurred when the normal control was impaired through the dissociation of the adult personality into two.

remains of postural control were present in their patients—which indicated that some connexion was still present with pre-spinal centres—the mass-reflex did not appear. In other words, in the course of phylogenetic evolution, a compound mechanism has been evolved, the parts of which stand to each other in a relation of dominance and subordination. But here the dominance appears to be only slightly reversible, as opposed to the cases of Perophora and of mental regression. Here the subordinate system is so thoroughly under the control of the other (presumably owing to certain structural relations and to innate physico-chemical peculiarities inherent in synapses concerned with inhibition), that it is apparently impossible to tilt the balance so as to make the subordinate system the dominant one for long together, so long as both are in organic connexion. It is only when the two systems are separated from each other that the real nature of the subordinate system can be studied as it exists apart from controlling influence from without. As indicated above, differential inhibition through fear may induce a short temporary reversal of dominance.[1]

Child has pointed out that a somewhat similar (and also simpler) relation subsists between the dominant and the subordinate regions in many low forms of animals, such for example as Planarians. Here, so long as the head region is exerting its dominant or controlling influence, other portions of the organism cannot form a head. But when this influence is removed, either by the amputation of the head or by the 'physiological isolation' of parts of the organism (by their removal, through growth, beyond the radius of influence of the head), then the most anterior part of the isolated region at once reacts by producing a head (Child, 1915*b*, p. 96 et seq.). In Head's spinal case, however, after isolation the subordinate system does not take on the characters of the dominant system, but assumes a form which is peculiar to itself.

[1] The views of Head and Riddoch have been recently criticized (e.g. 'Medical Science', vol. 4, 1921, pp. 141, 430). The fact of decerebrate rigidity, however, would, among others, equally well serve to illustrate the principle of neurological dominance and subordination, although here we remain without phylogenetic analogies.

We may now leave the nervous system and return to physiology. As an example in mammals, and one concerned only with the parts of one organ, the following will serve.

As is common knowledge, the testis in mammals consists of several functionally-distinct parts. Apart from blood-vessels and nerves there are (1) the germ-cells (spermatogonia, spermatocytes, spermatids, and spermatozoa), (2) the cells of Sertoli, (3) the interstitial cells or cells of Leydig, (4) connective-tissue cells. In the normal testis these exist in proportions which do not vary beyond narrow limits. Various agencies, however, will upset this balance. The germ-cells are the most susceptible. Exposure of the testis region to X-rays or to Mesothorium; or ligature or section of the vas deferens; or abnormal position in the organism, which can come about spontaneously as in natural cryptorchism or can be produced experimentally as in artificial cryptorchism or by transplantation, will bring about some degree of degeneration of the germ-cells. This is accompanied in every case by a hypertrophy of the interstitial cells. The cells of Sertoli are usually unaffected. It would appear that these latter are not cells capable of rapid multiplication. The chief competition is therefore between the germ-cells and the interstitial cells. The former are in some way dominant; when they are damaged, a check on the latter is removed, and their active increase results. Whereas removal of the testis to an abnormal environment usually results in the permanent disappearance of the germ-cells, X-ray treatment, if not very intense, only damages them temporarily. Later they regenerate, and finally come to have their old proportion once more. The increase in the number of interstitial cells only lasts until this regeneration starts, and is followed by a decrease. Finally, the normal equilibrium is re-attained.[1]

[1] See also R. Goldschmidt, 'Biol. Centralbl.', 36, 1916, p. 160. In Lepidopteran testes cultivated in tissue-culture, normal spermatogenesis occurs. But the germ-cells always die before the cells of the follicle. When this happens, the follicle-cells, which have till then remained normal, start at once to multiply at a rapid rate.

The germ-cells are thus, in normal circumstances, partially dominant over the interstitial cells, and are also more susceptible than they are. This is the same relation that we found to hold good between the zooid and stolon of Perophora. Furthermore, it appears that in the testis a similar relation is to be found between the interstitial cells in their turn and the connective tissue (and Sertoli cells). Transplanted testes, as we have said, first lose their germ-cells and show increase of interstitial tissue. Within a few months the Sertoli cells also degenerate and disappear (Steinach, Sand). We may take this to mean that these cells, while not increasing after the loss of the germ-cells because they are not a multiplicative type of cell, are slightly less resistant than the interstitial cells. Even these, however, are less resistant to unfavourable conditions than the connective tissue. After a longer or shorter period (usually several months) in the abnormal situation, the interstitial cells in their turn start to decrease in number, and now it is the connective-tissue cells which show a corresponding increase. Finally, the 'testis' comes to consist of nothing but connective tissue and blood-vessels. This is also seen in some few cases of cryptorchism.

We have thus a system in which there enter four variable sub-systems. One of these, for a reason which we can conjecture but not prove, does not increase when others decrease. The other three, however, are all in that state of dynamic equilibrium which we have seen in its simplest manifestation in Perophora. But this time they are arranged in a series, A being physiologically dominant over B, and B in its turn over C. Normally, therefore, the relative proportions of the three tissues are regulated according to the activity of A. When A is adversely affected B increases, but not C. C, however, increases when both A and B have been affected.

If such a type of system were to exist, it should follow that in some (abnormal) circumstances somewhat different conditions should obtain, and that a slightly different end-result should be brought about. As a matter of fact, in some of the transplantations of Sand, this did occur. In three cases both

germ-cells and interstitial cells disappeared, leaving only Sertoli cells and connective tissue. In one other case the germ-cells and Sertoli cells were much less affected than the interstitial tissue. This recalls the varying behaviour of the stolon-zooid system in Perophora according to the internal condition of the zooid. (See Lipschütz, 1919, Chap. IV, where full references are given.) Another view of an almost identical problem is given by the varying response of the mammalian ovary to different intensities of X-ray treatment (Lipschütz, 1919, Chap. V, p. 205).

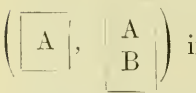
The conclusions we reached in discussing Detwiler's results (pp. 679–687) are of importance when we come to apply the principles of dominance, differential inhibition, and resorption to an explanation of the phenomena of metamorphosis. In metamorphosis, as I have pointed out elsewhere (Huxley, 1921 *b*), we have to think of the full-grown larva as consisting of two minor systems in competition with each other—the differentiated system of larval organs, and the developing system of adult organs. The two enter into a state of balance. This balance may be tilted in favour of the adult, or kept at the existing tilt which favours the larval system. It has often been maintained that the time of metamorphosis was determined by the production of a given relative quantity of some definite substance within the organism, e.g. thyroid secretion in the larvae of Amphibia. Such a concentration of a particular substance is often the effective agent in tilting the balance, but it is not the essential cause of metamorphosis. The essential cause of metamorphosis is that two mutually incompatible systems are in a state of dynamic physiological equilibrium within the same organism.

In Echinoderm metamorphosis the mechanism for upsetting the balance appears to be simpler than in Amphibia. Experiments of Runnström (1917) and of my own, an account of which is now in the press, indicate that exposure of the pluteus tissues to unfavourable agencies of various descriptions will lead to their dedifferentiation and partial resorption. In nature the actual chain of events leading to this result appears to be as

follows: the Echinus rudiment at the start grows concomitantly with the Pluteus. After a certain time, however, it becomes so large that its weight drags the larva to the bottom. Here the conditions, as regards both food and general environment, are unfavourable to the pluteus tissues; these begin to dedifferentiate, and as soon as they have passed a certain critical stage in the process the Echinus tissues become dominant and are able to develop further at the expense of the larval organization. In the broadest terms the balance in Amphibia is regulated mainly from within, in Echinoids mainly from without; but in both cases the possibility of the sudden change which we call metamorphosis depends on the co-existence of two systems in the same organism which are very closely balanced as regards physiological dominance.

To sum up, we may say that the facts of physiological dominance of inhibition of growth, of resorption, and of the state of balance which exists among the parts of any organism and is the dynamic expression of Roux's 'Kampf der Teile', are all intimately connected. As a matter of fact physiological dominance is rendered most obvious when it can be reversed, as in Perophora or in metamorphosis—and that is when the balance between sub-systems is very close.

The various examples discussed may perhaps be made clearer by the use of symbols. In every case let A = a dominant system; B a system normally subordinate to A; C one normally subordinate to B and also to A. An arrow ↓ indicates dominance, pointing towards the subordinate system. Brackets ( ) indicate subordinate condition. Dashes (A′, B′, &c.) indicate alteration of the system from its original condition to another. Erasure (A̸, B̸, &c.) indicates disappearance of a system by resorption. Suffixes (A_1, B_2, &c.) indicate homologous systems in order of age or size. Enclosure

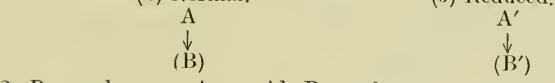


indicates passage to a non-functional state.

Plus sign (A+, B+, &c.) indicates increase of the system.

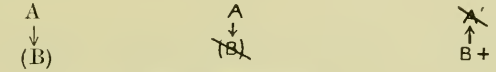
1. Clavellina. A = zooid, B = stolon.

(*a*) Normal. (*b*) Reduced.



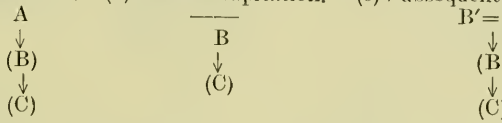
2. Perophora. A = zooid, B = stolon.

(*a*) Normal. (*b*) Starved, water changed. (*c*) Starved, water not changed.



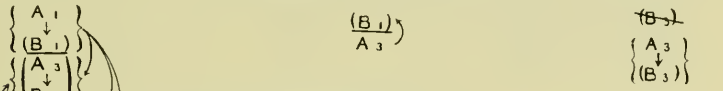
3. Planaria or single Stenostoma zooid. A = brain-region, B = pharynx-region, C = tail-region.

(*a*) Normal. (*b*) After decapitation. (*c*) Subsequent regeneration.

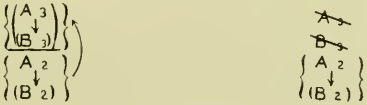


4. Stenostoma chain. A = brain-region, B = rest of zooid. A_1 B_1 = oldest, A_4 B_4 = youngest zooid.

(*a*) Normal. (*b*) Transection giving brain-region behind posterior brainless region of another zooid. (*c*) End-result from (*b*).

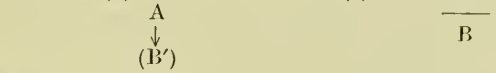


(*d*) Transection giving older zooid posterior to younger. (*e*) Result of (*d*).



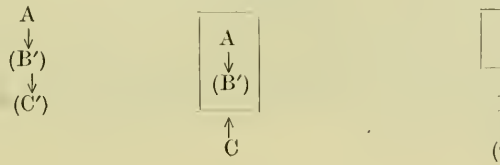
5. 'Spinal man.' A = cerebral centres, B = mass-reflex.

(*a*) Normal. (*b*) After transection of cord.



6. Mental regression. A = adult system, B = juvenile system, C = infantile system. (Only three systems given for simplicity's sake.)

(*a*) Normal. (*b*) After regression. (*c*) During recovery.



7. Testis. A = germ-cells, B = interstitial tissue, C = Connective tissue.

(*a*) Normal.	(*b*) Transplanted, after short period.	(*c*) Ditto, after longer period.
A	A	A
↓	B+	B
(B)	↓	C++
↓	(C+)	
(C)		

8. Amblystoma limb-buds. A_2 = transplanted limb-bud, A_1 = regenerated remains of limb-bud in original position.

(*a*) After transplantation to a distance of more than four segments.	(*b*) End-result from (*a*).	(*c*) After transplantation to a distance of less than four segments.	(*d*) End-result from (*c*).
A_2	A'_2	A_2	A'_2
		↓	
A_1	A'_1	(A_1)	A'_1

9. Metamorphosis of Echinoids. A = larval tissues, B = adult tissues.

(*a*) At the time when larva sinks to bottom.	(*b*) Shortly after.	(*c*) End-result.
A	(A′)	A′
↑↓	↑	
B	B	B′

9. Summary.

1. The social Ascidian Perophora viridis may dedifferentiate in either of two distinct ways, or by a mixed method : (*a*) by reduction to a spheroidal mass, as in Clavellina ; (*b*) by incipient reduction as in (*a*), but followed by total resorption into the stolon, which may grow during the process.

2. Resorption is due to the migration of the individual cells out of the tissues into the haemocoel.

3. In certain conditions the zooid maintains itself, in spite of food not being provided, at its original size and in perfect health. This it does by resorbing the stolon.

4. Experiments with dilute solutions of KCN show that resorption of the zooid occurs in slightly unfavourable conditions, which affect the sensitive zooid more than the less highly-organized stolon.

5. The results are to be explained as follows: (*a*) In the competition between zooid and stolon the zooid normally is dominant because metabolic processes take place at a greater rate in it than in the stolon. The stolon is therefore starved at the expense of the zooid. (*b*) The zooid is more susceptible than the stolon to toxic agencies. (*c*) In low concentrations of such agencies it is therefore affected while the stolon is not. (*d*) As a result it begins to dedifferentiate. Dedifferentiation is here accompanied by the migration of the cells out of the tissues. (*e*) The speed of its metabolic processes is now no longer greater than that of the stolon's. It is therefore now starved at the expense of the stolon. (*f*) Any cells migrating out of the tissues are removed by the normal circulation, by the stolon-circulation (irregular pulsation of the stolon), or by utilization as food by the stolon. As in chemical reactions where the end-products are removed, the reaction thus runs to its limit, i.e. to complete resorption of the zooid.

6. Stopping the circulation by means of KCl results in dedifferentiation accompanied by a much smaller degree of resorption.

7. At low temperatures (about 5° C.) some dedifferentiation occurs; but there is very little resorption, apparently owing to the cessation or slowing of the heart-beat.

8. Partial dedifferentiation is recorded in Amaroucium and Botryllus.

9. The significance for general biological problems of dominance due to high rate of metabolism, of differential susceptibility and of dedifferentiation, is discussed.

10. The similarity of certain psychological and neurological phenomena is noted (mental regression, alteration of spinal reflexes when freed from cerebral control, &c.).

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Illustrations.

All figures are drawn to scale with the Abbé Camera lucida. Otherwise they are semi-diagrammatic. All were drawn at table level; the magnification is indicated for each figure.

Fig. 1.—Clavellina type of reduction (×25). Two zooids, A and B, isolated without stolons. *a*. Day of operation. B has a trace of stolon-connexion. *b*. After forty-eight hours. Reduction started earlier in B. Both have formed short stolons, but that of B remains within the test. A has only just started to reduce. *c*. Advanced reduction (three days for B, four days for A). The stolon of B is large and lobulated, but has not emerged from the test. B is spheroidal and opaque, in stage 4–5. A is in stage 3, which it did not reach till after three days. Its stolon has grown, and is distended with cells. A's heart was beating slowly, B's had almost stopped.

Figs. 2 and 3.—Growth or maintenance of zooid at expense of stolon.

Fig. 2 (×80).—*a*. Immature zooid on day of operation. *b*. The same, perfect, after three days. The stolon has been much reduced both in length and breadth. A small bud had formed and been absorbed. *c*. After five days. Further reduction of the right end of stolon; zooid in first stage of reduction, which has led to a slight dilatation of the left end of the stolon. *d*. Stolon-tip from a similar system after three days, showing shrunken appearance.

Fig. 3 (×25).—*a*. A system on the day of operation. *b*. The same three days later. Zooid actively functional, stolon much drained in all dimensions.

Fig. 4.—Maintenance of bud following resorption of first zooid. *a*. (×80). Original zooid in stage 4 of reduction, after two days. Stolon healthy. *b*. (×80). After three days. Stage 4, but smaller; meanwhile a bud had formed to the left of the zooid, and by now was 50 per cent. larger in diameter than the zooid. *c*. (×40). After five days the zooid had disappeared. The remains of its test is seen. The right part of the stolon, to the right of the bud, has also been resorbed, and resorption is beginning in the other portion, as shown by the extent of its test. *d*., *e*. (×40). After seven days. In *d*. the tip of the stolon is shown contracted, in *e*. expanded with blood. *f*. After twelve days. The stolon has almost disappeared. The zooid is practically unchanged in size or development.

Figs. 5–15.—Resorption of zooids and growth of stolons.

Fig. 5 (×25).—Stolon-growth. *a*. A system on the day of operation. *b*. The same, but stolon only; four days later. The zooid was in stage 2 of reduction. A bud is seen on one stolon-branch. Note the test bridging concavities of the stolon.

Fig. 6 (×40).—Early stages of resorption. *a*. After two days. Zooid just reaching stage 3. The exhalant siphon is still slightly attached to the test. Faint traces of gills visible. The stolon-branch B and the tip of A represent new growth. *b*. Eight and a half hours later. The zooid is in stage 3–4, and has shrunk considerably; B has grown.

Fig. 7 (×64).—Zooid in stage 3–4 of reduction, showing heart and traces of inhalant siphon and stomach; note the double stolon-connexion.

Fig. 8 (×64).—Zooid in stage 4 of reduction. The heart is seen end on.

Fig. 9 (×64).—Later stages of reduction. *a*. Zooid in stage 4, after two days; ectoderm in places cubical. A stolon outgrowth had occurred. *b*. The same, ten hours later (test omitted); further shrinkage. Ectoderm all cuboidal. *c*. Fourteen hours later (test omitted); further shrinkage. A new stolon outgrowth has occurred. *d*. Forty-eight hours later (five days in all). It is now in stage 6 (after four days it had reached stage 5). The pale ovoid is probably the remains of the stomach. Note the slight reduction of the test. *e*. Twenty-four hours later (six days). Zooid portion smaller than stolon-connexion.

Fig. 10 (×64).—Zooid reaching stage 5. The stolon was attached to another zooid, and showed active circulation. It was hard to be sure whether the heart was beating.

Fig. 11 (×100).—Zooid in stage 5. Stolon as in 10. Zooid ectoderm cuboidal. Solid organ-remains fill most of the zooid.

Fig. 12 (×64).—*a*. Zooid in stage 4, after two days. *b*. The same, nine and a half hours later (from a different aspect). Stolon as in 10.

Fig. 13 (×64).—Zooid in stage 5 of reduction. Zooid of the same opacity as the stolon.

Fig. 14 (×64).—Zooid in stage 5-6. Opacity as in 13.

Fig. 15 (×64).—Zooid in stage 6. Some remains of organs visible.

Figs. 16-18.—Reduction at low temperature.

Fig. 16 (×32).—After eight days; early stage of reduction. Note considerable opacity combined with open siphons. Heart beating, but circulation only in right half of the stolon-connexion. Note a new stolonic outgrowth into the test of the zooid.

Fig. 17 (×64).—A similar zooid after eight days. Débris on siphons, which are open. Heart not beating, but visible.

Fig. 18 (×64).—Similar; but a slightly later stage of reduction. Depressions still mark the siphons. Heart beating, but very faintly.

Figs. 19-20.—Reduction in KCl solutions.

Fig. 19 (×25) (50 c.c. sea-water+4 c.c. m/2 KCl).—*a*. Early stage of reduction, after one day. Inhalant siphon-lobes of test separate from siphons. Outgrowths at the end of stolon. *b*. The same, twenty-four hours later. Cell-strands attach siphon-regions to test. No sign of internal organs. Stolon healthy.

Fig. 20 (×25) (50 c.c sea-water+8 c.c. m/2 KCl). Similar to 19, *b*., except that the stolon as well as the zooid has been adversely affected (shrinkage, cuboidal epithelium).

Figs. 21-3.—Reduction in KCN solutions.

Fig. 21 (×25).—In m/2,000 KCN. *a*. Before treatment. The zooid is a not quite developed bud. *b*. After twenty-four hours. Zooid in stage 3 of reduction. Stolon slightly shrunk, but crowded with cells, and with attempts at new growth.

Fig. 22 (×25).—In m/4,000 KCN. *a*. Before treatment. *b*. After forty-eight hours. Zooid much reduced. Stolon crowded with cells, but shrunken; no new growth.

Fig. 23 (×25).—In m/32,000 KCN. *a*. Before treatment. Zooid a not quite developed bud. *b*. After twenty-four hours. Zooid considerably reduced, stolon with clubbed ends and with new growth (within test only). *c*. In reversed position after forty-eight hours. Zooid much reduced. Stolon crowded with cells, and with new growth outside test.

Fig. 24 (×340).—To show pulsation of stolon. The same stolon-tip (*a*) expanded; (*b*) (less than a minute later), contracted. The position of

the test (*x*) did not change, and a space was left between it and the ectoderm when contraction occurred. Note the thickened epithelium in contraction, with irregular outline externally. Note the small outgrowth in the expanded state; this was not observed after contraction. The stolon remained for a few minutes contracted, then expanded in under a minute; and vice versa. The blood-cells are not figured.

Fig. 25 (×340).—A large lateral outgrowth, on the same stolon as that shown in fig. 24. The blood-cells are shown in the outgrowth itself, but only a few indicated elsewhere.

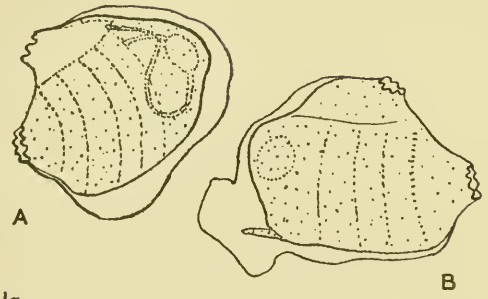
Fig. 26 (×340).—A normal growing stolon-tip. Note the columnar epithelium at the extreme tip. Close to the tip there are very few green blood-cells, the majority being white. Then comes a zone where a considerable proportion are green, and then one where they are in the majority. The circulation, though active, did not extend into the densely-packed region drawn.

Figs. 27 and 28.—Dedifferentiation in Amaroucium.

Fig. 27 (×80).—A young oozite in stage 2–3 of reduction. The dense anterior mass was orange-red. Portions of the intestine are seen below. Muscular contraction of the whole organism still took place at intervals.

Fig. 28 (×80).—A blastozooite dedifferentiated in weak alcohol, stage 3. Note the cell-masses outside the main body of the organism.

Huxley, del.



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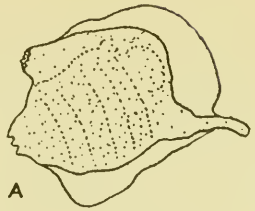

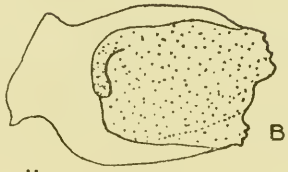

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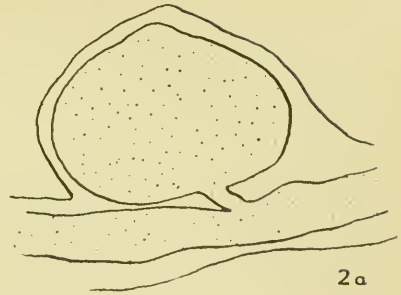

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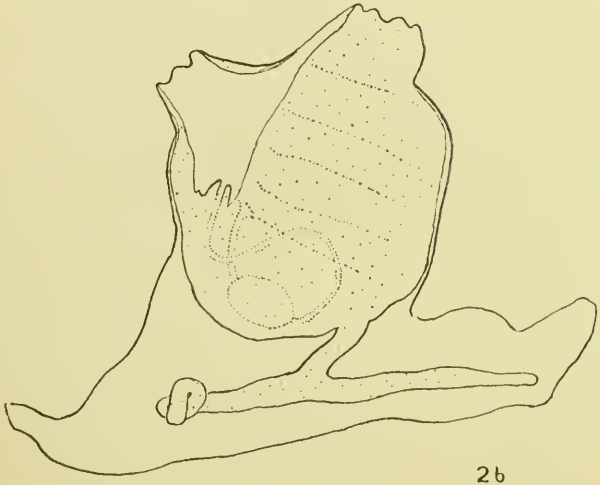

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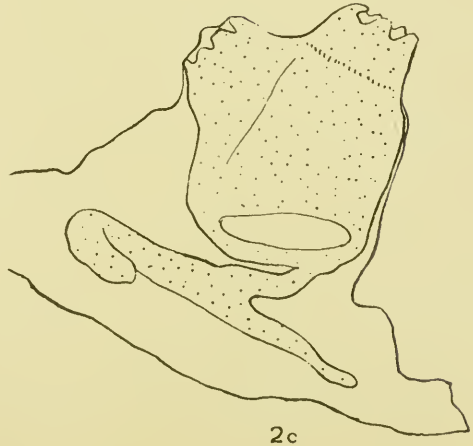

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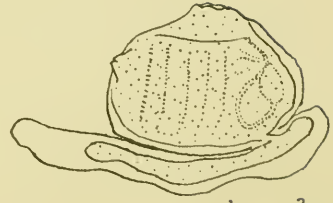

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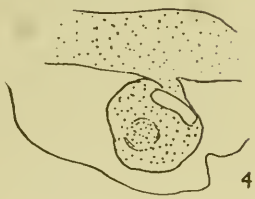

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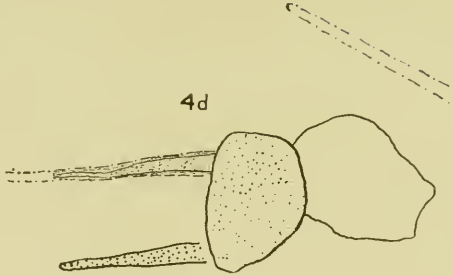

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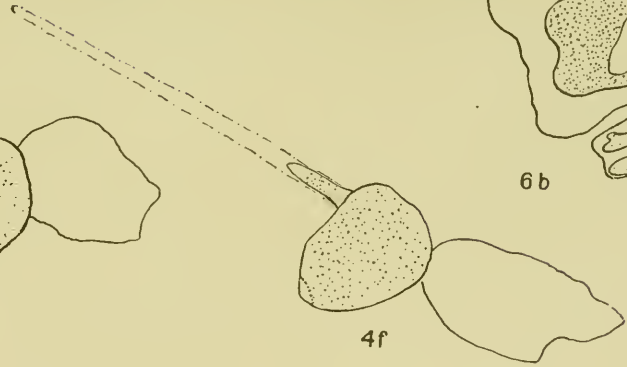

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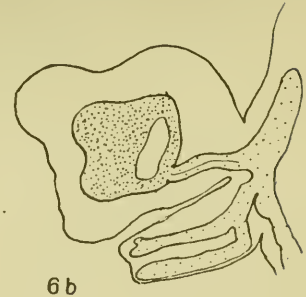

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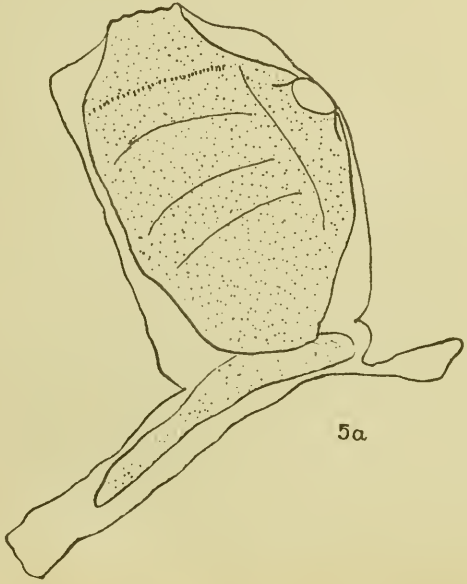

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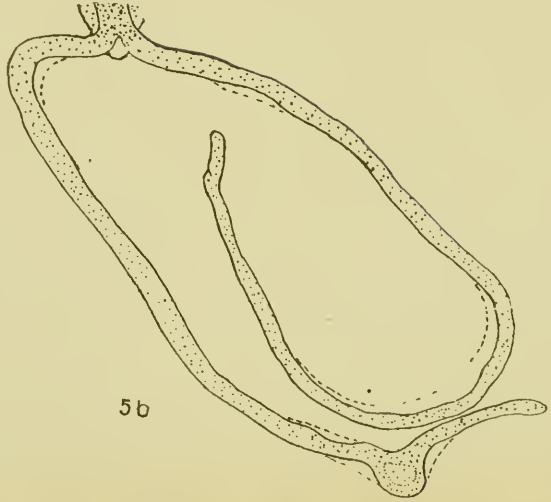

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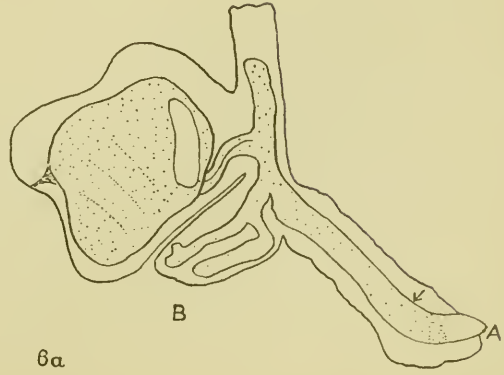

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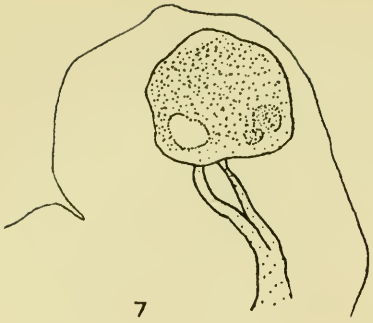

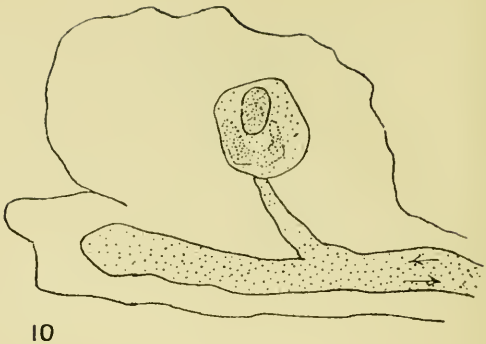

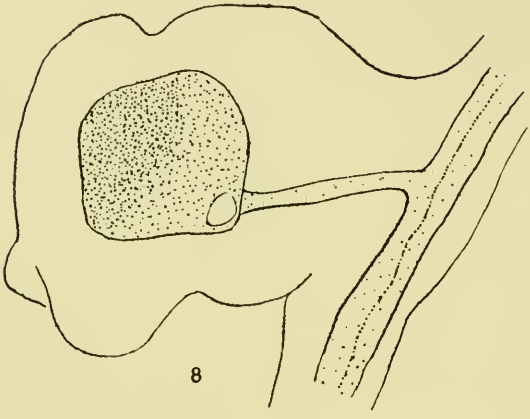

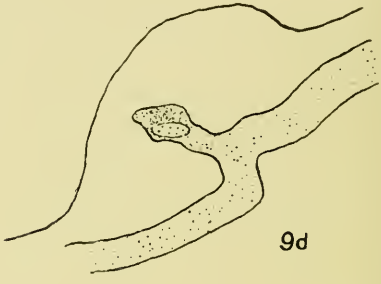

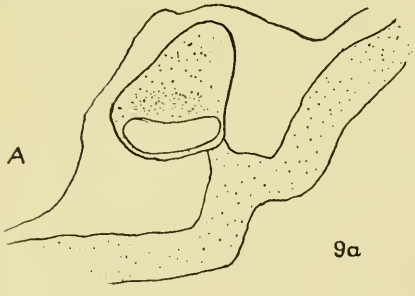

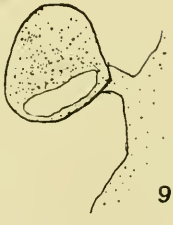

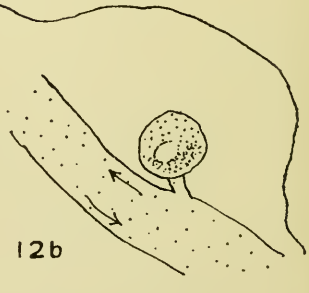

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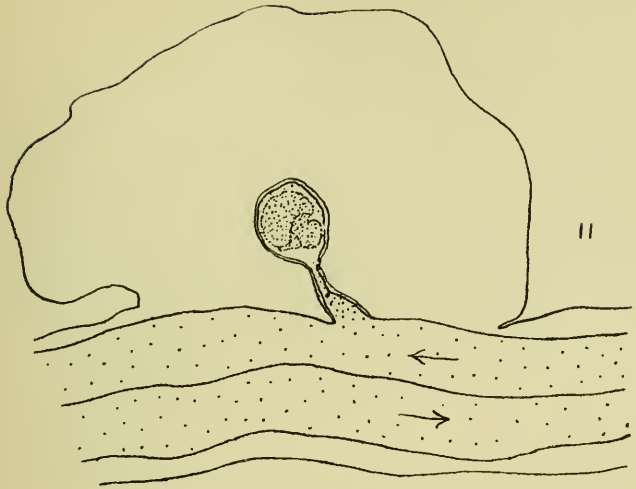
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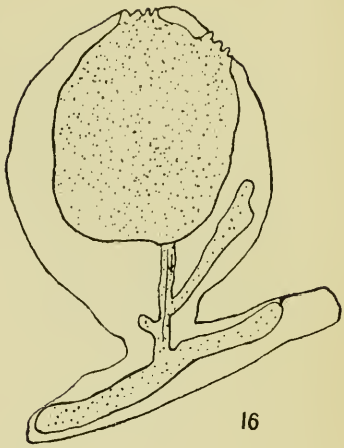
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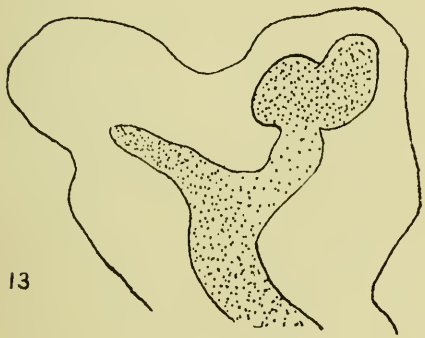
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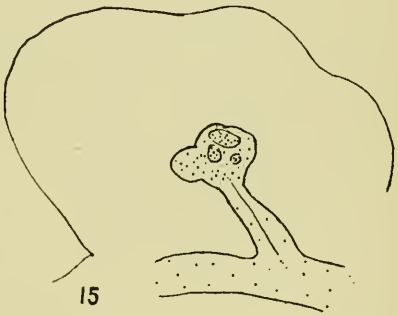
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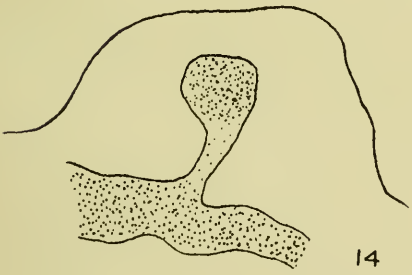
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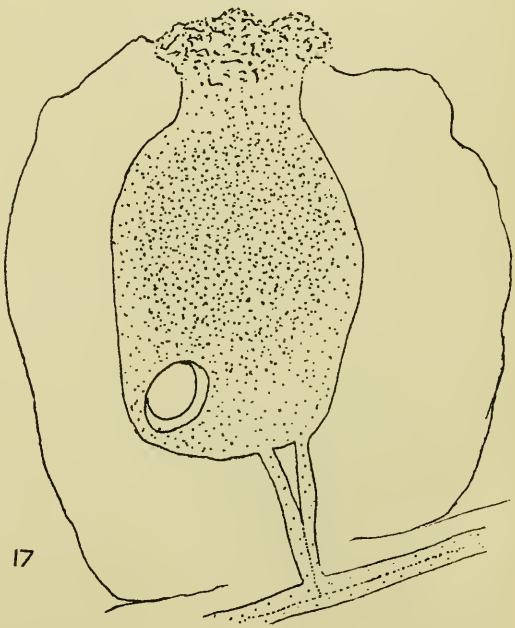
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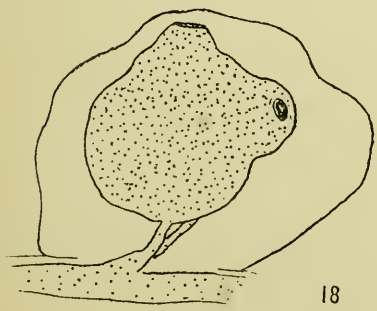
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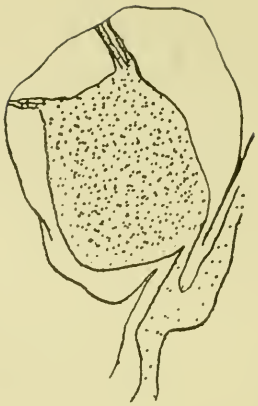

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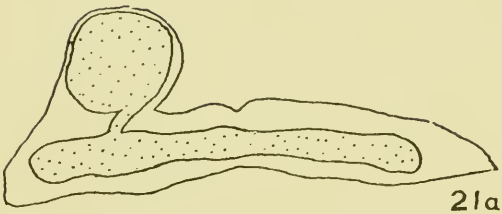

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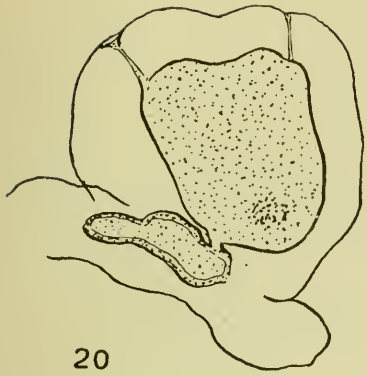

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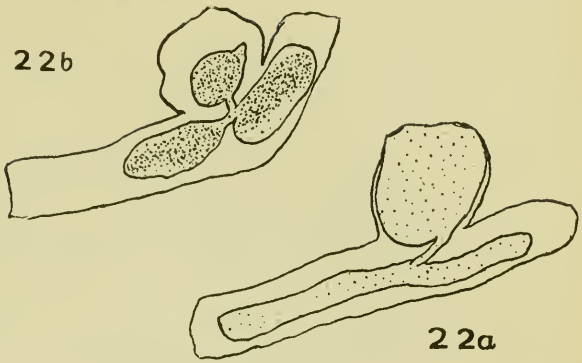

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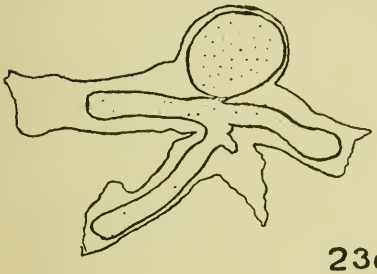

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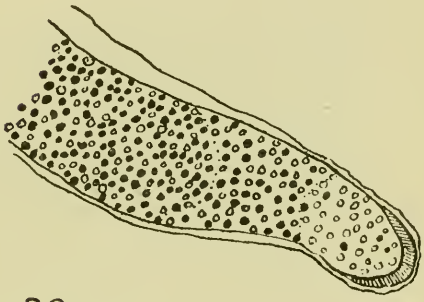

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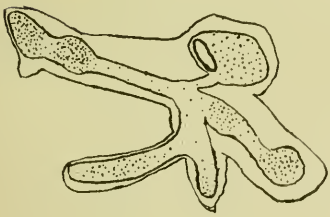

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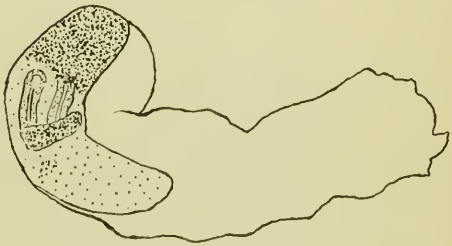

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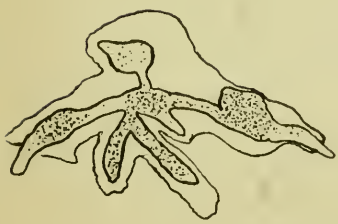

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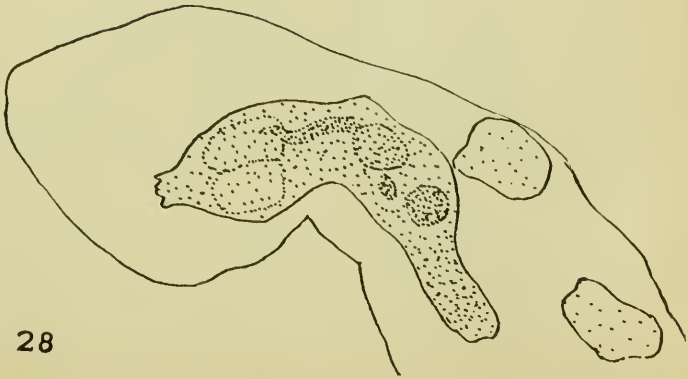

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Review

The Microtomist's Vade-Mecum, by Arthur Bolles Lee. 8th edition, edited by Professor J. Brontë Gatenby. London, J. A. Churchill, 1921. Price 28*s*. net.

This eighth edition of Mr. Bolles Lee's well-known *Microtomist's Vade-Mecum* has been edited and entirely revised by Professor J. Brontë Gatenby with the assistance of five collaborators.

Readers of this Journal will not be surprised to find that Professor Gatenby has himself written special sections dealing with chromatin, chromosomes, and cytoplasmic inclusions in which he gives us the full benefit of his thorough practical experience. Moreover, he has re-written the part on Mammalian Embryological methods. An innovation is the inclusion of two new methods for staining bacteria in tissues which will doubtless be very useful to biologists not versed in bacteriological technique. In the next edition we may hope to see mentioned the important ' Carmine Claudius ' method for differentiating yeasts, as well as Gram-positive bacteria in tissues.

Professor Gatenby is also personally responsible for a very valuable chapter on the cultivation of tissues ' in vitro '.

Zoologists will be grateful to Professor Bayliss for re-writing the chapter on Staining. Here we find an authoritative general account of the principles involved in the staining of living as well as dead cells. A careful reading of this lucid summary will save histologists from many pitfalls.

The chapters on Neurological Techniques have been to a great extent re-written by Dr. Da Fano. The additional directions given for the carrying out of the Bielschowsky and other complicated impregnation methods will be much appreciated.

For an important section embodying some of the new work in micro-chemistry on the lipoids and true fats and their differentiation we are indebted to Dr. W. Cramer, while Mr. J. T. Carter has revised the section on bone and teeth. In addition to the short account of the Protozoa given by

Mr. Bolles Lee in former editions, Dr. A. Drew includes many useful notes on the culture and staining of Amoeba, in which he has had much experience. The general arrangement of this section leaves something to be desired; for example, there are two paragraphs headed 'Flagellata' (1003 and 1033), and the fixation of Coccidia is considered in paragraph 1001, and again under Sporozoa in § 1031.

A new departure is the inclusion for beginners of a final chapter tabulating general procedure in the making of microscopical preparations.

It will be gathered that though the bulk of the volume remains little changed there has been considerable rearrangement of the contents. Some have been eliminated and much useful new matter has been added.

Professor Gatenby is to be congratulated on the success of his editorship. The appearance of this new edition of the familiar and indispensable vade-mecum will be heartily welcomed by all working zoologists.

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PRINTED IN ENGLAND
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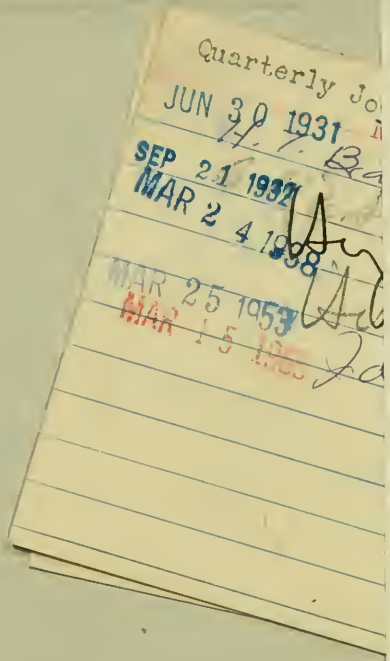
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