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EDITED BY

SIR RAY LANKESTER, K.C.B., M.A., D.Sc., LL.D., F.R.S.,

HONORARY FELLOW OF EXETER COLLEGE, OXFORD;
MEMBER OF THE INSTITUTE OF FRANCE (ASSOCIÉ ÉTRANGER DE L'ACADEMIE DES SCIENCES);
CORRESPONDENT OF THE IMPERIAL ACADEMY OF SCIENCES OF ST. PETERSBURG, AND OF THE
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LATE LINACRE PROFESSOR OF COMPARATIVE ANATOMY AND FELLOW OF MERTON COLLEGE, OXFORD;
EMERITUS PROFESSOR OF ZOOLOGY AND COMPARATIVE ANATOMY IN UNIVERSITY COLLEGE, UNIVERSITY OF LONDON.

WITH THE CO-OPERATION OF

ADAM SEDGWICK, M.A., F.R.S.,

FELLOW OF TRINITY COLLEGE, CAMBRIDGE, AND PROFESSOR OF ZOOLOGY IN THE IMPERIAL COLLEGE OF
SCIENCE AND TECHNOLOGY, LONDON;

SYDNEY J. HICKSON, M.A., F.R.S.,

BEYER PROFESSOR OF ZOOLOGY IN THE UNIVERSITY OF MANCHESTER;

E. A. MINCHIN, M.A., F.R.S.,

PROFESSOR OF PROTOZOOLOGY IN THE UNIVERSITY OF LONDON;

AND

GILBERT C. BOURNE, M.A., D.Sc., F.R.S.,

LINACRE PROFESSOR OF COMPARATIVE ANATOMY, AND FELLOW OF MERTON COLLEGE, OXFORD.

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**The Early Development of the Marsupialia, with
Special Reference to the Native Cat (*Dasyurus Viverrinus*).**

(Contributions to the Embryology of the Marsupialia, IV.)

By

J. P. Hill, D.Sc.,

Jodrell Professor of Zoology and Comparative Anatomy, University
of London, University College.

With Plates 1-9 and 2 Text-figs.

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INTRODUCTION.

“In mammalian embryology very many surprises are yet in store for us” (Hubrecht, '08).

THE present contribution contains an account of the principal results and conclusions at which I have arrived after a somewhat protracted and much interrupted study of an extensive collection of early developmental stages of Marsupials, ranging from the fertilised egg to the blastocyst in which the two primary germ layers are definitely established. I believe I am now able to give for the first time an account of early Marsupial ontogeny, based on the examination of an adequate material, and both consistent in itself and with what we know of the early development in the other two Mammalian sub-classes. The material at my disposal was obtained during my tenure of office in the University of Sydney, and with the aid of grants from the Royal Society and of a George Heriot Research Fellowship. It represents the proceeds of some eight years' collecting, and comprises a fairly complete series of stages of the native cat (*Dasyurus viverrinus*), together with a few early stages of other Marsupials, notably *Perameles* and *Macropus*.

Dasyurus proved in many ways a convenient subject for embryological purposes. It can readily be trapped in many districts in New South Wales; it lives and breeds fairly well in captivity, and though always somewhat intractable, it can, owing to its size, be easily handled, and so may be subjected

if necessary to daily examination.¹ But it has this great disadvantage, which it apparently shares with other Marsupials, that a very variable period intervenes between coitus and ovulation. As a consequence, the obtaining of any desired cleavage or early blastocyst stage is largely a matter of chance.² It is true that the changes which take place in the pouch, in correlation with ovulation and the events connected therewith, do afford in the case of late pregnant females some indication of the stage of development likely to be met with, but these changes are at first of too indefinite a character to be of much service beyond indicating that ovulation may have taken place.

Dasyurus breeds but once a year, the breeding season extending over the winter months—May to August. One remarkable feature in the reproduction of *Dasyurus*, to which I have directed attention in a previous paper (Hill, '00), may be again referred to here, and that is the fact that there is no correlation between the number of ova shed during ovulation and the accommodation available in the pouch. The normal number of teats present in the latter is six, though the presence of one or two supernumerary teats is not uncommon; the number of ova shed at one period is, as a rule, far in excess of the teat number. I have, for example, several records of the occurrence of from twenty to twenty-five eggs, two of twenty-eight, one of thirty, and one of as many as thirty-five! (twenty-three normal blastocysts and twelve

¹ *Perameles*, on the other hand, though quite common in many parts of the State, is by no means such a convenient type. It is much less easily trapped than *Dasyurus*, does not live nearly so well in captivity, and is particularly difficult to handle. I have to thank Mr. D. G. Stead, now of the Department of Fisheries, Sydney, for first directing my attention to the breeding habits of *Dasyurus*, and also for providing me with the first female from which I obtained segmenting eggs.

² For example, I obtained unsegmented ova from the uteri, four, five, six, seven and eight days after coitus, 2-celled eggs six and seven days after, 4-celled eggs eleven and eighteen days after. In one case the young were born eight days after the last observed act of coitus, in another sixteen days after, and in yet another twenty days after.

abnormal). There can be little doubt that *Dasyurus*, like various other Marsupials (e. g. *Perameles*, *Macropus*, etc.), has suffered a progressive reduction in the number of young reared, but even making due allowance for that, the excess in production of ova over requirements would still be remarkable enough. Whether this over-production is to be correlated in any way with the occurrence of abnormalities during early development or not, the fact remains that cleavage abnormalities are quite frequently met with in *Dasyurus*.

Technique.—As fixatives, I have employed for ovaries the fluids of Hermann, Flemming, Ohlmacher, and Zenker; for ova and early blastocysts, Hermann, Flemming, Perenyi, and especially picro-nitro-osmic acid (picro-nitric acid [Mayer] 96 c.c., 1 per cent. osmic acid 2 c.c., glac. acetic acid 2 c.c.); for later blastocysts, the last-named fluid especially, also picro-corrosive-acetic and corrosive-acetic.

To facilitate the handling of ova and early blastocysts during embedding, I found it convenient to attach each specimen separately to a small square of pig's foetal membrane by means of a dilute solution of photoxylin (1 to 2 per cent.). Orientation of the specimen was then easily effected during final embedding, under the low power of the microscope. The larger blastocysts were double-embedded in photoxylin and paraffin, the cavity of the blastocyst being tensely filled with the photoxylin solution by means of a hypodermic syringe fitted with a fine needle.

For the staining of sections, Heidenhain's iron-haematoxylin method proved the most satisfactory, and was almost exclusively employed. Entire portions of the blastocyst wall were stained either with Ehrlich's or Delafield's haematoxylin.

I am much indebted to Mr. L. Schaeffer, of the Anatomical Department of the University of Sydney, and to Mr. F. Pittock, of the Zoological Department, University College, for invaluable assistance in the preparation of the photomicrographs reproduced on Plates 1-5, and also to Mr. A. Cronin, of Sydney, and Miss M. Rhodes, for the drawings from their respective pencils reproduced on Plates 6 and 7.

To Miss V. Sheffield I am indebted for the original of fig. 63. To my friend Dr. F. P. Sandes, Sydney, I am indebted for kind help in the revision of certain parts of the manuscript.

CHAPTER I.—CRITICAL REVIEW OF PREVIOUS OBSERVATIONS ON
THE EARLY DEVELOPMENT OF THE MARSUPIALIA.

Apart from the very brief abstract of a short paper on the development of *Dasyurus*, which I read before Section D of the British Association in 1908 (included in Dr. Ashworth's Report, 'Nature,' vol. lxxviii), our knowledge of the processes of cleavage and germ-layer formation in the Marsupialia is based (1) on the well-known observations of the late Emil Selenka ('86) on the development of the Virginian opossum (*Didelphys marsupialis*), published in 1886 as Heft 4 of his classical 'Studien'; and (2) on those of W. H. Caldwell ('87) on the uterine ovum, and cleavage process in the native bear (*Phascolarctus cinereus*).

Selenka's account of the mode of origin of the germ-layers in *Didelphys* differs widely, as the sequel will show, from my description of the same in *Dasyurus*. Now *Didelphys* and *Dasyurus* are two marsupials, admittedly allied by the closest structural ties, and we should therefore not expect *à priori* that they would differ fundamentally in the details of their early ontogeny, however much they might diverge in respect of the details of their embryonal nutritional arrangements.

Furthermore, we might reasonably hope, in view of the generally admitted relationships of the Marsupialia, that a knowledge of their early development would aid us in the interpretation of that of Eutheria, or, at least, that their early developmental phenomena would be readily comparable with those of Eutheria. It cannot be said that Selenka's observations realise either of these expectations. "Which-ever view is taken of Selenka's description of the opossum," writes Assheton ('98, p. 254), "many obvious difficulties remain for the solution of which no satisfactory suggestion can as yet be offered."

As concerns my own observations, I venture to think it is possible to bring them into line with what we know of the early ontogeny in the other two mammalian sub-classes, and I have attempted to do so in the concluding chapter of this paper, with what success the reader can judge, whilst as regards the divergence between Selenka's results and my own, I am perfectly convinced that the explanation thereof is to be found in the fact that the whole of Selenka's early material was derived from but two pregnant females, and that much of it consequently consisted of eggs which had failed to develop normally. From the one female, killed 5 days after coition, he obtained one egg in the 2-celled stage, one with about twenty cells and nine unfertilised ova. From the second, killed 5 days 8 hours after coition, he obtained "ausser zwei tauben, 14 befruchtete Eier nämlich je ein Ei mit 4, 8, 42, 68 Zellen, eine junge und eine ältere Gastrula mit noch dicker Eiweisschicht und endlich acht auch gleichen Entwicklungsstufe stehende weit grössere Keimblasen, deren Wand noch grösstentheils einschichtig war" ('86, p. 112). Selenka recognised that the last-mentioned blastocyst "die normale Entwicklungsphase repräsentiren," since he found as a rule that all the embryos from one uterus were in the same developmental stage. Nevertheless he proceeded to describe the segmenting eggs and the two "gastrulæ" which lagged so far behind the blastocysts, as if they were perfectly normal developmental stages. He does, indeed, question whether or not the 42-celled stage is normal, but decides in the affirmative, "denn wenn ich von zwei Zweifelhaften Fällen absehe, so habe ich niemals Eier aus den ersten Tag aufgefunden, welche auf irgend welche Anomalie der Entwicklung hinwiesen." This, however, can hardly be accepted as a satisfactory reason for his conclusion, since apart from the other eggs of the same batch, he had but the two eggs from the other female for comparison, viz. the 2-celled egg (and even that is, in my view, not quite normal), and the 20-celled egg, which is stated to have suffered in preparation. With the exception of the two eggs just mentioned, all the crucial

early stages (ranging from the 4-celled stage to the completed blastocyst), on whose examination Selenka based his account of germ-layer formation in *Didelphys*, would thus appear to have been derived from a single female.¹ No wonder it is impossible to reconcile his description either with what we know of germ-layer formation in the Prototheria and Eutheria or with my account of the same in *Dasyurus*.

My own experience with the latter has shown me that no reliance whatever is to be placed on segmenting eggs or blastocysts which exhibit marked retardation in their stage of development as compared with others from the same uterus, and also that batches of eggs or blastocysts in which there is marked variation in the stage of development attained should likewise be rejected. Abnormalities in the process of cleavage and of blastocyst formation are by no means uncommon in *Dasyurus*, and during the earlier stages of my own work I spent much time and labour on the investigation of just such abnormal material as that on which Selenka, no doubt unwittingly, but I feel bound to add, with an utter disregard for caution, based his account of the early development of *Didelphys*.

I propose now, before passing to my own observations, to give a short critical account of Selenka's observations, my comments being enclosed in square brackets.

The uterine ovum of *Didelphys* is enclosed by (1) a relatively thin "granulosamembran," formed by the transformation of a layer of follicular cells [really the shell-membrane, first correctly interpreted by Caldwell ('87) and formed in the Fallopian tube]; (2) a laminated layer of albumen, semitransparent; (3) a zona radiata, not always recognisable [in my experience invariably distinct].

Cleavage begins in the uterus, is holoblastic, and at first equal. A 2-celled stage is figured (Taf. xvii, fig. 3) [not quite normal as regards the relations of the blastomeres], and also a 4-celled stage [normal in appearance except for the

¹ The collection of my own early material of *Dasyurus* has involved the slaughter of over seven dozen females.

enormous thickness of the albumen layer], in which the four equal-sized blastomeres are radially arranged round a cleavage cavity and are conical in form, their upper ends being more pointed, their lower ends thicker and richer in yolk-material. The nucleus of each is excentric, being situated nearer the upper pole. [This description is applicable word for word to the 4-celled stage of *Dasyurus*.]

An 8-celled stage (fig. 6) is next described, seven of the blastomeres being equal in size and one being smaller. They are arranged somewhat irregularly in two circles. [This stage I regard as abnormal both in respect of the arrangement of the blastomeres and the occurrence of irregularity amongst them.] Selenka (p. 119) thought it probable that the third cleavage planes cut the first two at right angles and divided each of the first four blastomeres into a smaller ectodermal cell and a larger more granular entodermal, but states that he was unable to establish this owing to the opacity of the albumen-layer. [My observations show that it is the fourth cleavage in *Dasyurus*, not the third, which is equatorial, unequal, and qualitative, and that even then the cells formed are not ectodermal and entodermal in significance. The albumen is normally never opaque.]

A 20-celled stage is mentioned, but not described, since it suffered in preparation. It is said to have a large entoderm cell in the cleavage cavity. [A statement of very doubtful value, since the blastomeres were admittedly pressed together and probably displaced by the shrunken egg-membranes.]

The next stage described is a spherical "gastrula" (Taf. xvii, figs. 7, 8), composed of forty-two cells with an open "blastopore" at the vegetative pole, a smaller opening at the animal pole, and a large "ur-entoderm" cell in the cleavage-cavity, just inside the "blastopore." The wall of the "gastrula" consists of cells graduated in size; those in the region of the blastopore are the largest and richest in deutoplasm, those at the opposite pole are the smallest and most transparent. [This is a very characteristic stage in the formation of the blastocyst, with which I am quite familiar in *Dasyurus*. Selenka's speci-

men, judging from *Dasyurus*, is normal as regards the constitution of its wall and the occurrence of an opening at each pole. The lower opening, however, has no blastoporic significance, but, like the upper, owes its presence to the mode of formation of the blastocyst-wall by the spreading of the blastomeres towards the poles of the sphere formed by the egg-envelopes. Selenka's blastopore simply marks the last point of closure. This specimen I hold to be abnormal from the presence of the so-called "urentoderm" cell in its interior. I figure (Pl. 3, fig. 37) a section of a fairly comparable and undoubtedly abnormal blastocyst of *Dasyurus* in which there is also present in the blastocyst cavity a large free cell. Here this latter is unquestionably a blastomere of the lower hemisphere, which, having failed to divide, has become enclosed by the spreading of its neighbours. Selenka's "urentodermzelle" I regard as a similarly displaced blastomere.]

A 68-celled "gastrula" (figs. 9 and 10) is next described. It is essentially similar to the preceding, only the "blastopore" has closed.

The succeeding stage (fig. 11) is a somewhat older "gastrula," in which gastrulation is said to be still in progress, since over the lower pole, in the region of the now closed blastopore, it is no longer possible to say which cells belong to the ectoderm, which to the entoderm. The latter layer is described as being several cells thick in the blastoporic region, and as in course of spreading round inside the ectodermal wall of the "gastrula" towards the upper or animal pole. [This specimen is undoubtedly abnormal, at all events there is no comparable stage in *Dasyurus*. It is difficult to obtain a clear idea of Selenka's conception of the mode of origin of the germ-layers, but he evidently held (cf. pp. 116 and 119) that the large yolk-rich cells of the lower ("blastoporic") pole constitute the anlage of the entoderm, and that they become inturned at the "blastopore" and proliferate to form the definitive entoderm, which then gradually extends round to the animal pole, in contact with the inner surface of the wall of the gastrula, that wall forming the ectoderm. He appa-

rently did not regard the "urentodermzelle" as the sole progenitor of the entoderm, but simply as an entoderm-cell precociously inturned from the "blastoporic" margin.

This view of Selenka, however, lands us in the predicament of having to regard the embryonal area as differentiating over the vegetative hemisphere, since in the next stage the "blastopore" is described as being situated excentrically in that area. Either Selenka's determination of the poles in the 42-celled blastocyst is wrong, or the entoderm does not originate as he describes it. My own observations force me to accept the latter alternative. In his paper Selenka gets over the difficulty very easily by altering the orientation of his figures. On Taf. xvii, the figures of sections of blastocysts are so placed that the "blastopore" is below, next the bottom of the plate. These figures I hold to be correctly orientated. On Taf. xviii, the figures are inverted, so that the "blastopore" is above; as the result the animal pole of fig. 11, Taf. xvii, becomes the vegetative pole of the stage next described (fig. 2, Taf. xviii).]

The stage just referred to, described as an "eiförmige gastrula," is represented in a drawing made from the fresh specimen as lying quite free in a large perivitelline space enclosed by a very thick layer of albumen, outside which is the "granulosa-membran." In section (fig. 2) a mass of entoderm is seen to reach the surface at one pole (marked *bl.*) uppermost in the figure, whilst other entodermal cells are shown spreading from this towards the lower pole. The ectoderm of the wall is represented as composed of definitely cubical cells. [The presence of a large perivitelline space, by itself stamps this specimen as not normal. The sectional figure must be schematic.]

The last of Selenka's early stages to which reference need be made here is formed by eight "gastrulæ" (blastocysts), reckoned as ten hours after the commencement of cleavage [a reckoning I consider of no value] (Taf. xviii, figs. 3 and 4). The embryonal area is now distinguishable by the larger size of its ectodermal cells. The entoderm is unilaminar, and has

extended beyond the limits of the embryonal area. The position of the "blastopore" is said to be marked in all by a mass of coagulum attached to the wall, and in three by a definite opening as well. It is situated excentrically in the embryonal area. [Except for the "blastopore" and the presence of a thick layer of albumen, this blastocyst stage is quite comparable with the corresponding one in *Dasyurus*; the latter, however, is considerably larger. Of Selenka's early material, I think it is these blastocysts alone which had any chance of giving origin to normal embryos.]

W. H. Caldwell, who, as Balfour student, visited Australia in 1883-4, obtained a very rich collection of early marsupial material, of which, unfortunately, no adequate account has ever been published. He gave, however, in his introductory paper on the 'Embryology of the Monotremata and Marsupialia' ('87), an account of the structure of the ovum, both ovarian and uterine, in *Phascolarctus*, and he showed that the ovum during its passage down the Fallopian tube becomes enclosed outside the albumen layer in "a thin transparent membrane, .0015 mm. thick," which he homologised with the shell-membrane of the monotreme egg. This important discovery of the existence of a shell-membrane in the Marsupialia I can fully confirm. I am, however, unable to accept his interpretation of the internal structure of the ovum of *Phascolarctus*, or his remarkable statement that cleavage in that form is of the meroblastic type. Cleavage is not described in detail, nor is any account given of the mode of origin of the germ-layers.

CHAPTER II.—THE OVUM OF *DASYURUS*.

1. Structure of the Ovarian Ovum.

The full-grown ovarian ovum of *Dasyurus* (Pl. 1, fig. 1) appears as a rounded, or more usually, ovalish cell, the diameter of which varies in section in ten eggs measured from $\cdot 28 \times \cdot 126$ mm. to $\cdot 27 \times \cdot 26$ mm. (average, $\cdot 24$ mm.), and is therefore large relatively to the ova of Eutheria. It

is enclosed by a thin, but very definite refractive membrane or zona (vitelline membrane of Caldwell) of an approximate thickness of $\cdot 002$ mm. (fig. 1, *z.p.*), on which the cells of the discus proligerus (fig. 1, *d.p.*) directly abut, a differentiated corona radiata and syncytial layer being absent. It appears to be identical in its relations and optical characters with the membrane investing the monotreme ovum, and never shows in section any trace of radial striations (though I believe I have detected an extremely faint appearance of such in the fresh zona), or of the extension into it of protoplasmic processes from the adjacent cells of the discus proligerus, such as Caldwell figures in the case of the ovum of *Phascolarctus* (cf. his Pl. 29, fig. 5). Within the zona the peripheral cytoplasm of the ovum is differentiated to form an exceedingly thin but distinct bounding layer or egg-membrane (vitelline membrane, *sensu stricto*).

The cytoplasmic body of the ovum exhibits a very obvious and striking differentiation into two regions in correspondence with the presence in it of two definitely localised varieties of deutoplasmic material, respectively granular and fluid. Peripherally it consists of a relatively narrow cytoplasmic zone of practically uniform width, dense and finely granular in appearance owing to the presence in it of numerous particles of deutoplasmic nature. This we may distinguish as the formative zone (fig. 1, *f.z.*). In it lies embedded the large vesicular nucleus (about $\cdot 06 \times \cdot 03$ mm. in diam.). Centrally and forming the main bulk of the ovum is a mass of greatly vacuolated cytoplasm presenting the appearance of a clear wide-meshed reticulum. Its framework is coarser peripherally where it passes over without definite limit into the formative zone, with which it is structurally identical, but much finer and wider-meshed centrally, so fine, indeed, that it almost invariably breaks down under the action of fixatives, and appears in sections as an irregular space, perhaps crossed by a few fine interlacing strands (fig. 1, *d.z.*). The meshes of this reticulum are occupied by a clear fluid which must be held to constitute the central deutoplasm of the egg. We

may accordingly designate this central reticular area as the deutoplasmic zone.

If we pass now from the full-grown to the ripe ovarian ovum (Pl. 1, figs. 2 and 3), i. e. an ovum in which either the first polar spindle has appeared or the first polar body has already been separated off, it at once becomes evident that important changes have occurred in the disposition and relative proportions of the two constituent regions of the egg-cytoplasm. The full-grown ovum is of the centrolecithal type, the central deutoplasmic zone forming its main bulk and being completely surrounded by the thin formative zone. The ripe ovum, on the other hand, exhibits an obvious and unmistakable polarity, and is of the telolecithal type, as the following facts show. The cytoplasmic body evidently consists of the same two regions as form that of the full-grown ovum, but here the dense formative region now forms its main bulk, and no longer surrounds the clear deutoplasmic region as a uniform peripheral layer. It has not only increased considerably in amount as compared with that of the full-grown egg, and at the expense apparently of the more peripheral coarser portion of the deutoplasmic zone, but it has undergone polar segregation, with the result that it now occupies rather more than one hemisphere of the egg as a dense finely granular mass, with vacuoles of varying size sparsely scattered through it (figs. 2 and 3, *f.z.*). It accordingly defines one of the ovular poles. The opposite pole is just as markedly characterised by the presence immediately below it of a more or less rounded clear mass, eccentrically situated, and composed of an extremely fine cytoplasmic reticulum with wide fluid-filled meshes. It is completely surrounded by formative cytoplasm (though over the polar region the enclosing layer is so extremely thin that it here almost reaches the surface), and its cytoplasmic framework is perfectly continuous with the same, the line of junction of the two being abrupt and well defined. So delicate, however, is this framework that it breaks down more or less completely under the action of fixatives of such

excellence even as the fluids of Flemming and Hermann, and thus in sections usually all that represent it are a few irregular cytoplasmic strands crossing a large, sharply defined clear space (figs. 2 and 3, *d.z.*). The mass in question has thus all the characters of the deutoplasmic zone of the full-grown ovum, and it must undoubtedly be held to represent the central portion of that which has not been utilised in the upbuilding of the formative cytoplasm, and which has been forced to take up an excentric position immediately below the polar region of one hemisphere, owing to the increase of the formative cytoplasm and its segregation in the other hemisphere.

The ripe ovum of *Dasyurus* thus possesses a polarity which in its way is equally as striking as that of the Monotreme egg. Towards the one pole the main mass of the ovum is composed of dense, slightly vacuolated formative cytoplasm, in which the polar spindle is situated peripherally, but nearer the equator than the formative pole. Toward the opposite pole and practically reaching the surface is a rounded mass of greatly vacuolated deutoplasmic cytoplasm. Roughly, the formative cytoplasm constitutes about two-thirds of the bulk of the ripe egg, the deutoplasmic the remaining third. Such being the structure of the ripe ovarian egg, if we classify it at all, we must place it, it seems to me, with eggs of the telolecithal type. My view of the significance of this marked polar differentiation of the constituent materials of the ripe ovum of *Dasyurus* I shall presently indicate. Meantime I would lay special emphasis on the fact that the eccentric mass of deutoplasmic cytoplasm represents material, surplus deutoplasmic material which has not been utilised in the upbuilding of the formative cytoplasm.

The fact of the occurrence in the Eutherian ovum of a polar differentiation of its constituent materials is now definitely established, thanks especially to the valuable researches of Prof. O. Van der Stricht and his pupils—H. Lams and the late J. Doorme. In this connection I wish to refer here in some detail to the extremely interesting obser-

vations of Van der Stricht [’03, ’05] on the structure and polarity of the ovum of the bat (*Vesperugo noctula*), since these observations are in essential agreement with my own on the ovum of *Dasyurus*, and enable me to affirm that the polar differentiation herein recorded for the first time for the Marsupial ovum is attained as the result of vitellogenic processes, which essentially correspond with those of the ovum of the bat. Van der Stricht, as is well known, has made a special study of the process of vitellogenesis in the Eutherian ovum, and is, indeed, at the present time the foremost authority on this particular subject, so that his views are worthy of all respect.

Study of the oöcyte of *Vesperugo* during the period of growth shows, according to Van der Stricht, that “a un moment donné du développement du jeune œuf, les boyaux et amas vitellogènes [derived, according to him, from ‘une couche vitellogène, mitochondriale,’ present in the young oöcyte in the first stage of growth] disparaissent au profit du vitellus, dont la structure pseudo-alvéolaire s’accroît graduellement.” The full-grown oöcyte at the stage just prior to the appearance of the first polar spindle is characterised by the presence of this “pseudo-alveolar structure” throughout the extent of its cytoplasmic body. The alveoli or vacuoles are of variable size, are filled by a clear liquid, and “correspondent incontestablement au deutoplasma de l’œuf. A ce stade du développement de l’oöcyte, ce vitellus nutritif, auquel s’ajoutent bientôt des granulations graisseuses, est répandu uniformément dans toutes les profondeurs du cytoplasme. Nulle part on ne constate une zone deutoplasmique distincte d’une zone de vitellus plastique.” In *Dasyurus* the stage in vitellogenesis which almost exactly corresponds with that of the full-grown oöcyte of *Vesperugo* just described is seen in oöcytes not quite full-grown. In fig. 4 is shown an oöcyte of *Dasyurus* ($\cdot 26 \times \cdot 20$ mm. in diameter), in which the same pseudo-alveolar structure as described by Van der Stricht for the *Vesperugo* oöcyte is perfectly distinct. Here, however, fatty particles are not

apparent, and the peripheral portion of the cytoplasm tends to be free from vacuoles. In *Dasyurus* the formation of these deutoplasmic vacuoles begins in oöcytes about .2 mm. or less in diameter. This characteristic "pseudo-alveolar" stage is followed in both *Vesperugo* and *Dasyurus* by one in which there is recognisable in the cytoplasmic body of the ovum a differentiation into a dense peripheral zone and a central vacuolated area. In *Vesperugo* this stage is attained about the time of appearance of the first polar spindle, whilst in *Dasyurus* it is attained somewhat earlier, always prior to the formation of the latter. So close is the agreement between the two forms that Van der Stricht's description of the bat's egg at the time of appearance of the first polar spindle might equally well be applied to the full-grown ovum of *Dasyurus*. He writes ['03, p. 43] : "Vers l'époque de l'apparition du premier fuseau de maturation, le vitellus prend un autre aspect. La partie centrale deutoplasmique conserve une structure pseudo-alvéolaire, mais dans le voisinage immédiat du premier fuseau et dans toute l'étendue de la couche périphérique du protoplasme, apparaît une mince zone de vitellus compact et dense, plus ou moins homogène où les vésicules claires font défaut. . . . A ce moment, on distingue dans l'oöcyte de *V. noctula* une zone centrale très étendue, riche en deutoplasme et une zone corticale très mince, riche en vitellus plastique." This centrolecithal phase, as we may term it, is followed in *Vesperugo* during fertilisation and the separation of the second polar body by a telolecithal phase characterised by a distinct polarity. "La zone de vitellus plastique s'épaissit encore, mais surtout à un pôle de l'œuf, à celui opposé au pôle où se détachent les deux globules polaires. Ce pôle, où s'accumule graduellement le vitellus formateur, mérite le nom de pôle animal. Il est opposé au pôle d'expulsion des globules polaires, vers lequel est refoulé le deutoplasme, et qui se comporte désormais comme le pôle végétatif. Pendant que les deux pronucléus mâle et femelle se forment, le vitellus plastique augmente graduellement en abondance au pôle

animal, tandis qu'il diminue au pôle végétatif, et le deutoplasme, parsemé d'un plus grand nombre de boules graisseuses, constitue une masse sphérique excentrique, voisine des deux globules polaires" (Van der Stricht, '03, pp. 44-45). It is evident, then, that the fertilised ovum of *Vesperugo* exhibits a polarity comparable with that of the ripe ovarian ovum of *Dasyurus*, and that the vitellogenetic processes in the ova of these two widely separated forms proceed along lines almost identical, at all events so far as their broad outlines are concerned. In both we find during growth a progressive vacuolisation of the egg-cytoplasm consequent on the elaboration of a deutoplasmic fluid. In both, the "pseudo-alveolar" condition so engendered is followed by one in which there is recognisable a differentiation into a peripheral "formative" zone rich in deutoplasmic granules, and a central "deutoplasmic" zone rich in fluid yolk, and finally in both there occurs a segregation of the granular "formative" and fluid yolk-constituents to opposite regions of the egg, with resulting attainment of a definite polarity. In view of the close general agreement in the vitellogenetic processes, and in the constitution of the ova in *Vesperugo* and *Dasyurus*, it might be expected that the poles would accurately correspond, but such is not the case if Van der Stricht's determination of the poles in the ovum of *Vesperugo* is correct. In the latter, according to Van der Stricht, the deutoplasm is located at that pole from which the polar bodies are given off; at the opposite pole the "plastic" vitellus accumulates, and close to it the two pronuclei unite and the first cleavage spindle is formed. Accordingly Van der Stricht concludes that "le premier pôle correspond au pôle végétatif, le second au pôle animal des œufs à deutoplasme polaire (O. Hertwig)." In *Dasyurus*, on the other hand, I am perfectly convinced (and adequate reason for my conviction will be forthcoming in the course of my description of the processes of cleavage and germ-layer formation) that the pole of the ripe ovum in relation to the mass of deutoplasmic cytoplasm is not the vegetative pole, but represents morphologically the upper or

animal pole of the egg, the opposite pole in relation to which the formative cytoplasm is situated being the lower or vegetative. The deutoplasmic cytoplasm thus lies in the upper hemisphere, whilst the formative cytoplasm occupies the lower. If Van der Stricht's determination of the poles of the ovum of *Vesperugo* be accepted, then we must conclude that the poles of the *Dasyurus* ovum are exactly reversed as compared with those of the bat's egg. In this connection it may be recalled that Lams and Doorme ['07] have demonstrated the occurrence in *Cavia* of an actual reversal of the original polarity of the ovum, prior to the beginning of cleavage. These facts may well give us pause before we proceed to attach other than a purely secondary significance to the exact location of the formative and deutoplasmic constituents in the Metatherian and Eutherian ovum. But besides this apparent difference in the location of the deutoplasmic constituents of the ova of *Dasyurus* and *Vesperugo*, there exists yet another which concerns the fate of these constituents in the respective eggs. In *Vesperugo*, Van der Stricht shows that the "deutoplasm" remains an integral part of the egg, and retains its polar distribution in the blastomeres up to at least the 4-celled stage.¹ In *Dasyurus*, on the other hand, the fate of the deutoplasmic mass is a very different, and, indeed, a very remarkable one. It does not remain an integral part of the segmenting egg as in *Vesperugo*, but prior to the completion of the first cleavage furrow it becomes bodily separated off, apparently by a process of abstriction, from the formative cytoplasm as a clear rounded mass which takes no further direct part in the developmental processes. As soon as its elimination is effected, the remainder of the cytoplasmic body of the ovum, formed of the formative cytoplasm alone, divides into the first two equal-sized blastomeres, the first cleavage plane being coincident with the polar diameter and at right angles to the plane of separation of the deutoplasmic mass, or "yolk-body" as we may term it (Pl. 2, figs. 14-16, 19, *y.b.*), so that it is this formative zone of the

¹ Vide, however, "Addendum" (p. 121).

ovum which is alone concerned in the production of the embryo and its foetal membranes.

We have but to recall the conclusion already reached that the clear vacuolated zone at the upper pole of the ripe ovum of *Dasyurus* consists of surplus material, mainly in the form of fluid of deutoplasmic nature which has not been utilised in the upbuilding of the formative cytoplasm, and the significance of this remarkable and, so far as the Mammalian ovum is concerned, absolutely unique occurrence becomes at once manifest.¹ We have to do here with an actual elimination of surplus deutoplasmic material by the Marsupial ovum—a phenomenon only paralleled elsewhere, so far as I am aware, and even then but distantly, by the curious temporary separation of the so-called yolk-lobe which occurs during the cleavage of the yolk-laden eggs of certain Molluscs (*Nassa*, *Ilyanassa*, *Modiolaria*, *Aplysia*, *Dentalium*) and Annelids (*Myzostoma*, *Chætopterus*). In these forms cleavage of the ovum into the first two blastomeres is accompanied by the separation of a portion of the ovular substance in the form of a non-nucleated mass or so-called yolk-lobe. This latter, which has been shown to be connected with the formation of determined organanlagen, reunites with one of the two blastomeres, and then the same process of abstriction and reunion recurs at the second cleavage.² We have here evidently a purely adaptive phenomenon, the object of which no doubt is to permit of the total cleavage of the yolk-laden ovum on what are presumably the old ancestral lines, and I believe a comparable explanation will be found applicable to the elimination of surplus yolk-material by the Marsupial ovum.

As regards the significance of the occurrence of the deutoplasmic zone in the ovum of *Dasyurus*, holding the views that I do as to the phylogeny of the Marsupialia (viz. that the Metatheria and Eutheria are the divergent branches of a

¹ Vide "Addendum" (p. 121), in which reference is made to the discovery by Prof. Van der Stricht of the elimination of deutoplasm in the ovum of *Vesperugo*.

² Cf. Korschelt u. Heider, 'Lehrbuch d. vergl. Entwicklungsgeschichte,' Lief. 3, p. 107, 1909.

common stock, itself of Prototherian derivation), and bearing in mind the occurrence of an undoubted representative of the shell round the Marsupial ovum, I venture to see in the fluid-material of the deutoplasmic zone the partial and vestigial equivalent of the yolk-mass of the monotreme egg. In other words, I would regard the deutoplasmic fluid as the product of an abortive attempt at the formation of such a solid yolk-mass. The objection will no doubt be forthcoming that this interpretation cannot possibly be correct since the supposed equivalent of the yolk-mass in the *Dasyure* ovum is located, on my own showing, at the wrong pole—at the upper instead of at the lower. But its precise location does not seem to me to be a matter to which we need attach any great importance, since it has doubtless been adaptively determined in correlation with the special character of the cleavage process.

The belief that the minute yolk-poor ovum of the Eutheria is no pure primarily holoblastic one, but that it has only secondarily arrived at the total type of cleavage as the result of the all but complete loss of the yolk ancestrally present in it, consequent on the substitution of the intra-uterine mode of development for the old oviparous habit, is now widely held amongst Mammalian embryologists. Hubrecht, however, is an exception, wedded as he is to a belief in the direct derivation of the Eutheria from Protetrapodous ancestors with yolk-poor, holoblastic eggs. Whether the interpretation I have put forward, viz. that the non-formative or deutoplasmic zone of the *Dasyure* ovum is the reduced and partial equivalent of the yolk-mass of the Monotreme egg, be accepted or not, I venture to think that my discovery of an actual elimination of deutoplasmic material by the Marsupial ovum affords a striking confirmation of the truth of the prevailing conception as to the phylogeny of the Eutherian ovum, and I further venture to think that the facts I have brought forward in the preceding pages justify us in regarding the ripe ovarian ovum of *Dasyurus* as being potentially of the yolk-laden, telolecithal type, and the uterine ovum, by bodily casting out the superfluous part of its deutoplasm, as becoming at the same time

secondarily homolecithal and secondarily holoblastic. The Marsupial ovum presents itself to my mind as the victim of tendencies conditioned by its ancestry, and in particular it appears as if its inherited tendency to elaborate yolk had not yet been brought into accurate correlation with the other changes (reduction in size, intra-uterine development), which it has undergone in the course of phylogeny. As the consequence it manufactures more yolk than it can utilise, and so finds itself under the necessity of getting rid of the surplus. Whether or not a comparable elimination of deutoplasmic material occurs in the ova of other Marsupials, future investigation must decide. I should be quite prepared to find variation in this regard, correlated perhaps with the size of the egg. In the case of *Phascolarctus*, Caldwell gives the diameter of the ovum as $\cdot 17$ mm., and his figure of a (horizontal?) section of the uterine ovum (here produced as text-fig. 1, p. 27) shows a differentiation of the cytoplasmic body of that into vacuolated and granular zones quite comparable with that of the *Dasyurus* ovum. From the few measurements of ova of other marsupials that I have been able to make, it would appear that the ovum of *Trichosurus* approximates in size to that of *Dasyurus*, whilst that of *Perameles* and probably also that of *Macropus* are smaller. From Selenka's figure I have calculated that the ovum of *Didelphys* measures about $\cdot 13 \times \cdot 12$ mm. in diameter. In the smaller ova it is quite likely that yolk-formation may not proceed so far as in the relatively large ovum of *Dasyurus*.

2. Maturation and Ovulation.

The details of the maturation process have not been fully worked out, owing to lack of material. As in the Eutheria (Sobotta, Van der Stricht, Lams and Doorme, and others), the first polar body is separated off in the ovary, the second apparently in the upper part of the Fallopian tube where entrance of the sperm takes place. The first polar figure (late anaphase observed, fig. 5) lies in the formative cyto-

plasm, close below and at right angles to the zona. Its exact site is subject to some slight variation, and is best described as adjacent to the equatorial region of the egg, sometimes nearer the lower pole, more usually, perhaps, nearer the upper. Centrosomes and polar radiations were not observed. The heterotypical chromosomes (gemini) have the form of somewhat irregular, more or less angular granules. I have not been able to determine their number. The figure is barrel-shaped, and almost as broad as long, measuring $\cdot 015 \times \cdot 013$ mm. The first polar body (fig. 6, *p.b.*¹) is small relatively to the size of the egg, its diameters varying round $\cdot 03 \times \cdot 01$ mm., and its shape is that of a flattened bi-convex disc. In uterine eggs there is some evidence pointing to the probability of its having undergone division.

The second polar spindle (figs. 3 and 7) lies immediately subjacent to the first polar body in the fully ripe ovarian ovum. It is shorter than the first, measuring $\cdot 013$ mm., and much narrower. The second polar body measures about $\cdot 015 \times \cdot 01$ mm. in diameter, and is thus smaller than the first. I have only seen the second polar body in uterine ova, and therefore can only presume that it is separated off in the upper part of the Fallopian tube, subsequently to the penetration of the sperm, as in Eutheria.

Ovulation takes place irrespective of whether copulation has occurred or not, and it is a fact worthy of record that, even if the ova be not fertilised, the pouch and mammary glands undergo the same series of growth changes as are characteristic of, at all events, the earlier stages of normal pregnancy.

The follicular cells of the discus proligerus investing the ovum are already in the ripe follicle in a state of disruption, and I believe they separate completely from the ovum at the moment of dehiscence, so that, except for the zona, the ova are quite naked when they enter the tube. I have no evidence of the existence outside the zona of a layer of proalbumen such as Caldwell describes round the ovum of *Phascolaretus*. Apparently the ova are shed almost simultaneously, and they

must pass with considerable rapidity down the tubes to the uteri where cleavage begins, for I have only once found a tubal ovum, and that one had evidently been retarded for some reason, and was polyspermic.

3. The Secondary Egg-membranes: Albumen and Shell-membrane.

During the passage of the ovum down the tube it is fertilised, and becomes enclosed externally to the zona by two secondary layers formed as secretions by the cells of the oviducal lining. First of all, the ovum becomes surrounded by a transparent to semi-transparent laminated layer of albumen, .015 to .022 mm. in thickness, composed of numerous very delicate concentric lamellæ, and having, normally, numbers of sperms imbedded in it (figs. 8-11, *alb., sp.*). Then outside the albumen layer there is laid down a definite, but at first very thin, double-contoured membrane (figs. 8 and 10, *s.m.*), which, following Caldwell, I have no hesitation in homologising with the shell-membrane of the Monotreme egg. Caldwell in 1887 described and figured a definite membrane enclosing the uterine ovum of *Phascolarctus*, externally to, and quite distinct from the albumen, which he interpreted as the representative of the shell-membrane of the Monotremata, but owing apparently to the fact that Selenka altogether failed to recognise its true nature in *Didelphys*, since he regarded it as a derivative of the follicular epithelium, and termed it the "granulosa-membran," this highly significant discovery of Caldwell has been largely ignored. Such a membrane is constantly present and easily recognisable in all the Marsupials (*Dasyurus*, *Perameles*, *Trichosurus*, *Macropus*, *Petrogale*, *Phascologale*, *Acrobates*, *Phascolarctus*, *Bettongia*), of which I have had the opportunity of studying early developmental stages. It is laid down in the Fallopian tube, is perfectly distinct from the albumen, and increases in thickness in the uterus, and if it has not the significance which Caldwell has suggested, then I must leave it to those

who decline to accept Caldwell's interpretation to put forward an alternative one, since I am unable to do so.

The shell-membrane of *Dasyurus* (Pl. 1, figs. 8-11; Pl. 2, figs. 17, 18, *s.m.*) is a transparent, perfectly homogeneous layer, highly refractive in character and of a faint yellowish tint. When fully formed it possesses firm, resistant properties, recalling those of chitin, and is doubtless composed of a keratin base. It is distinguishable at once from the albumen by its optical characters and staining reactions, so that there is not the slightest justification for the supposition that it may represent simply the specially differentiated outermost portion of that layer. In ova which have just passed into the uterus (fig. 10) the shell-membrane is extremely delicate, its thickness being only about $\cdot 0016$ mm., but even before cleavage begins it has increased to $\cdot 002$ mm. (fig. 12); in the 2-celled stage (fig. 18) it has reached $\cdot 005$ mm., in the 4-celled stage (fig. 22) $\cdot 0072$ mm., whilst in the 16-celled stage (figs. 24-26) it has practically attained its maximum thickness, viz., $\cdot 0075$ - $\cdot 008$ mm. Caldwell's measurements in the case of *Phascolarctus* agree closely with the above (shell of unsegmented ovum from the uterus, $\cdot 0015$ mm. thick, that of the $\cdot 3$ mm. "ovum," $\cdot 01$ mm.). Its presence renders the thorough penetration of ova and early blastocysts with paraffin a capricious and frequently troublesome operation, and its resistant shell-like nature becomes only too obvious in the process of section-cutting, since it cracks with the utmost readiness (cf. Pl. 3, figs. 32, 37).

The occurrence of a shell-membrane round the Marsupial ovum is a feature of considerable phyletic significance, as I need hardly point out. It shows us that the ancestors of the Metatheria must have been oviparous, or must themselves have come from an oviparous stock, which there is no valid reason for supposing was other than Prototherian in its characters. It also renders untenable the views of Hubrecht to the effect that the Metatheria are the descendants of Eutheria, whilst the Eutheria themselves have been directly derived from some presumed viviparous group of hypothetical Prote-

trapods, unless we are to suppose that the Metatheria are even now on the way to acquire secondarily the oviparous habit, much in the same way as the Monotremes, according to Hubrecht, have long since succeeded in doing.

The occurrence of a shell-membrane round the Marsupial ovum has also an important ontogenetic significance in relation to the mode of formation of the blastocyst, as I shall endeavour presently to show.

4. The Uterine Ovum.

The unsegmented ovum from the uterus (figs. 8-13) consists of the following parts:

(1) The shell-membrane externally, $\cdot 0016$ - $\cdot 002$ mm. in thickness.

(2) The laminated layer of albumen, $\cdot 015$ - $\cdot 022$ mm. or more in thickness.

(3) The zona, about $\cdot 0016$ mm. in thickness.

(4) The perivitelline space, between the zona and the ovum, occupied by a clear fluid which coagulates under the action of certain fixatives, e. g. Hermann's fluid (fig. 11, *p.s.*), and which has diffused in from the uterus. The minute polar bodies lie in this space, usually nearer the upper pole than the lower.

(5) The ovum proper.

The entire egg is spherical in form, and varies in diameter in the fresh state from about $\cdot 3$ mm. to $\cdot 36$ mm. (average about $\cdot 32$ mm.).

The ovum itself is ovoidal, its polar diameter always slightly exceeding the equatorial. Its average diametrical measurements in the fresh state run about $\cdot 25 \times \cdot 24$ mm., though I have records of ova measuring as much as $\cdot 3 \times \cdot 29$ mm., and I find that there is an undoubted slight variation in the size of the ova of even one and the same batch, as well as in those from different females.

The uterine ovum exhibits the same marked polarity as

characterises the ripe ovarian ovum (the upper pole being marked by the vacuolated deutoplasmic zone (figs. 8-11, *d.z.*), and so far as its cytoplasmic body is concerned it shows no essential difference from that.

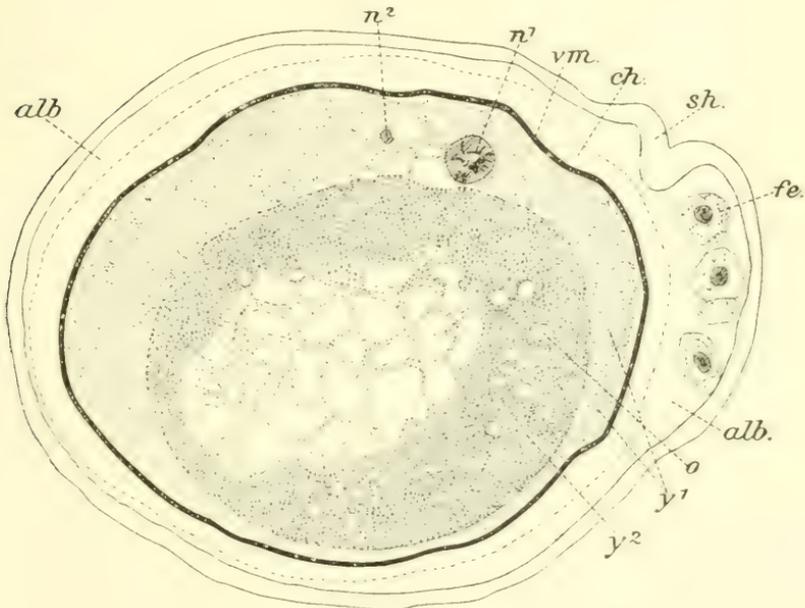
Examined fresh in normal salt solution, the formative cytoplasm forming the bulk of the ovum appears dense, finely granular, and of a very faint lightish-brown tint, its opacity being such that the two pronuclei situated in its central region can just be made out. In section, this central region is distinguishable from the peripheral zone by its uniform, more finely granular character and by the absence of the fluid-filled vacuolar spaces which are generally present in the latter figs. 10 and 12). The deutoplasmic zone at the upper pole, which is only partially visible in the entire egg owing to the way in which it is enclosed by the formative cytoplasm (figs. 8, 9, *d.z.*), presents a characteristically clear or semi-transparent vacuolated appearance in the fresh state, but may have embedded in it a small dense mass (fig. 8, cf. also figs. 11 and 14), evidently formed by the transformation of a portion of its fluid constituent into the solid state, and so to be regarded as comparable with a bit of formative cytoplasm.

In most of the unsegmented uterine ova at my disposal the male and female pronuclei have attained approximately the same size and lie in proximity in the central more homogeneous region of the formative cytoplasm (figs. 10-12). The transformation of the sperm-head into the male pronucleus probably takes place during the passage of the ovum down the tube, and was not observed, and I am as yet uncertain whether the pronuclei unite to form a single cleavage nucleus or give origin directly to the chromosomes of the first cleavage figure.

Caldwell figures ('87, Pl. 30, fig. 5) a section through the uterine ovum of *Phascolarctus* which I reproduce here as Text-fig. 1, in order to facilitate comparison with my figs. 11 and 12, with which it shows an essential agreement, apart from the presence of follicular cells in the albumen which I have never observed in *Dasyurus*, and making allowance for the

difference in sectional plane. The figure is stated to represent "the seventeenth section of a vertical longitudinal series of thirty-five sections through the segmenting ovum, containing two nuclei, taken from the uterus and measuring .17 mm. in diameter." Caldwell has, I think, fallen into several errors in his interpretation of the structural features seen in this

TEXT-FIG. 1.



Section of uterine ovum of *Phascolaretus cinereus*.
(After Caldwell.)

figure. In the first place, the sectional plane appears to me not to be vertical as in my own figs. 11 and 12, but horizontal, and to have passed through the lower portion of the deutoplasmic zone, shown in the figure as a central markedly vacuolated area. Then there is no evidence to be derived from the figure in support of the description of the ovum as segmenting. The part inside the zona (*vm.*) labelled *y*¹ and described as "protoplasm with finest yolk-granules," I would

interpret simply as coagulum in the perivitelline space, whilst the so-called "segmentation nuclei" (n_1, n_2) situated in it are probably the polar bodies or their derivatives. The part labelled γ_2 , and designated "white yolk," I would regard as the ovum itself. It exhibits an obvious differentiation into a central vacuolated area and a peripheral, dense, granular zone with scattered vacuoles, and I think there can be little doubt but that the former corresponds to the deutoplasmic zone of the *Dasyure* ovum, the latter to the formative zone. It is these errors of interpretation apparently which misled Caldwell into making the statement, now widely quoted in the text-books, that cleavage in *Phascolarctus* is of the meroblastic type.

CHAPTER III.—CLEAVAGE AND FORMATION OF THE BLASTOCYST.

I. Cleavage.

Cleavage begins in the uterus as in *Didelphys*, *Phascolarctus*, and no doubt *Marsupials* in general. The first externally visible step towards it consists, as already described, in the elimination by abstriction of the deutoplasmic zone at the upper pole. The yolk-body so formed appears as a definitely limited, clear, rounded mass which lies in contact with the slightly concave upper surface of the formative remainder of the ovum. It is quite colourless and transparent except for the frequent occurrence in it of a small, more or less irregular opaque mass, representing probably a condensation product of its fluid material (cf. Pl. , figs. 8, 14, *y.b.*). Consisting as it does of a very delicate cytoplasmic reticulum with fluid-filled meshes it is extremely fragile, and is seen to advantage only in fresh material (figs. 14 and 19, *y.b.*). It takes no direct part in the later developmental processes though during the formation of the blastocyst it becomes enclosed in the blastocyst cavity and finally undergoes disintegration therein, its substance becoming added to the fluid which fills the same, so that it may be said, in this indirect way, to fulfil, after all, its original nutritional destiny. Separation

tion of the yolk-body is rapidly followed by the completion of the division of the formative remainder of the ovum into the first two blastomeres, the plane of division being coincident with the polar diameter or egg-axis and at right angles to the plane of separation of the yolk-body (Pl. 2, fig. 14). I obtained relatively little material between the stage of the unsegmented ovum with two equal-sized pronuclei seen in fig. 12 and the 2-celled stage (fig. 14), both of which are well represented in my material, so that it would appear that the separation of the yolk-body and the division of the formative remainder of the ovum are effected with considerable rapidity. Fig. 13 shows, however, a section of an unsegmented ovum in which the chromosomes of the metaphase of the first cleavage figure are visible in the central region of the formative cytoplasm, but situated, it is worthy of note, nearer the future upper pole than the lower pole. The deutoplasmic zone (*d.z.*) still forms an integral part of the egg, and there is no sign of commencing abstriction. I have also sections of ova in a still more advanced stage of the first cleavage, in which the daughter-nuclei have but recently been constituted and are still quite minute, and the cleavage furrow is well marked on the surface of the egg. In these ova the yolk-body is already separated, so that we may conclude with a fair degree of certainty that its elimination about coincides with the first appearance of the cleavage furrow.

Figs. 14-16 show the 2-celled stage, respectively in side, lower polar, and end views. The blastomeres are of approximately equal size and otherwise quite similar. Selenka also found the same to be the case in *Didelphys*, though in the single specimen of the 2-celled stage he had for examination (Taf. xvii, fig. 3) the blastomeres are displaced and somewhat shrunken. Each blastomere has much the shape of a hemisphere from which a wedge-shaped segment has been sliced off, a form readily accounted for when we take account of the effect of the elimination of the deutoplasmic zone. After that event, the formative remainder of the ovum has the form of a sphere from which a somewhat bi-convex lens-

shaped piece has been gouged out at the upper pole. Consequently, when it divides along its polar diameter, the resulting blastomeres will have the form of hemispheres with obliquely truncated upper surfaces or ends, which will be proportionately thicker than the lower ends. In correlation therewith we find the nucleus of each blastomere situated slightly excentrically, rather nearer the upper than the lower pole (fig. 18). The rounded yolk-body lies partly enclosed between the upper truncated surfaces of the blastomeres.

Two-celled eggs are shown in vertical section in figs. 17 and 18. The cytoplasm of the blastomeres exhibits a well-marked differentiation into two zones corresponding to that already seen in the formative cytoplasm of the unsegmented egg, only much more accentuated, viz. a dense, fine-grained perinuclear zone, and a less dense, more vacuolated peripheral zone, in which there is present a coarse, irregular network of deeply staining strands, recalling the framework of mitochondrial origin described by Van der Stricht ('04, '05) in the human ovum and that of *Vesperugo*. We have here in this differentiation of the cytoplasm, evidence of the occurrence of an intense metabolic activity which has resulted in a marked increase in the amount of deutoplasmic material present in the blastomeres as compared with that found in the ovarian egg or even in the unsegmented uterine egg. The blastomeres consequently present a somewhat dense opaque appearance when examined in the fresh state, their nuclei being partially obscured from view. Amongst the Eutheria, various observers (Sobotta, Van der Stricht, Lams and Doorne) have described a similar increase in the deutoplasmic contents of the egg after its passage into the Fallopian tube or uterus.

The second cleavage plane is also vertical and at right angles to the first. The resulting four equal-sized blastomeres viewed from the side (Pl. 2, fig. 19) are seen to be ovalish in outline, their lower ends being slightly narrower and more pointed than their upper ends, which diverge somewhat to enclose the lower part of the yolk-body. Seen from one of

the poles, in optical section (figs. 20, 21), they appear triangular with rounded corners and centrally directed apices. The space occupying the polar diameter, which they enclose is the cleavage cavity. The blastomeres are now somewhat less opaque than those of the 2-celled stage, so that their nuclei, excentrically situated nearer their upper ends and enclosed in the central granular zone of the cytoplasm, can now be fairly distinctly made out in the fresh egg.

The arrangement of the blastomeres at this stage is exceedingly characteristic, and is identical with that of the blastomeres in the corresponding stage of *Amphioxus* or the frog, but is quite different from that normal for the 4-celled stage of the *Eutheria*. They lie disposed radially or meridionally around the polar diameter, occupied by the cleavage cavity, their thicker upper ends partially surrounding the yolk-body. Selenka figures a precisely similar arrangement in his 4-celled stage of *Didelphys*, so that we may conclude it holds good for the Marsupials in general.

Whilst, then, in Marsupials the first two cleavage planes are vertical or meridional, and at right angles to each other, and the first four blastomeres are arranged radially around the polar diameter (radial type of cleavage), in the *Eutheria* such is never the case, at all events normally, so far as is known. In the *Eutheria* the first four blastomeres form, or tend to form, a definite cross-shaped group, as the result apparently of the independent division of the first two blastomeres in two different planes at right angles to each other, the division planes being meridional in the one, equatorial in the other.¹ This pronounced difference in the spatial relations of the first four blastomeres in the *Metatheria* and *Eutheria* is a feature of the very greatest interest and importance, since it is correlated with and in part conditions the marked dissimilarity which we meet with in the later developmental occurrences in the two groups, in particular in the mode of formation of the blastocyst in the two.

¹ Compare in this connection Assheton's remarks ('09, pp. 232-233), which have appeared since this chapter was written.

Moreover, so far as the Eutheria are concerned, it affords us, I believe, a striking and hitherto unrecognised example of a phenomenon to which Lillie ('99) has directed attention, viz. adaptation in cleavage.

Fig. 22 shows a horizontal section through the 4-celled stage, and fig. 23 a vertical section of the same. The blastomeres in their cytoplasmic characters essentially resemble those of the 2-celled stage, but the peripheral deutoplasmic network is here more strongly developed, and it is especially worthy of note that it is more marked towards the lower poles of the blastomeres (fig. 23), as also appears to be the case in the 2-celled stage. The shell-membrane measures in thickness $\cdot 0072$ mm.

The next succeeding (third) cleavages are again meridional, each of the four blastomeres becoming subdivided vertically into two, not necessarily synchronously. Fig. 53. Pl. 6, shows a side view, and fig. 54 a view from the lower pole of a 6-celled egg, two of the blastomeres of the 4-celled stage having divided before the other two. The blastomeres have moved apart, and now form an open ring approximately equatorial in position, and surrounding the central cleavage space, the upper opening of which is occupied by the yolk-body. I have failed to obtain a perfectly normal 8-celled stage, nevertheless the evidence clearly shows that the first three cleavage generations in *Dasyurus* are meridional and equal, and that the resulting eight equal-sized blastomeres form an equatorial ring in contact with the inner surface of the sphere formed by the zona and shell-membrane.

Whilst, then, the first three cleavage generations are meridional and equal, the succeeding divisions (fourth cleavage generation), on the contrary, are equatorial and unequal, each of the eight blastomeres becoming divided into a smaller, more transparent upper cell, with relatively little deutoplasm, and a larger, more opaque lower cell with more abundant deutoplasmic contents. In this way there is formed an exceedingly characteristic 16-celled stage, consisting of two

superimposed rings, each of eight cells. The upper ring of smaller and clearer cells partially encloses the yolk body, and is situated entirely in the upper hemisphere of the sphere formed by the egg-envelopes. The lower ring of larger, more opaque cells lies approximately in the equatorial region of the said sphere. This 16-celled stage is figured in fig. 55, Pl. 6, as seen from the side, and in fig. 56 as seen from the upper pole, both figures being taken from a spirit egg .37 mm. in diameter. The marked differences in the cells of the two rings are well brought out in the micro-photographs reproduced as figs. 24, 25, and 26, Pl. 2. Figs. 24 and 25 represent horizontal sections of an egg .38 mm. in diameter, the former showing the eight cells of the lower ring, and the latter the eight cells of the upper ring. Fig. 26 shows a vertical section through an egg also of a diameter of .38 mm., but with seventeen cells, one of the original eight cells of the upper ring having divided and one being in process of division. The section passes through the yolk-body (*y.b.*), which is seen as a faintly outlined structure lying in contact with the zona between the two cells of the upper ring (*f.c.*).

The shell-membrane in eggs of this 16-celled stage has attained a thickness of .0075 mm., and the albumen layer has been almost completely absorbed, so that the zona now lies practically in apposition with the shell-membrane, the two together forming a firm resistant sphere, to the inner surface of which the blastomeres are closely applied. The separation between the zona and shell-membrane seen in the figures is largely, if not wholly, artificial.

The average measurements of the cells of the two rings in the .38 mm. egg, figured in figs. 24 and 25, are as follows :

	Upper ring cells.	Lower ring cells.
Diameter06 × .058 mm.	.09 × .064 mm.
Vertical height095 mm.	.115 mm.
Nucleus0165 mm.	.02 mm.

These measurements demonstrate at a glance the distinct difference in size which exists between the cells of the two rings, whilst the cytoplasmic differences between them are

equally evident from an inspection of the micro-photographs, figs. 24-26. In the larger cells of the lower ring (fig. 24, *tr.ect.*) the nucleus (rich in chromatin and nucleolated) is surrounded by a perinuclear zone of clearer, coarsely vacuolar cytoplasm, outside of which is a densely granular deutoplasmic zone, which extends to within a short distance of the periphery of the cell-body. In the smaller cells of the upper ring (fig. 25, *f.c.*) the cytoplasm is coarsely reticular, with a tendency to compactness round the nucleus, and its contained deutoplasmic material is spare in amount as compared with that of the lower cells, being mainly located in a quite narrow peripheral zone. The upper cells thus appear relatively clear as compared with the dense, opaque-looking lower cells (fig. 26).

It becomes evident, then, that we have to do here, in this fourth cleavage generation, with an unequal qualitative division of the cytoplasm of the blastomeres of the 8-celled stage. Just such a division as this we should expect if the deutoplasmic material were mainly aggregated towards the lower poles of the dividing cells. The evidence shows that this is actually the case. In the 2-celled and especially in the 4-celled eggs we have already seen that the deutoplasmic network is already most strongly developed towards the lower poles of the blastomeres. This polar concentration of the deutoplasm reaches its maximum in blastomeres of the 8-celled stage, and confers on these an obvious polarity. Although I failed to obtain normal examples of the latter stage, I have fortunately been able to observe the characters of the blastomeres in sections of eggs with twelve, thirteen, and fourteen cells respectively.

In the 12-celled egg (Pl. 6, fig. 57), measuring $\cdot 38$ mm. in diameter, four of the eight original blastomeres are still undivided; the remaining four have undergone division unequally and qualitatively, one but recently, so that $4 + (4 \times 2) = 12$. The undivided blastomeres are large (average diameter, $\cdot 11 \times \cdot 076$ mm.) and ovoidal in form, their lower ends being thicker than their upper, and they exhibit a well-

marked polarity. The nucleus lies excentrically in the upper half of the cell, just above the equator, and is surrounded by a finely granular zone of cytoplasm, outside which is a thin irregular ring of deutoplasmic material. The cytoplasm of the apical part of the cell is clear and relatively free from deutoplasm; that of the lower half, on the other hand, is so rich in deutoplasm as to appear quite dense and opaque. The conclusion is therefore justified that the blastomeres of the 8-celled stage possess a definite polarity, which has been acquired as the result of the progressive concentration of deutoplasmic material at their vegetative poles during the cleavage process. Division, in the equatorial plane, of cells so constituted must necessarily be unequal and qualitative, so far at least as the cytoplasm is concerned.

In the 13-celled stage three of the original eight blastomeres are in process of division, and five have already divided unequally and qualitatively, so that $3 + (5 \times 2) = 13$, and in the 14-celled stage two of the original blastomeres are in division and six have already divided: $2 + (6 \times 2) = 14$.

The significance to be attached to this characteristic unequal and qualitative division of the blastomeres of the 8-celled stage to form two superimposed cell-rings, markedly differentiated from each other, we shall presently consider. Meantime I may categorically state the conclusions I have reached in regard thereto. The wall of the blastocyst in *Dasyurus* is at its first origin, and for some considerable time thereafter, unilaminar throughout its entire extent, and I regard the upper cell-ring of the 16-celled stage as giving origin to the formative or embryonal region of the unilaminar wall, the lower cell-ring as furnishing the extra-embryonal or non-formative remainder of the same. I shall therefore refer to the upper cell-ring and its derivatives as formative or embryonal, and to the lower cell-ring and its derivatives as non-formative or extra-embryonal.

The formative or embryonal region furnishes the embryonal ectoderm and the entire entoderm of the vesicle, and I accordingly conclude that it is the homologue of the embryonal knot

or inner cell-mass of the Eutherian blastocyst. The non-formative or extra-embryonal region directly gives origin to the outer extra-embryonal layer of the bilaminar blastocyst wall, i. e. to that layer which in the Sauropsida and Prototheria is ordinarily termed the extra-embryonal ectoderm. I regard it as such, and as the homologue of the so-called trophoblast (or as I prefer to term it, the "trophoblastic ectoderm" or "tropho-ectoderm") of the Eutherian blastocyst.

A word or two here before concluding this section by way of summary, as to the condition of the enclosing egg-envelopes. During the sojourn of the egg in the uterus the albumen is gradually resorbed, and by about the 16-cell stage it has all but completely disappeared, thus permitting the zona to come into direct apposition with the inner surface of the shell-membrane. The shell-membrane itself increases very considerably in thickness during cleavage, and by the 16-celled stage had practically reached its maximum, viz. .0075-.008 mm., i. e. it is nearly five times thicker than that of the ovum which has just entered the uterus. The thickened shell-membrane by itself is firm and resistant, and it becomes still more so by the application of the zona to its inner surface, the two together forming a spherical supporting case round the segmenting egg, to the inner surface of which the blastomeres become closely applied.

The existence of such a firm supporting envelope round the Marsupial egg is, in my view, a feature of very great ontogenetic significance, and one which must be taken into account in any comparison of the early developmental occurrences in the Metatheria and Eutheria. As the sequel will show, the mode of formation of the blastocyst in these two sub-classes is fundamentally different, and in my opinion the explanation of this difference is to be found in the retention by the Metatheria of a relatively thick resistant shell-membrane, and its complete disappearance amongst the Eutheria.

2. Formation of the Blastocyst.

It is characteristic of the Marsupial that the cleavage-cells proceed directly to form the wall of the blastocyst, without the intervention of a morula stage, as in the Eutheria.

The fifth cleavages are meridional, each of the eight cells of the two rings of the 16-celled stage becoming subdivided vertically into two, so that there results a 32-celled stage consisting of two rings, each composed of sixteen cells. As might be expected, the smaller less yolk-rich cells of the upper ring tend to divide more rapidly than the larger yolk-laden cells of the lower ring, but the difference in the rate of division of the two is only slight. I have, for example, sections of a 17-celled stage (that already referred to, fig. 26) consisting of nine formative cells ($= 6 + [1 \times 2] + 1$ in division) and eight non-formative cells, and also of a 31-celled stage (Pl. 6, fig. 59, seen from lower pole; cf. also fig. 60, showing a side view of another 31-celled egg, both eggs $\cdot 375$ mm. in diameter), consisting of sixteen formative and fifteen non-formative cells, of which one is in process of division. But I have also preparations of 32-celled eggs with an equal number of formative and non-formative cells, showing that the latter may make up their leeway, the former resting meantime. On the other hand, the cells of the two rings may divide more irregularly, as evidenced by a stage of about forty-two cells, consisting approximately of twenty-three formative cells ($= 9 + [7 \times 2]$) and nineteen non-formative ($= 13 + [3 \times 2]$). Whatever the rate of division, the important point is that the division planes are always radial to the surface, so that all the resulting blastomeres retain a superficial position in contact with the inner surface of the supporting sphere formed by the zona and shell-membrane. In apposition with the continuous surface afforded by that, the blastomeres, continuing to divide, gradually spread round towards the poles, the descendants of the upper or formative cell-ring gradually extending towards the upper pole marked by the yolk-body, whilst those of the

lower or non-formative cell-ring similarly spread towards the lower pole. As the blastomeres divide and spread they become smaller and more flattened, and gradually cohere together, and so in this way they eventually give origin to a complete unilaminar layer lining the inner surface of the sphere formed by the egg-envelopes. It is this unilaminar layer which constitutes the wall of the blastocyst.

The just completed blastocyst of *Dasyurus* is a spherical fluid-filled vesicle measuring about .4 mm. in diameter (Pl. 3, figs. 27-29, Pl. 6, figs. 61, 62), and invested externally by the thin zona and the shell-membrane (.0075-.0078 mm. in thickness). The albumen layer has completely disappeared, and the shell-membrane, zona, and cellular wall are from without inwards in intimate apposition. The smallest complete vesicles which I have examined measure .39 mm. in diameter (figs. 27, 61), and in one of these I find the cellular wall consists approximately of about 108 cells. In four other eggs of the same diameter and from the same female the wall of the blastocyst is as yet incomplete at the lower pole (fig. 31, *l.p.*), and in these, rough counts of the cells yielded the following respective numbers—89, 93, 121, 128. In another also incomplete blastocyst of the same batch, .41 mm. in diameter (fig. 32), the cellular wall consists of about 130 cells. The largest complete blastocyst in this same batch measured .49 mm. in diameter, so that we have a range of variation in size of the just completed blastocyst extending from .39 to .49 mm.

The unilaminar wall of the blastocyst consists of a continuous layer of more or less flattened polygonal cells (figs. 27-29, 61, 62) lying in intimate contact with the zona, itself closely applied to the shell-membrane. Over the lower hemisphere the non-formative cells are on the whole larger and plumper than the formative cells of the upper hemisphere, and in surface examination they appear somewhat denser owing to the fact that they possess much more marked perinuclear zones of dense cytoplasm than do the formative cells (cf. fig. 63, representing a .6 mm. vesicle). In sections, however, this latter difference is much less obvious, indeed,

is hardly, if at all, detectable, so that one has to depend partly on the relative thickness of the cells, partly, and, indeed, mainly, on the yolk-body in determining which hemisphere is which.

The blastocyst cavity is tensely filled by a coagulable fluid derived from that poured into the uterine lumen through the secretory activity of the uterine glands. Also situated in the blastocyst cavity, in contact with the inner surface of the wall in the region of the upper pole, is the spherical yolk-body (fig. 29, *y.b.*). It becomes overgrown and enclosed in the blastocyst cavity as the result of the completion of the cellular wall over the upper polar region, much in the same sort of way as the yolk in the meroblastic egg becomes enclosed by the peripheral growth of the blastoderm. In the majority of my sections of early blastocysts the yolk-body has been dragged away from contact with the formative cells through the coagulation of the albuminous blastocystic fluid, and lies more or less remote from the wall enclosed by the coagulum, except on the side next the upper hemisphere (fig. 31, *y.b.*, *c.g.*). In two instances, one of which is shown in fig. 32, I find the yolk-body had become so firmly attached to one of the formative cells that the coagulum formed during fixation failed to detach it, and only succeeded in drawing it out to a pear-shape.

The yolk-body, it may here be mentioned, persists for a considerable time in the blastocyst cavity; I have found it shrunken indeed, but still recognisable, in relation to the embryonal area in vesicles 4.5-6 mm. in diameter. And there may even appear within it peripherally, irregular strands which stain deeply with iron-haematoxylin and which recall those forming the peripheral deutoplasmic network of the early blastomeres. Eventually, however, it seems to disappear, its substance passing into the blastocystic fluid, so that, as already remarked, it fulfils in this indirect way its original destiny.

Normally the cavity of the just completed blastocyst contains no cellular elements whatever. In one otherwise perfectly normal blastocyst (.39 mm. diam.) I find present,

however, a small spheroidal body .028 mm. in diameter, composed of glassy-looking cytoplasm enclosing a central deeply staining granule. This I interpret as a cell or cell-fragment which has been accidentally separated off from the wall, and which has undergone degeneration. In later blastocysts such cellular bodies exhibiting more or less evident signs of degeneration are of fairly common occurrence. They are of no morphological significance.

Selenka's "Blastopore."—Normally the wall of the blastocyst is first completed over the upper hemisphere, in correspondence with the fact that the formative cells not only divide somewhat more rapidly than the non-formative but have a smaller extent of surface to cover, since the upper cell-ring from which they are derived lies about midway between the upper pole of the sphere formed by the egg-envelopes and the equator of the same, whilst the lower cell-ring from which the non-formative cells arise is approximately equatorial in position. We thus meet with stages in the formation of the blastocystic wall such as are represented in surface view on Pl. 3, fig. 30, and in section in figs. 31 and 32, in which the blastocystic cavity, prior to the completion of the cellular wall over the lower polar region, is more or less widely open below. There can be no doubt, I think, but that this opening corresponds to that observed by Selenka in his 42-celled "gastrula" of *Didelphys* and regarded by him as the blastopore, since he believed the entoderm arose from its lips. My observations conclusively show that it has no connection whatever with the entoderm, this layer arising from the formative region of the upper hemisphere, and that it is a mere temporary opening of no morphological significance, blastoporic or other. Prior to the completion of the wall at the upper pole a corresponding opening is temporarily present there also. Both owe their existence to the characteristic way in which the blastocyst wall is formed by the spreading of the products of division of the two cell-rings of the 16-celled stage towards opposite poles in contact with the surface provided by the enclosing egg-envelopes.

I have met with one specimen, an incomplete blastocyst .39 mm. in diameter (belonging to the same batch as the other blastocysts referred to in this section¹), in which the lower hemisphere would appear to have been completed before the upper, for the yolk-body lies in contact with the zona in the region where the cellular wall is as yet absent, and that the yolk-body has not been secondarily displaced is proved by a micro-photograph of the specimen in my possession (taken immediately after its transference to the fixing solution), in which the yolk-body is seen to lie at the unclosed pole in exactly the same position as in the sections.

In connection with this exceptional specimen, it may be recalled that Selenka, in his 68-celled "gastrula" of *Didelphys* (fig. 10, Taf. xvii), figures the wall as complete at the lower pole, the "blastopore" having already closed, but as still incomplete at the upper pole, there being present a small opening leading into the blastocyst cavity. In the 42-celled "gastrula" (fig. 8, Taf. xvii) this same opening and the "blastopore" as well are present. The occurrence of these openings at opposite poles, and the general agreement in the constitution of the blastocyst wall (larger, more yolk-rich cells at lower pole, smaller, less yolk-rich cells at upper), in the corresponding stages in *Didelphys* and *Dasyurus* justify the conclusion that the blastocyst of the former develops in the same way as does that of the latter. It is worthy of remark, however, that the just completed blastocyst of *Didelphys* appears to be considerably smaller than that of *Dasyurus*. Selenka unfortunately gives no measurements of his early stages, but I have calculated from the figure, the magnification of which is given, that the 68-celled blastocyst has a diameter of about .137 mm. The corresponding stage of *Dasyurus* measures about .39 mm., and is therefore nearly three times as large.

¹ This batch, from female 2 B. 16. vii. '01, comprised altogether twenty-eight eggs, of which some eighteen were normal complete and incomplete blastocysts (.39-.49 mm. in diameter) and ten abnormal, four of these being unsegmented ova.

Selenka's Urentodermzelle.—Whilst the 42- and 68-celled blastocysts described by Selenka may be regarded as normal so far as the occurrence of polar openings and the constitution of their wall are concerned, I hold them to be abnormal in respect of the presence in each of a single large yolk-laden cell, regarded by Selenka as entodermal in significance. It is well to point out that Selenka was not able actually to determine the fate of this cell; he merely presumed that it took part in the formation of the definitive entoderm. No such cell occurs in normal blastocysts of *Dasyurus* at any stage of development, and in my opinion Selenka's "urentodermzelle" is none other than a retarded and displaced blastomere, i. e. a blastomere which has failed for some reason to divide, and which has become secondarily enclosed by the products of division of its fellows, and I am strengthened in this interpretation by the occurrence in an abnormal blastocyst of *Dasyurus* of just such a large cell as that observed by Selenka. The vesicle in question is one of the batch already referred to, and measured $\cdot 397$ mm. in diameter. The cellular wall (fig. 37) is apparently normal, but is incomplete at one spot, and the gap so left is occupied by a large binucleated cell, rich in deutoplasm and measuring $\cdot 12 \times \cdot 072$ mm. (fig. 37, *abn.*). This cell corresponds in its size and cytoplasmic characters with a non-formative blastomere of about the 16-celled stage, and I regard it simply as a blastomere which has failed to undergo normal division. In another abnormal blastocyst ($\cdot 39$ mm. diam.) from the same batch, the cellular wall appears complete and normal, but the blastocyst cavity contains a group of about sixteen spherical cells averaging about $\cdot 032$ mm. in diameter, and in yet another abnormal egg of the same diameter and batch there is present an incomplete layer of flattened cells over one hemisphere, and towards the opposite pole of the egg-sphere there occurs a group of spherical cells of variable size and some of them multinucleate. In this abnormal egg it appears as if the formative cells had divided in fairly normal fashion, whilst the non-formative cells had failed to do so.

CHAPTER IV.—GROWTH OF THE BLASTOCYST AND DIFFERENTIATION OF THE EMBRYONAL ECTODERM AND THE ENTODERM.

1. Growth of the Blastocyst.

In the preceding chapter we have seen that the cleavage process in *Dasyurus* results in the formation of a small spherical vesicle, about .4 mm. in diameter, which consists, internally to the investment formed by the apposed zona and shell-membrane, simply of a cellular wall, unilaminar throughout its entire extent, and enclosing a fluid-filled cavity normally devoid of any cellular elements. The stage of the just completed blastocyst is followed by a period of active growth of the same, and it is a noteworthy feature in the development of *Dasyurus* that during this time the blastocyst undergoes no essential structural change, but remains unilaminar until it has reached a diameter of from 4.5 to 5.5 mm. Even during cleavage, the egg of *Dasyurus* increases in diameter, partly owing to the thickening of the shell membrane, partly, and, indeed, mainly, as the result of the accumulation of uterine fluid under pressure within the egg-envelopes, but the increase due to these causes combined is relatively insignificant, being only about .1 mm. As soon, however, as the cellular wall of the blastocyst is completed, rapid growth sets in, under the influence of the hydrostatic pressure of the fluid, which tensely fills the blastocyst cavity, with the result that the small relatively thick-walled blastocyst becomes converted into a large extremely thin-walled vesicle, but beyond becoming very attenuated, the cellular wall during this period of active growth undergoes no essential change, and retains its unilaminar character until the blastocyst, as already mentioned, has reached a diameter of from 4.5 to 5.5 mm. In vesicles of about this size there become differentiated from the formative cells of the upper hemisphere the embryonal ectoderm and the entoderm, and this latter layer then gradually spreads round inside the non-formative (extra-embryonal ectodermal) layer of the lower hemisphere so as to

form a complete lining to the blastocyst, which thereby becomes bilaminar. Such a marked enlargement of the blastocyst prior to the differentiation of the embryonal ectoderm and entoderm as is here described for *Dasyurus* does not apparently occur, so far as known, in other Marsupials: in *Perameles*, for example, the embryonal ectoderm and the entoderm are in process of differentiation in vesicles a little over 1 mm. in diameter (v. p. 77), in *Macropus* these two layers are already fully established in a vesicle only .8 mm. in diameter (v. p. 79), and much the same holds good for *Trichosurus* and *Petrogale*. It is paralleled by the marked growth which in the Monotremes follows the completion of the blastocyst and which precedes the appearance of embryonal differentiation. It must be remembered, however, that the growing blastocyst in the Monotreme is bilaminar and not unilaminar as in *Dasyurus*, owing to the fact that the entoderm is established as a complete layer at a very much earlier period than is the case in the latter. I am nevertheless inclined to regard the attainment by the *Dasyurus* blastocyst of a large size, prior to the differentiation of the embryonal ectoderm and the entoderm, as a more primitive condition than that found in other Marsupials. The pronounced hypertrophy which the uteri of *Dasyurus* undergo during the early stages of gestation, an hypertrophy which appears to be proportionately greater than that met with in other forms,¹ is no doubt to be correlated with the presence in them of such a considerable number of actively growing blastocysts.

Selenka states (Heft 5, p. 180) that he examined seven blastocysts of *Dasyurus* " $\frac{3}{4}$ mm." in diameter, taken from a female fifteen days after copulation. He describes their structure as follows: "Man unterscheidet (1) eine sehr zarte äussere, homogene Haut (Granulosamembran), (2)

¹ For example, the uteri of a female (5, 18. vii. '01) from which I obtained twenty-one normal vesicles, 4.5-6 mm. in diameter, with the embryonal area definitely established, measured as follows: Left uterus, 4.5 × 4.7 × 1.4 cm. (fourteen vesicles); right uterus, 4.5 × 4.2 × 1.45 cm. (seven vesicles and one shrivelled).

darunter ein Lager von Ektodermzellen, welche im Gebiete des Embryonalschildes prismatisch, am gegenüberliegenden Pole nahezu kubisch, im übrigen abgeplattet erscheinen, (3) ein inneres zusammenhängendes Lager von abgeflachten Entodermzellen." This description, apart from the reference to the thin shell-membrane, is entirely inapplicable to blastocysts of *Dasyurus* of the mentioned size which I have studied.

I have examined a practically complete series of vesicles of *Dasyurus* ranging from .4 mm. to 4 mm. in diameter and all of them without exception are unilaminar.

Of vesicles under 1 mm. diameter I possess serial sections of more than two dozen, ranging from .5 mm. to .8 mm. in diameter, and obtained from three different females. These differ structurally in no essential respect from the just completed blastocysts. A surface view of a blastocyst .6 mm. in diameter is shown in fig. 63, Pl. 6; in this the difference in the cytoplasmic characters of the cells of opposite hemispheres is clearly brought out, the non-formative cells of the lower hemisphere having much more marked perinuclear zones of dense cytoplasm (deutoplasm) than the formative cells of the upper hemisphere; moreover, the former cells tend to be of larger superficial extent than the latter. Fig. 34, Pl. 3, represents a section of a blastocyst .57 mm. in diameter, and fig. 35 a section of one .73 mm. in diameter. These blastocysts differ in no essential way from the .43 mm. blastocyst represented in fig. 33. As in the latter, the cellular wall is unilaminar throughout, but both it and the shell-membrane have undergone considerable attenuation. Moreover in these blastocysts, apart from the clue afforded by the shrivelled yolk-body, it is practically impossible to determine from the sections which is morphologically the upper hemisphere and which the lower. In fig. 36, from a .6 mm. blastocyst, on the other hand, the cells of the hemisphere opposite the yolk-body (*y.b.*) are larger than those of the hemisphere adjacent to which that body is situated. In the .57 mm. blastocyst the shell-membrane has a thickness of .0052 mm., in the .73 mm. blastocyst it measures .0045 mm., and in a .84 mm. blastocyst

·0026 mm. The zona is now no longer recognisable as an independent membrane. In blastocysts of this stage of growth a variable number of small spherical cells or cell-fragments are frequently met with in the blastocyst cavity, usually lying in contact with the inner aspect of the cellular wall (fig. 34, *i.c.*). In some blastocysts such structures are absent, in others one or two may be present, in yet others numbers of them may occur. They may be definitely nucleated, but this is exceptional; more usually they contain one or more deeply staining granules (of chromatin?), or are devoid of such. They are of no morphological importance, and I think there can be no doubt that they represent cells or fragments of cells which have been separated off from the cellular wall during the process of active growth. They are of common occurrence in later blastocysts, and it is possible the so-called "yolk-balls" observed by Selenka in *Didelphys* are of the same nature.

If we pass now to vesicles from 1 to 3 or 3·5 mm. in diameter, we find the wall still unilaminar, but considerably more attenuated than it is in the blastocysts last referred to. In a vesicle with a diameter of 1·24 mm. the shell-membrane has a thickness of about ·0015 mm., whilst the cellular wall has a thickness of only ·0045 mm. In a 3·5 mm. vesicle the shell-membrane measures about ·0012 mm., whilst the cellular wall ranges from ·0018 to ·0048 mm. in thickness. A small portion of the wall of a vesicle, 2·4 mm. in diameter, is shown in Pl. 6, fig. 64. In these later vesicles I have failed to detect, either in surface examination of the vesicles *in toto* or in sections, any regional differences between the cells indicative of a differentiation of the wall into upper or formative, and lower or non-formative, hemispheres. Everywhere the wall is composed of flattened, extremely attenuated cells, polygonal in surface view, and all apparently of the same character. It might therefore be supposed that the polarity, which is recognisable in early blastocysts, and which is dependent on the pronounced differences existent between the cells of the upper and lower rings of the 16-celled stage, is of no funda-

mental importance, since it apparently becomes lost at an early period during the growth of the blastocyst. Such an assumption, however, would be very wide of the mark, as I hope to demonstrate in the next section of this paper, and, indeed, in view of the facts already set forth, is an altogether improbable one.

Reappearance of Polar Differentiation in the Blastocyst Wall.—Following on the period of what may be termed the preliminary growth of the blastocyst, in the course of which the original polar differentiation in the blastocyst wall apparently becomes obliterated, is an extremely interesting one, during which that differentiation again becomes manifest. In view of the fact (1) that the fourth cleavage in *Dasyurus* is of the nature of a qualitative cytoplasmic division, and (2) that approximately one half or rather less of the unilaminar vesicle wall is formed from the eight smaller and less yolk-rich cells of the upper ring of the 16-celled stage, and its remainder from the eight larger more yolk-rich cells of the lower ring, it thus becomes a question of the first importance to determine if we can the significance of that differentiation.

Amongst the Eutheria, it has been conclusively shown by various observers (Van Beneden, Heape, Hubrecht, Assheton, and others) that there occurs during cleavage an early separation of the blastomeres into two more or less distinctly differentiated groups, one of which eventually, by a process of overgrowth, completely encloses the other. The peripheral cell-group or layer forms the outer extra-embryonal layer of the wall of the later blastocyst (the trophoblast of Hubrecht, or trophoblastic ectoderm as I prefer to term it). It therefore takes no direct part in the formation of the embryo, and may be distinguished as non-formative. The enclosed cell-group, termed the inner cell-mass or embryonal knot, gives rise, on the other hand, to the embryonal ectoderm as well as to the entire entoderm of the vesicle, and may accordingly be distinguished as formative. May it not be, then, that we have here at the fourth cleavage in *Dasyurus* a separation of the

blastomeres into two determinate cell-groups, respectively formative and non-formative in significance, entirely comparable with, and, indeed, even more distinct than that which occurs during cleavage in the Eutheria? I venture to think that the evidence brought forward in this paper conclusively justifies an answer in the affirmative to that question.

If we assume that the upper cell-ring of the 16-celled stage in *Dasyurus* is formative in destiny and the lower cell-ring non-formative, then we might naturally expect to find in the unilaminar wall of the later blastocyst some differentiation indicative of its origin from two distinct cell-groups, and indicative at the same time of the future embryonal and extra-embryonal regions. Now just such a differentiation does, as a matter of fact, become evident in vesicles 3·5 to 4·5 mm. in diameter. We have already seen that the wall in early blastocysts ·4 to ·8 mm. in diameter exhibits a well-marked polar differentiation in correspondence with its mode of origin from the differentiated cell-rings of the 16-celled stage, its upper hemisphere or thereabouts consisting of smaller cells, poor in deutoplasm, its remainder of larger cells, rich in deutoplasm. In later blastocysts, 1–3 mm. or more in diameter, it is no longer possible to recognise this distinction—at all events I have failed to observe it—but if we pass to blastocysts 4·5 mm. in diameter, in which the wall is still unilaminar, we find on careful examination of the entire vesicle under a low power that there is now present a definite continuous line, which encircles the vesicle in the equatorial region so as to divide its wall into two hemispherical areas (Pl. 4, fig. 38, *j.l.*). If we remove and stain a portion of the wall of such a vesicle, including this line, and examine it microscopically (figs. 42–46), it becomes apparent at once, from the disposition of the cells on either side of the line, that we have to do with a sutural line or line of junction produced by the meeting of two sets of cells, which are pursuing their own independent courses of growth and division. The cells never cross the demarcation line from the one side to the other, but remain strictly confined

to their own territory, so that we are justified in regarding the vesicle wall as composed of two independently growing zones. Now the existence of two such independent zones in the unilaminar wall is, to my mind, only intelligible on the view that they are the products of two originally distinct, predetermined cell-groups, and if this be admitted, then I think we are justified in concluding, in view of the facts already set forth, that the two zones in question are derived, the one from the upper cell-ring of the 16-celled stage, the other from the lower ring; that, in other words, they represent respectively the upper and lower hemispheres of the early blastocysts.

If, now, we find that the embryonal ectoderm and the entoderm arise from one of these two regions of the unilaminar wall, whilst the other directly forms the outer extra-embryonal layer of the later (bilaminar) vesicle, then we must designate the former region as the upper or formative, and the latter as the lower or non-formative. Further, bearing in mind the characters of the cells of the two rings of the 16-celled stage, I think we are justified in holding that the formative region is derived from the ring of smaller, less yolk-rich cells, and the non-formative region from the ring of larger, more yolk-rich cells, even if it is impossible to demonstrate an actual genetic continuity between the constituent cells of these two rings and those forming the independently growing areas of the later blastocyst. I have recently re-examined a series of vesicles, measuring 1.5-1.8 mm. in diameter, obtained from a female killed in 1906, and I have so far found it impossible, either in the entire vesicle or in portions of the wall stained and mounted on the flat, to distinguish between the cells over opposite hemispheres. Thus the only actual guide we have for the determination of the poles in such vesicles is the yolk-body, and though the latter is liable to displacement, it is worthy of record that I have several times found it in relation to the formative area in vesicles 4.5-6 mm. in diameter, but never in relation to the non-formative region. This evidence is, therefore, so far as it goes, confirmatory of

the conclusion reached above, viz. that the formative hemisphere is derived from the smaller-celled ring of the 16-celled stage. On that conclusion is based my interpretation of the poles in the unsegmented ovum, and of the two cell-rings of the 16-celled stage as respectively upper and lower.

Of vesicles over 1 mm. in diameter, the smallest in which I have been able to detect the sutural line above referred to measure 3.25 mm. in diameter. In three lots of vesicles, 3.5 mm. in diameter from three different females, I have failed to recognise it, whilst in two other lots, respectively 3.75 mm. (average) and 4 mm. in diameter, the line appears to be in course of differentiation as in the 3.25 mm. vesicles. A portion of the wall of one of the 3.5 mm. vesicles just referred to is shown in Pl. 4, fig. 41, and a portion of the wall of the 3.25 mm. stage, including the sutural line, in fig. 42. Both vesicles were fixed in the same fluid, viz. picro-nitro-osmic acid. Comparison of the two figures reveals the existence, quite apart from the presence of the junctional line in fig. 42, and its absence in fig. 41, of certain more or less obvious differences between them. In fig. 41 the cells are larger, and their cytoplasmic bodies are inconspicuous, being fairly homogeneous and lightly staining. In fig. 42, on the contrary, the cell-bodies are strongly marked, the cytoplasm being distinguishable into a lighter-staining peripheral zone, and a much more deeply staining perinuclear zone, showing evidence of intense metabolic activity. This latter zone is more or less vacuolated, and contains, besides larger lightly staining granules, numerous smaller ones of varying size, stained brown by the osmic acid of the fixative. In the 4 mm. vesicles the cells show precisely the same characters; in the 3.75 mm. vesicles, which were fixed in a picro-corrosive-acetic fluid, the granules are absent from the cytoplasm, otherwise the cells are similar to those of the other two. Mitotic figures are common. The sutural line is recognisable in all three sets of vesicles (3.25, 3.75, and 4 mm.) (fig. 42, *j.l.*), but I cannot be certain that it runs continuously round, and it appears to have a rather more sinuous course than in later blastocysts. The cells of the two regions

of the blastocyst wall, separated by the sutural line, differ somewhat in their characters. On one side of the line (fig. 42, *tr.ect.*) the cells appear to be on the whole slightly larger, and of more uniform size than they are on the other, and they also stain somewhat more deeply. Comparison with later blastocysts shows that the region of more uniform cells is non-formative, that of less uniform, formative. At this stage, however, the differences between the cells of the two regions are as yet so little pronounced that it is practically impossible in the absence of the sutural line to say to which hemisphere an isolated piece of the wall should be referred.

I am inclined to regard the sutural line in these vesicles as being in course of differentiation, and judging from the disposition of the cells on either side of it, I think its appearance is to be correlated with the marked increase in the mitotic activity of the cells of the two hemispheres which sets in in vesicles of 3-4 mm. diameter. The preliminary increase in size of the blastocyst up to about the 3 mm. stage might be described as of a passive character, i. e. it does not appear to be effected as the result of the very active division of the wall-cells, but is characterised rather by a minimum of mitotic division and a maximum of increase in surface extent of the cells, due to excessive stretching consequent on the rapid imbibition of uterine fluid. Once, however, the requisite size has been attained, the cells of the unilaminar wall commence to divide actively, and doubtless as the outcome of that wave of activity, the sutural line makes its appearance between the two groups of independently growing cells.

On the inner surface of the blastocyst wall, especially in the region of the formative hemisphere, there are present in these vesicles numbers of small deeply staining cells of spherical form, and containing osmicated granules similar to those in the wall-cells. They may occur singly or in groups, and appear to me to be of the same nature as the internal cells of the earlier blastocyst. In addition to these cells, there are present clusters of cytoplasmic spheres, staining similarly to the spherical cells, and apparently of the nature of fragmenta-

tion products formed either directly from the wall-cells or from these internal cells.

2. Differentiation of the Embryonal Ectoderm and the Entoderm.

After the preliminary growth in size of the blastocyst is completed, the next most important step in the progressive development of the latter is that just dealt with, involving the appearance of the sutural line, with resulting re-establishment of polar differentiation in the blastocyst wall. Following on that, we have the extremely important period during which the embryonal ectoderm and the entoderm become definitely established.

For the investigation of the earlier phases of this critical period I have had at my disposal a large number of unilaminar blastocysts derived from three females, distinguished in my notebooks as β , 25 . vii . '01, with fifteen vesicles of a maximum diameter of 4.5 mm.; 8 . vii . '99, with twelve vesicles, 4.5 mm. in diameter; and 6 . vii . '04, with twenty-two vesicles, 4.5 and 5 mm. in diameter. These three lots of vesicles may for descriptive purposes be designated as '01, '99, and '04 respectively.

The '01 vesicles are distinctly less advanced than the other two. The sutural line is now, at all events, definitely continuous, and can readily be made out in the intact vesicle with the aid of a low-power lens (Pl. 4, fig. 38, *j.l.*), but the differences between the cellular constituents of the two hemispheres which it separates are much less obvious than they are in the '99 and '04 vesicles. Here, again, one hemisphere forming half or perhaps rather more of the entire vesicle is distinguished from the other by the greater uniformity and the slightly deeper staining character of its constituent cells (figs. 43 and 44, *tr. ect.*). This hemisphere, subsequent stages show, is the lower or non-formative hemisphere. It is characterised especially by the striking uniformity in the size of its cells. Over the opposite hemisphere, the upper or formative one (figs. 43 and 44, *f.a.*), the

cells are more variable in size, the nuclei thus appearing less uniformly and less closely arranged, and they stain, on the whole, somewhat less deeply than those of the lower hemisphere. The non-formative cells are on the average smaller than the largest of the formative cells, but they are more uniform in size, and their nuclei thus lie at more regular distances apart, and appear more closely packed. They are also richer in deutoplasmic material, and so stain rather more deeply than the formative cells. Sections show that the cellular wall is unilaminar throughout its extent, and that, whilst it is somewhat thicker than that of 3.5 mm. vesicles, it is still very attenuated, its thickness, including the shell-membrane, ranging from .004 to .008 mm. I have examined a number of series of sections taken through portions of the wall known to include the sutural line, and find it quite impossible to locate the position of the latter; indeed, I cannot certainly distinguish between the formative and non-formative regions.

In the blastocyst cavity, lying in contact with the inner surface of the wall, and most abundant in the region of the formative hemisphere, there are present numbers of deeply staining spherical cells with relatively small nuclei similar to those described in connection with the 3.25 mm. vesicles. They occur singly or in groups, and may appear quite normal or may show more or less evident signs of degeneration. Their nuclei may stain deeply and homogeneously, or may be represented by one or two deeply staining granules, vacuoles may occur in their cytoplasm, and spherical cytoplasmic masses of very variable size, with or without deeply staining granules of chromatin, may occur along with them. In sections and preparations of the wall of these and other 4.5 mm. vesicles there are to be found, in both the formative and non-formative hemispheres, small localised areas from which such spherical cells are being proliferated off in numbers together. Pl. 5, fig. 47, from the formative hemisphere of an .04 vesicle shows one of the most marked examples of such proliferative activity that I have encountered. A similar but smaller proliferative

area occurs on the non-formative hemisphere of the same vesicle.

These spherical cells are, I am convinced, of no morphological importance, and are destined sooner or later to degenerate. They have certainly nothing to do with the entoderm, the parent-cells of that layer arising exclusively from the formative hemisphere and not from cells such as these, which are budded off from both hemispheres. The fact that they are, in unilaminar vesicles, more numerous over the formative hemisphere may perhaps be taken as an indication of the greater mitotic activity of the formative as compared with the non-formative cells.

The Primitive Entodermal Cells.—Following closely on the stage represented by these '01 blastocysts is the extremely important one constituted by the '99 and '04 vesicles before referred to. This stage is the crucial one in primary germ-layer formation, and marks the transition from the unilaminar to the bilaminar condition, since in it the entodermal cells are not only distinctly recognisable as constituents of the formative region, but are to be seen both in actual process of separation from the latter and as definitely internal cells, frequently provided with, and even connected together by, pseudopodial-like processes of their cell-bodies. Such cells are already present in the '01 vesicles (fig. 71), and probably also in the blastocysts in which the sutural line first makes its appearance, but are much less conspicuous than in these older blastocysts.

The '99 blastocysts are distinctly more advanced than the '01 batch and are just a little earlier than the '04 lot. The former measured, as already mentioned, 4.5 mm. in diameter, the latter 4.5 and 5 mm. (the majority being of the latter size). In my notes on the intact '99 vesicles I find it stated that one hemisphere, forming rather less than half of the entire extent of the vesicle wall, appeared somewhat denser than the other, the sutural line marking the division between the two. I naturally inferred at the time that the denser hemisphere corresponded to the embryonal region of the

Eutherian blastocyst and the less dense to the extra-embryonal region of the same, but just the reverse proves to hold true for the '04 vesicles, the formative hemisphere in these appearing less dense than the non-formative. I cannot now test my former inference by direct observation since I do not appear to have any of the '99 vesicles left intact, but amongst my *in toto* preparations of the vesicle wall I find one labelled as from the "lower pole" which unmistakably belongs to the formative hemisphere, hence I conclude that the denser and slightly smaller region which I originally regarded as formative is really non-formative, a conclusion which brings the '99 vesicles into agreement with the '04 batch.

In these latter vesicles the sutural line and the two regions of the wall can be quite readily made out on careful examination under a low power with transmitted light. The one region appears slightly denser (darker) and has more closely arranged nuclei (*i. e.* is composed of smaller cells) than the other. On the average this denser region appears to be rather the less extensive of the two; the two regions may be about equal; on the other hand the denser may be the smaller. Examination of stained preparations of the wall demonstrates that the darker hemisphere is non-formative, the lighter, formative. It would therefore seem that in certain of these '04 vesicles the formative region has grown more rapidly than the non-formative.

In stained preparations of the wall both of the '99 and '04 vesicles, the differences between the two hemispheres are now so well marked that there is no difficulty in referring even an isolated fragment to its proper region. The non-formative hemisphere differs in no essential way from that of the '01 vesicles, and as in these, is readily distinguishable from the formative by the much greater uniformity in the size and staining properties of its cells (*fig. 45*), as well as by the fact that there are no primitive entodermal cells such as occur in relation to the formative hemisphere, in connection with it. Its constituent cells are on the average distinctly smaller than

the largest of the formative; their nuclei lie nearer each other, with the result that in surface examination of the blastocyst the non-formative region appears rather denser than the formative. In *in toto* preparations of the wall the former usually stains darker than the latter (fig. 45), but this is not always the case; in fig. 46, from an '04 vesicle, there is practically no difference in this respect between the two regions; in yet others of my preparations of '99 vesicles the formative region has stained more deeply than the non-formative.

The formative hemisphere in the earlier blastocysts of this particular developmental stage was described (*ante*, p. 51) as differing from the non-formative in that its constituent cells were much less uniform in character than those of the latter. This same feature, but in much enhanced degree, characterises the formative region of the vesicles under consideration, for it can now be definitely stated that the latter region is constituted by cells of two distinct varieties, viz. (1) more lightly staining cells which form the chief constituent of the formative region, its basis so to speak, and which are on the average larger than those of the other variety, and (2), a less numerous series of cells, distinctly smaller than the largest cells of the former variety, and with denser, more granular and more deeply staining cytoplasm, and frequently met with in mitotic division (*cf.* Pl. 6, fig. 65). The two varieties of cells are intermingled promiscuously, the smaller cells occurring singly and in groups but in a quite irregular fashion, so that here and there we meet with patches of the wall composed exclusively of the larger cells.

The evidence presently to be adduced shows that the larger cells furnish the embryonal ectoderm, and that the smaller cells give origin to the primitive entodermal cells from which the definitive entoderm arises. The smaller cells may therefore be regarded as entodermal mother-cells. Whether these latter cells are progressively formed from the larger cells simply by division, or whether the two varieties become definitely differentiated from each other at a particular stage in

development, must for the present be left an open question. Of the actual existence in the unilaminar formative region of these '99 and '04 blastocysts of two varieties of cells, respectively ectodermal and entodermal in significance, there can be no doubt. In preparations of the formative region, however, whilst one can without hesitation identify certain cells as being in all probability of ectodermal significance and others as prospectively entodermal (cf. figs. 65, 66), it must be admitted that one is often in doubt as to whether one is dealing with small ectodermal cells or with genuine entodermal mother-cells. It is, therefore, hardly to be wondered at that I have not yet been able to satisfactorily determine at what precise period the entodermal mother-cells first become differentiated, though judging from the facts that in the earliest vesicles in which the sutural line is recognisable one region of the wall already differs from the other in the less uniform size of its constituent cells, and that internally situated entodermal cells are already present in small numbers in the '01 vesicles (fig. 71), I incline to the belief that it will probably be found to about coincide with the first appearance of the sutural line. To this question I may perhaps be able to return at some future time.

In addition to the presence of these entodermal mother-cells, which enter directly into its constitution, the formative region of the '99 and '04 blastocysts is characterised by the occurrence on its inner surface of definitely internal cells, which generally agree with the former cells as regards size and staining properties and are evidently related to them. It is these internally situated cells which directly give origin to the definitive entoderm of the later blastocysts, and one need, therefore, have no hesitation in applying to them the designation of primitive entodermal cells. They are exclusively found in relation to the formative hemisphere, and appear in *in toto* preparations as flattened, darkly staining cells closely applied to the inner surface of the unilaminar wall, and disposed quite irregularly, singly, and in groups. They vary greatly in number in blastocysts of even the same batch, but on the

whole are most abundant in the '04 series, and they also exhibit a remarkable range of variation in shape. They may have a perfectly distinct oval or rounded outline (figs. 67, 71, 72), or, as is more frequently the case, they may lack a determinate form and appear quite like amœboid cells owing to their possession of cytoplasmic processes of markedly pseudopodial-like character (fig. 69). Frequently, indeed, the cells are connected together by the anastomosing of these processes, so that we have formed in this way the beginnings at least, of a cellular reticulum (figs. 68, 69, 70).

The question now arises, How do these primitive entodermal cells originate from the small, darkly staining cells of the unilaminar formative region designated in the foregoing as the entodermal mother-cells? I can find no evidence that the primitive entodermal cells are formed by the division of the mother-cells in planes tangential to the surface; on the contrary, all the evidence shows that we have to do here with an actual inward migration of the mother-cells, with or without previous mitotic division, such inward migration being the outcome of the assumption by the mother-cells, or their division products, of amœboid properties; in other words, the evidence shows that the formation of the entoderm is effected here not by simple delamination (using that term in the sense in which it was originally employed by Lankester), but by a process involving the inward migration, with or without previous division, of certain cells (entodermal mother-cells) of the unilaminar parent layer, a process comparable with that found in certain Invertebrates (Hydroids) and distinguished by Metschnikoff as "gemischte Delamination."

In this connection it has to be remembered that the cells of the unilaminar wall of the blastocyst are under considerable hydrostatic pressure, and, in correlation therewith, tend to be tangentially flattened, though the flattening in this stage is much less than in the earlier blastocysts. From a series of measurements made from an '04 vesicle, I find that over the formative region the ratio of the breadth to the thickness of the cells varies from 6 : 1 to 2 : 1, and even to 3 : 2. On the

whole cells of the type indicated by the ratio 6 : 1 predominate, and we should hardly expect to find such cells dividing tangentially. In fact, the only undoubted examples of such division I have met with occur in the single abnormal vesicle present in the '04 batch. In this particular vesicle, which had a diameter of 3 mm. and was thus smaller than the others, there was present on what appeared to correspond to the formative hemisphere of the normal blastocyst a well-defined and conspicuous ovalish patch, $1.23 \times .99$ mm. in diameter.¹ Sections show that over this area the cells of the unilaminar wall are much enlarged and more or less cubical in form, their thickness varying from .012 to .019 mm. These cubical cells exhibit distinct evidence of tangential division, both past and in progress. But in normal vesicles, whilst mitotic figures are quite commonly met with in the cells of the formative region (in which, indeed, they are more numerous than in those of the non-formative region), I have failed to find in my sections after long-continued searching even a single spindle disposed directly at right angles to the shell-membrane; the mitotic spindles lie disposed either tangentially to the surface or obliquely thereto.

For the determination of the mode of origin of the primitive entodermal cells, it is absolutely necessary to study both in *toto* preparations of the formative region, i.e. small portions of the unilaminar wall stained and mounted on the flat, and sections of the same. Sections alone are, on the whole, distinctly disappointing so far as the question under discussion is concerned, and, indeed, give one an altogether inadequate idea of the primitive entodermal cells themselves, seeing that practically all one can make out is that

¹ Curiously enough, amongst the '99 vesicles there also occurred a single small one, likewise 3 mm. in diameter, and with a thickened patch 1.28×1 mm. in diameter, quite similar in its character to that described in the text. I am as yet uncertain whether the thickened area in these two vesicles represents the whole of the formative hemisphere of normal blastocysts or only a hypertrophied part of the same, or whether, indeed, it may not represent the retarded non-formative hemisphere.

there are present, in close apposition with the inner surface of the unilaminar wall, small, darkly staining cells, apparently quite isolated from each other and usually of flattened form (figs. 73, 74, 76, *ent.*). One has only to glance at a well-stained in toto preparation of the formative region (cf. fig. 70) to realise how inadequate such a description of the primitive entoderm cells really is.

Sections nevertheless do yield valuable information on certain points. Besides affording the negative evidence of the absence of tangential divisions and the positive evidence that the primitive entodermal cells are actually internal (figs. 73, 74, 76), they show that growth of the wall in thickness has already set in, and that it is most marked over the formative region, though the thickness attained by the cells is as yet very unequal (figs. 73-76). Measurements taken from an '04 vesicle show that over the non-formative region (fig. 77) the cells vary in thickness from '006 to '009 mm., whilst over the formative region the range of variation is greater, viz. from '006 to '013 mm., so that we may conclude that the latter region is on the average thicker than the former (cf. figs. 73-76, with fig. 77 depicting a small portion of the non-formative region). It is still impossible to determine the position of the sutural line, even in sections of fragments of the wall known to contain it.

The entodermal mother-cells are not very readily recognisable in sections. In fig. 75, however, which is drawn from an accurately transverse section through the formative region of an '04 vesicle, there is depicted what is undoubtedly an entodermal mother-cell (*ent.*). The interesting point about this particular cell is that its cell-body, whilst still intercalated between the adjoining cells of the unilaminar wall, has extended inwards so as to directly underlie one of the wall-cells. Division of such a cell as this would necessarily result in the production of an internally situated cell with all the relations of one of the primitive entodermal type. The inwardly projecting spheroidal cell situated immediately to the left (in the figure) of the one just referred to, I also

regard as an entodermal mother-cell. Cells of this type are not infrequently met with in sections; they usually stain somewhat deeply, and are often found in mitosis.

The evidence obtainable from the study of *in toto* preparations conclusively proves that some at all events of the primitive entodermal cells are actually derived from the entodermal mother-cells much in the way suggested above, whilst others of the primitive entodermal cells are directly formed from mother-cells which bodily migrate inwards.

Fig. 65, Pl. 6, represents a small portion of the formative region of an '04 vesicle viewed from the inner surface. In the centre of the figure, surrounded by the larger, lighter staining (ectodermal) cells of the wall, is a smaller cell in the telophases of division, the cytoplasm of which is granular and stains deeply. That cell unmistakably forms a constituent of the unilaminar wall. I regard it as an entodermal mother-cell. Fig. 66 shows another cell of the same character in the anaphases of division, which likewise forms a constituent of the unilaminar wall, but which differs from the corresponding cell in fig. 65 in that its cytoplasmic body has extended out on one side (lower in the figure), so as to directly underlie part of an adjacent ectodermal cell. In other words we have here a surface view of the condition represented in section in fig. 75, only the entodermal mother-cell depicted therein is not actually in process of division. Fig. 67, taken from the same preparation as fig. 65, shows what I take to be the end result of the division of such a cell as is represented in the two preceding figures. Here we see two small deeply staining cells towards the centre of the figure, which from their disposition and agreement in size and cytological characters are manifestly sister-cells, and the products of division of just such an entodermal mother-cell as is represented in fig. 65, or, better, fig. 66. The one cell (upper in the figure) is more angular in form and manifestly still lies in the unilaminar wall; the other (lower in the figure) is ovalish in form and is no longer a constituent of the unilaminar wall, but is on the contrary a free cell, definitely internal both to the

latter and to its sister-cell. It is, in fact, a primitive entodermal cell, as comparison with fig. 68 proves, and that it has been formed by the division of a mother-cell situated in the unilaminar wall can hardly, I think, be doubted. Its sister-cell, which is still a constituent of the wall, would presumably have migrated inwards some time later.

It is to be noted that the primitive entodermal cell referred to above and those depicted in figs. 71 and 72 are definitely contoured, ovalish and rounded cells, entirely devoid of processes. In these respects they differ markedly from the entodermal cells shown in figs. 68-70, which are very variable in form owing to their possession of more or less elongated pseudopodial-like processes. It might therefore be inferred that the formation of these processes only takes place after the entodermal cells have become definitely internal. Such an inference, however, would be incorrect, for I have abundant evidence showing that such processes may be given off from the entodermal mother-cells whilst they are still constituents of the wall. In *in toto* preparations, it is often difficult to determine with certainty whether a particular entodermal cell still enters into the constitution of the unilaminar wall or not. In the portion of the formative region of a '04 vesicle depicted in fig. 70, however, I am satisfied that all the entodermal cells therein shown (they are readily distinguishable by their smaller size and more deeply staining character) are, with the possible exception of the one on the extreme right, at least partially intercalated between the larger ectodermal cells of the wall. Some of them are entirely situated in the wall; others have extended inwards in varying degree so as to partially underlie the ectodermal cells. It is these latter entodermal cells in particular which exhibit the cytoplasmic processes above referred to. As the figure shows, these processes have all the characters of pseudopodia; they vary in size, form, and number from cell to cell, individual processes may be reticulate and their finer prolongations may anastomose with those of others, and they are formed of cytoplasm, less dense and rather less deeply staining than that of the

cell-bodies from which they arise. Attention may be specially directed to the cell towards the left of the figure (marked *ent.*). Here we have an entodermal cell whose cytoplasmic body is evidently still partially intercalated between the cells of the wall, but which is, at the same time, prolonged inwards (towards the left) so as to underlie the adjoining ectodermal cell. From this inward prolongation there are given off two slender processes, one short and tapering, the other very much longer; this latter, after becoming very attenuated, gradually widens to form an irregular fan-shaped expansion, sucker-like in appearance, and produced into several slender threads, which is situated adjacent to the nucleus of the ectodermal cell on the extreme left. Then from the right side of the same cell there is given off a small inwardly projecting bulbous lobe which may well be the start of just such another process as arises from the left side. Processes of the peculiar sucker-like type just described, formed of a slender elongated stem and a distal expanded extremity from which delicate filamentous prolongations are given off, are abundantly met with in preparations, and strikingly recall the pseudopodia of various Rhizopoda. They are seen in connection with other entodermal cells in fig. 70, and with many of those in fig. 68. I regard them as veritable pseudopodia. Towards the right side of fig. 70 the two entodermal cells there situated stand in direct protoplasmic continuity by means of two slender connecting threads, whilst the upper of these two cells is again joined by a very fine process to the irregular pseudopodial expansion which arises from one of the two entodermal cells situated nearer the middle of the figure, and that same expansion is directly connected with the second of the two entodermal cells just mentioned, so that we have here established the beginning of a cell-network, prior to the complete emancipation of its constituent entodermal elements from the unilaminar wall. We have, then, clear evidence that the entodermal elements in *Dasyurus*, prior to their separation from the unilaminar formative region are capable of exhibiting amœboid activity, since not only may

they send lobose prolongations of their cytoplasmic bodies inwards below the adjacent ectodermal cells, but they may emit more or less elongated processes of indubitable pseudopodial character, which similarly lie in contact with the inner surface of the wall-cells. Furthermore, we have evidence that these pseudopodial processes may anastomose with each other so as to initiate the formation of an entodermal reticulum, whilst the cells from which they arise are still constituents of the unilaminar wall—an especially noteworthy phenomenon. Certain of the primitive entodermal cells, as we have seen, are at first devoid of such processes, but since they all eventually form part of a continuous reticulum, it is evident that the entodermal elements are capable of emitting pseudopodial processes as well after as before their separation from the formative region.

Finally, in view of the fact that the entodermal mother-cells depicted in fig. 70 are not actually in process of division, and therein differ from those of figs. 65 and 66, we may conclude that the formation of the primitive entodermal cells is effected either with or without the previous division of the mother-cells.

If we admit, as I think on the evidence we must admit, that the entodermal cells in *Dasyurus* are endowed with amoeboid properties, then we are relieved of any further difficulty in regard to the mechanism of their inward migration from the unilaminar wall. Doubtless, in the case of those entodermal mother-cells which do not undergo division, the precocious formation of the above-described pseudopodial processes which spread out from the cells like so many suckers considerably facilitates their direct detachment from amongst the cells of the wall. In the case of those primitive entodermal cells which originate as the direct products of division of the mother-cells, it no doubt depends on a variety of circumstances (e. g. actual form of the dividing cell, direction of the spindle, etc.) whether they exhibit amoeboid activity precociously (i. e. before their actual separation), or only at a later period.

The entoderm varies considerably in its degree of diffe-

rentiation in different vesicles of this stage, and even in different parts of the formative region of one and the same vesicle. In some vesicles there are relatively few primitive entodermal cells, in others they are much more abundant. Fig. 68, from the formative region of an '04 vesicle, shows a typical patch of them and illustrates very well the highest stage of differentiation which they attain in these vesicles. The entodermal cells therein depicted all appear to be definitely internal, and it is especially worthy of note that the portion of the unilaminar wall in relation to them is composed exclusively of the larger, lighter staining cells. It is these cells which directly form the embryonal ectoderm of the blastocysts next to be described. The entodermal cells are obviously amœboid in character (observe especially the cells near the middle of the figure), and are in active process of linking themselves together into a cellular reticulum. In fig. 69 is shown a small portion of the formative region of another '04 vesicle. A single entodermal mother-cell in process of division occurs in position in the unilaminar wall, which is otherwise composed of ectodermal cells, whilst internally there are present three entodermal cells, already linked together by their pseudopodial processes. The two lowermost cells afford especially striking examples of amœboid activity, the elongated pseudopodial process of the cell on the left terminating in a well-marked reticulation in definite continuity with the corresponding, but shorter and thicker process of the cell on the right.

3. Establishment of the Definitive Embryonal Area.

Following directly on the stage represented by the '04 blastocysts described in the preceding section is one designated in my list as 5, 18. vii. 01 and referred to here as 5, '01. It comprises twenty-two blastocysts obtained from a female killed fifteen days after coition and all normal, with the exception of one which was shrivelled, and all in precisely

the same stage of development. They measured from 4.5 to 6 mm. in diameter.

In this stage the formative region of the preceding blastocysts has become transformed into the definitive embryonal area (embryonic shield, Hubrecht) as the result of the completion of that process of inward migration of the entodermal mother-cells which we saw in progress in the vesicles last described, and the consequent establishment of the entoderm as a continuous cell-layer underlying and independent of the embryonal ectoderm constituted by the larger passive cells of the original unilaminar formative layer.

In the entire blastocyst (Pl. 4, fig. 39) the embryonal area is quite obvious to the naked eye as the more opaque, hemispherical region, forming rather less than half the entire extent of the vesicle wall; the larger remainder of the same is formed by the much more transparent, non-formative or extra-embryonal region. Sections of the entire blastocyst show (1) that the embryonal area is bilaminar over its entire extent, its outer layer consisting of embryonal ectoderm, already somewhat thickened, its much thinner inner layer consisting of entoderm, partly still in the form of a cellular reticulum, and (2) that the extra-embryonal region is still unilaminar throughout and composed of a relatively thin layer of flattened cells (extra-embryonal or trophoblastic ectoderm, trophoblast [Hubrecht])¹ (Pl. 8, fig. 78). The entoderm is co-extensive at this stage with the embryonal ectoderm, and terminates in a wavy, irregularly thickened, free edge (Pl. 5, fig. 49), which over most of its extent either directly underlies or extends very slightly beyond the line of junction between the embryonal and extra-embryonal ectoderm. The junctional line is thus not very easily seen. In fig. 48, however,

¹ In consonance with my conviction that this layer is homologous both with the so-called trophoblast of Eutheria and the extra-embryonal ectoderm of Prototheria, and in view of the theoretical signification which Hubrecht now insists should be attached to the term "trophoblast," and which I am wholly unable to accept, I venture to suggest as an alternative name for this layer that of "tropho-ectoderm."

a small portion of the line shows with sufficient distinctness, I think, to demonstrate its identity with that of the preceding stage.

The vesicle wall in all my sections of this stage appears to be somewhat thinner than that of the '04 blastocysts, but apart from this apparently variational difference the present blastocysts are almost exactly intermediate between the latter and those next to be described.

The embryonal ectoderm (fig. 78, *emb. ect.*) appears in section fairly uniformly thickened, though its cells are still of the flattened type. In surface view in in toto preparations (cf. fig. 48), they exhibit the same polygonal form and lightly staining qualities as the larger cells of the formative region of the '04 blastocysts, which we have already identified as prospective embryonal ectodermal cells. The junctional line between the embryonal ectoderm and the extra-embryonal is now for the first time readily distinguishable in sections (fig. 78). The extra-embryonal ectoderm (tropho-ectoderm) (Pl. 5, figs. 48 and 49, Pl. 8, fig. 78, *tr. ect.*) differs in no essential respect from the corresponding layer in the '04 blastocysts.

The entoderm in these blastocysts is exceedingly closely adherent to the inner surface of the embryonal ectoderm and cannot be removed therefrom by artificial means. It varies slightly in its character in different vesicles and in different parts of its extent in the same vesicle. Mostly it appears as a continuous thin cell-layer (figs. 49 and 78, *ent.*), but here and there patches occur in which the cells form a reticulum quite similar to that shown in fig. 68 of the preceding stage.

The next stage (designated in my list as 8.vi.01), and the last of *Dasyurus* that need be described in the present communication, comprises eleven vesicles (5-5.5 mm. in diameter), in which the embryonal area is conspicuous and distinctly in advance of that of the preceding vesicles, but is still devoid of any trace of embryonal differentiation (Pl. 4, fig. 40; Pl. 8, fig. 79).

The embryonal area is hemispherical in form (its greatest

diameter varying from 3.5 to 4 mm.) in all except two of the blastocysts, in which it is elongate, with longer and shorter diameters. It occupies about a third or less of the entire extent of the vesicle wall, and thus appears relatively smaller than that of the preceding (5, '01) vesicles. The entoderm now extends for a distance of about 1 mm. beyond the limits of the area, so that in the entire vesicle (fig. 40) three zones differing in opacity are distinguishable, viz. the dense hemispherical zone at the upper pole, constituted by the embryonal area; below that, a less dense, narrow annular zone, formed of extra-embryonal ectoderm and the underlying peripheral extension of the entoderm; and finally, the still less dense hemispherical area, forming the lower hemisphere of the blastocyst and constituted solely by extra-embryonal ectoderm. Thus approximately the upper half of the blastocyst is bilaminar, the lower half unilaminar. Sections show that the embryonal ectoderm (fig. 79, *emb. ect.*) is now a quite thick layer of approximately cubical cells, whilst the extra-embryonal ectoderm (*tr. ect.*) is formed of relatively thin flattened cells. The line of junction between the two is perfectly obvious, both in sections (fig. 79) and in surface view (Pl. 5, fig. 50). The embryonal ectodermal cells, though much thicker than the extra-embryonal, are of less superficial extent; their nuclei therefore lie closer together than those of the latter, moreover they are larger, stain more deeply, and are more frequently found in division, all of which facts testify to the much greater growth-activity of the embryonal as compared with the extra-embryonal ectoderm at this stage of development (cf. fig. 50, *emb. ect.* and *tr. ect.*; in the preparation from which this micro-photograph was made the entoderm underlying the embryonal ectoderm has been removed, whilst it is still partially present over the extra-embryonal ectoderm).

The entoderm (fig. 79, *ent.*) over the region of the embryonal area is readily separable as a quite thin membrane, and is then seen to consist of squamous cells, polygonal in outline, and either in direct apposition by their edges or connected together by minute cytoplasmic processes. Beyond the

embryonal area, however, its peripheral extension below the extra-embryonal ectoderm is much less easily separable in the intact condition (cf. fig. 50), because of its greater delicacy due to the fact that it has here largely the form of a cellular reticulum. In this extra-embryonal region the entodermal cells are frequently found in mitosis. It would appear, then, that the entoderm is first laid down in the region of the embryonal area as a cellular reticulum, which later becomes transformed into a continuous cell-membrane, and that its peripheral extension over the inner surface of the extra-embryonal ectoderm is the result of the growth and activity of its own constituent cells.

This peripheral growth continues until there is formed eventually a complete entodermal lining to the blastocyst cavity. The rate of growth appears to be somewhat variable. In a series of primitive streak vesicles (6-6.75 mm. in diameter) the lower third of the wall is, I find, still unilaminar. In another series of vesicles of the same developmental stage (4.5-6 mm. in diameter) a unilaminar area is present at the lower pole, varying from $1 \times .5$ mm. in diameter to as much as 4 mm. Even in vesicles 7-7.5 mm. in diameter a unilaminar patch may still occur at the lower pole, but in vesicles 8.5 mm. in diameter (stage of flat embryo) the entodermal lining appears always to be complete.

The Origin of the Entoderm in Eutheria.—The remarkable facts relative to the origin of the entoderm in *Dasyurus* which I have been able to place on record in the preceding pages, thanks to the large size attained by the blastocyst prior to the differentiation of the formative germ-layers and to the circumstance that the formative cells are not arranged, as they are in Eutheria, in the form of a more or less compact cell-mass, but constitute a thin unilaminar cell-layer of relatively great extent which can easily be cut up with scissors, and which, after staining and mounting on the flat can be examined under the highest powers, throw, it seems to me, a new and unexpected light on the mammalian entoderm, and at the same time help to fill the considerable

gap which has hitherto existed in our knowledge of its early ontogenesis. Although the mode of origin of the entoderm in *Dasyurus* would appear, in the present state of our knowledge, to find its closest parallel, not amongst vertebrates, but in certain invertebrates (cf. the mode of origin of the entodermal cells from the wall of the blastula in *Hydra* as described by Brauer¹), the observations of Assheton ('94) on the early history of the entoderm in the rabbit, when viewed in the light of the foregoing, seem to me to afford ground for the belief that phenomena comparable with those here recorded for *Dasyurus* will eventually be recognised as occurring also in *Eutheria*.

Hubrecht ('08), in his recent treatise on early Mammalian ontogeny, deals very briefly with the question of the origin of the entoderm in the latter group, merely stating that "from the inner cell-mass arises by delamination a separate lower layer which we designate as the entoderm of the embryo. These entoderm cells wander in radial direction along the inner surface of the trophoblast, which in many cases is thus soon transformed into a didermic structure. . . . When the entoderm has separated off by delamination from the embryonic knob, the remaining cells of the latter form the 'embryonic ectoderm,' which is thus situated between the entoderm and the trophoblast."

Assheton, in the paper just referred to, has given a careful account of the first appearance of the entodermal cells in the rabbit, and of what he believes to be the mode of their peripheral extension below the trophoblastic wall of the blastocyst. He shows that the inner cell-mass, at first spherical, gradually, as the blastocyst enlarges, flattens out below the "covering layer" of the trophoblast until it forms an approximately circular plate "nowhere more than two cells thick." During the process of flattening, cells are seen to jut out from the periphery of the mass; these eventually separate, and appear as rounded cells scattered irregularly over the inner surface of the trophoblast and "extending

¹ 'Zeitschr. f. wiss. Zool.,' Bd. lii, 1891.

over an arc of about 60° from the upper pole in all directions." These "straggling" cells, as Assheton terms them, as well as the innermost cells of the now flattened inner cell-mass, are regarded as hypoblastic and the outermost cells of the same as epiblastic (embryonic epiblast). "The hypoblast, as a perfectly definite layer, is formed by the time the blastodermic vesicle measures .5 mm. in diameter, that is, about the 102nd hour after coition. It is not, however, as yet by any means a continuous membrane; it is a network or fenestrated membrane. For this reason, in section it appears to be represented by isolated cells lying beneath the embryonic disc (v. fig. 29, *Hy.*)" (cf. *Dasyurus*). In considering the question how the peripherally situated ("straggling") entodermal cells, which are undoubtedly derived from the inner cell-mass, "apparently wander round the inside of the blastodermic vesicle," he reaches the conclusion that this is not the result of amoeboid activity or growth "in the sense of migration" on the part of these cells, but "is only an apparent growth round produced by the more rapid growth of a zone of the [trophoblastic] wall of the vesicle immediately surrounding the embryonic disc, in which zone the marginal cells of the inner mass lie." He is unable to find any evidence of the production of pseudopodial processes by these peripheral entodermal cells, the majority of them appearing at first to be quite isolated from each other and approximately spherical. "Certain of the cells here and there are connected by threads of protoplasm, but this, I think, is not a sign of pseudopodic activity, but merely indicates the final stage in division between the two cells." By the sixth day the hypoblast of the embryonic disc has assumed the form of a continuous membrane, composed of completely flattened cells, whilst the peripheral hypoblast cells have become more numerous, and "many of them, possibly all of them, are now undoubtedly connected by more or less fine protoplasmic threads." Such, in brief, is Assheton's account of the early history of the entoderm in the rabbit; it presents obvious points of agreement with my

own for *Dasyurus*, and I venture to think the agreement is even greater than would appear from Assheton's conclusions. In adopting the view that the more active growth of the region of the blastocyst wall immediately surrounding the inner cell-mass is the sole causal agent in effecting the separation and peripheral spreading of the entodermal cells, I cannot but feel, in view of his own description and figures and of my own results, that he has attributed a much too exclusive importance to that phenomenon and a much too passive rôle to the entodermal cells themselves. In *Dasyurus* the inward migration and the later peripheral spreading of the entodermal cells is effected without any such marked unequal growth of the blastocyst wall as occurs, according to Assheton, in the rabbit, as the direct outcome of their own inherent activity, and I believe the possession of a like activity characterises the entodermal cells of the rabbit. The evidence of Assheton's own fig. 40, which shows in surface view a portion of the vesicle wall with the peripheral entodermal cells in relation thereto, and which should be compared with my figs. 68 and 69, conclusively demonstrates, to my mind, the possession by these cells of amœboid properties, and thus support is afforded for the belief that the separation of the entodermal cells from the formative cell group (inner cell-mass) is here also the expression of an actual migration. Whether or not the strands of protoplasm which Assheton ('08, '09) describes as present in the sheep, pig, ferret, and goat, connecting the inner lining of the inner mass to the wall of the blastocyst, and which he interprets as tending "to show that the inner lining of the inner mass is of common origin with the wall of the blastocyst," are of any significance in the present connection, I cannot certainly determine.

4. Summary.

The results and conclusions set forth in the preceding pages of this chapter may be summarised as follows:

- (1) The unilaminar wall of the blastocyst of *Dasyurus* con-

sists of two regions distinct in origin and in destiny, viz. an upper or formative region, derived from the upper cell-ring of the 16-celled stage, and destined to furnish the embryonal ectoderm and the entoderm and a lower or non-formative region derived from the lower cell-ring of the mentioned stage, and destined to form directly the extra-embryonal or trophoblastic ectoderm (tropho-ectoderm) of the bilaminar vesicle.

(2) The formative region, unlike the non-formative, is constituted by cells of two varieties, viz.: (i) a more numerous series of larger, lighter-staining cells destined to form the embryonal ectoderm, and (ii) a less numerous series of smaller, more granular, and more deeply staining cells, destined to give origin to the entoderm and hence distinguishable as the entodermal mother-cells.

(3) The entodermal mother-cells, either without or subsequently to division, bodily migrate inwards from amongst the larger cells of the unilaminar wall and so come to lie in contact with the inner surface of the latter. They thus give origin to the primitive entodermal cells from which the definitive entoderm arises. The larger passive cells, which alone form the unilaminar wall after the inward migration of the entodermal cells is completed, constitute the embryonal ectoderm.

(4) The entodermal cells as well before as after their migration from the unilaminar wall are capable of exhibiting amoeboid activity and of emitting pseudopodial processes, by the anastomosing of which there is eventually formed a cellular entodermal reticulum underlying, and at first co-extensive with, the embryonal ectoderm.

(5) The definitive entoderm thus owes its character as a connected cell-layer primarily to the formation of secondary anastomoses between the pseudopodial processes emitted by the primitive entodermal cells (or entodermal mother-cells).

(6) The assumption by the entodermal cells of amoeboid properties whilst they are still constituents of the unilaminar

wall affords an intelligible explanation of the mechanism of their inward migration.

(7) The entoderm is first laid down below the formative or embryonal region of the blastocyst; thence it extends gradually by its own growth round the inner surface of the unilaminar non-formative region so as to form eventually a complete entodermal lining to the blastocyst cavity. In this way the blastocyst wall becomes bilaminar throughout.

(8) The bilaminar blastocyst consists of two regions, respectively embryonal and extra-embryonal. The embryonal region (embryonal area) is constituted by an outer layer of embryonal ectoderm and the underlying portion of the entoderm, and the extra-embryonal, of the extra-embryonal or trophoblastic ectoderm (tropho-ectoderm), which is separated from the embryonal by a well-marked junctional line, together with the underlying portion of the entoderm, which is perfectly continuous with that below the embryonal ectoderm.

(9) The formative or embryonal region of the blastocyst in *Dasyurus* is from the first freely exposed, and at no time during the developmental period dealt with in this paper does there exist any cellular layer externally to it, i. e. a covering layer of trophoblast (Deckschicht, Rauber's layer) is absent and there is no entypy of the primary germ-layers (cf. p. 111).

CHAPTER V.—SOME EARLY STAGES OF PERAMELES AND MACROPUS.

The early material of *Perameles* and *Macropus* at my disposal comprises only a small number of stages, but is of special importance, since it enables me to demonstrate that so far as these particular stages are concerned, the early developmental phenomena in these forms are essentially the same as in *Dasyurus*, and thus affords ground for the belief that there is one common type of early development throughout the series of the Marsupialia. Moreover, it is of interest since it reveals the existence of what might be termed

specific differences in the early development of these Marsupials, especially in regard to the time of appearance of the entoderm. In *Dasyurus*, it will be remembered, the primitive entoderm cells first become definitely recognisable as internally situated cells in vesicles 4.5 mm. in diameter. In *Perameles* they occur in vesicles just over 1 mm. in diameter, while in *Macropus* they are already present in a blastocyst only .35 mm. in diameter, so that it would appear that the entoderm is differentiated much earlier in the higher, more specialised types than in the more generalised forms. This difference in time of appearance of the entoderm is perhaps to be correlated with a difference in size of the ovarian ova in the three genera mentioned.

1. *Perameles*.

The earliest material of *Perameles* I possess consists of two eggs of *P. obesula*, which I owe to the skill and enthusiasm of my friend Mr. S. J. M. Moreau, of Sydney. Egg A measures .23 mm. in diameter, and egg B, .24 × .23 mm. The former consists of thirty-two cells, the latter of thirty. In both the shell-membrane has partially collapsed, but the general plan of arrangement of the blastomeres can still fairly readily be made out. Fig. 51, Pl. 3, represents a micro-photograph of a section of egg B, the better of the two. It shows the shell-membrane (nearly .005 mm. thick) externally, considerable remains of the albumen between that and the deeply stained zona, and then, closely applied to the inner surface of the latter, the blastomeres arranged in the form of an inverted Ω , so as to enclose a central space, open below as the figure stands. This latter opening extends through the series, and it seems probable that there was a corresponding one opposite to it in the intact egg. Evidently we have here a stage in the formation of the blastocyst, in which the blastomeres are in course of spreading towards one or both of the poles of the sphere formed by the egg-envelopes,

just as happens in the corresponding stage of *Dasyurus* (cf. fig. 51 with fig. 31, though the latter represents a somewhat older stage in *Dasyurus*). The blastocyst-wall here appears relatively more extensive than in the 32-celled stage of *Dasyurus*, an apparent difference which may perhaps be accounted for by the difference in size of the respective eggs (.24 mm. as compared with .36 mm.). The blastomeres situated adjacent to the opening and those on the right side of the figure tend to be more flattened and of greater superficial extent than the remainder, but I can recognise no difference in the cytological characters of the cells. The space or cleavage cavity enclosed by the blastomeres is partly occupied by a granular coagulum, and towards the opening there is present a lightly staining reticular mass, which recalls the yolk-body of *Dasyurus*, though I am not prepared to affirm that it is of that significance. The fixation of the specimen is not quite perfect.

My next stage of *Perameles* is constituted by a blastocyst of *P. nasuta*, for which I am again indebted to Mr. Moreau measuring in the preserved condition .29 × .26 mm. Fig. 52, Pl. 3, shows a section of this blastocyst. Structurally, it corresponds in all essential respects with the .43 mm. blastocyst of *Dasyurus*, figured on the same plate (fig. 33). The blastocyst wall is complete and unilaminar throughout. It is distinguishable into two regions, a more extensive region over which the cells are large and flattened and a less extensive, composed of smaller but thicker cells (left side of fig. 52). In the early blastocysts of *Dasyurus*, it may be recalled, the evidence showed that the region of more flattened cells is formative in significance, that of more bulky cells, non-formative. It is possible the same holds good for this *Perameles* blastocyst. On the other hand, the structural condition of the stage next to be described rather supports the view that the smaller region, composed of plumper cells, is in this case formative. That view seems to me the more probable of the two, but there is a considerable difference in size between the present blastocyst and those next available, so that it is

impossible to decide this point with certainty. The blastocyst cavity is partly occupied by coagulum. There are no cells present in it, but the question of the presence of a yolk-body must remain open. The shell-membrane ($\cdot 0045$ mm. in thickness) and zona are in close apposition.

Following this early blastocyst, I have three vesicles of *P. nasuta*, two of them measuring $1\cdot 3$ mm. in diameter, the other $1\cdot 1$ mm. In their stage of development they agree pretty closely with the $4\cdot 5$ – 5 mm. vesicles of *Dasyurus*, referred to in the preceding pages under the designation 6, '04, the entoderm being in process of differentiation. The formative region was readily distinguishable in the intact vesicles as a darker patch occupying about three eighths of the surface extent of the wall. In section (Pl. 8, figs. 80, 81) it is characterised by its greater thickness as compared with the non-formative or trophoblastic region, and by the presence below it of numbers of primitive entodermal cells. Compared with the corresponding stage in *Dasyurus*, the chief difference consists in the relatively much greater thickness of the cells of the formative region in the *Perameles* vesicle. The latter cells are here already more or less definitely cubical in shape, their thickness varying from $\cdot 09$ mm. to $\cdot 015$ mm., and altogether they form a layer of a much more uniformly thickened character than that of the 6, '04 vesicles of *Dasyurus*. The trophoblastic ectoderm (figs. 80, 81, *tr. ect.*) is composed of somewhat flattened cells, varying in thickness from $\cdot 005$ to $\cdot 008$ mm.

The primitive entodermal cells (figs. 80, 81, *ent.*) are present below the formative region in fair abundance, more especially around the periphery of the same, which may thus appear somewhat thickened (fig. 81). The cells vary in size from $\cdot 01 \times \cdot 007$ mm. to $\cdot 024 \times \cdot 009$ mm., and they stain on the whole somewhat more deeply than the formative cells, to whose under-surface they are closely applied. They occur singly and in groups. Mitotic figures are frequently met with in the cells of the formative area (observe the obliquely disposed figure in one of the formative cells in fig. 81), and

they also occur in the primitive entodermal cells. Examination of the sections leaves no doubt in one's mind as to the source of the entodermal cells. They are undoubtedly derived from the formative region of the vesicle wall. The shell-membrane has a thickness of about $\cdot 0027$ mm.

2. *Macropus*.

Of *Macropus* the earliest stage I have examined is a blastocyst of *M. ruficollis*, $\cdot 25 \times \cdot 21$ mm. in diameter. It is not in a quite perfect state of preservation, but is in a sufficiently good condition to enable me to say that the wall is complete and unilaminar throughout, just as in the $\cdot 29 \times \cdot 26$ mm. blastocyst of *Perameles*. The shell-membrane has a thickness of about $\cdot 005$ mm., and there are still remains of the albumen between it and the zona.

My next stage (figs. 82-85) is a blastocyst of the same species, $\cdot 35$ mm. in diameter. It unfortunately suffered in preparation, but practically the whole of the formative area of the blastocyst wall and part of the trophoblastic ectoderm are comprised in the sections (Pl. 9, fig. 82), so that it is still possible to make out its chief structural features. In its stage of development this blastocyst closely agrees with the last described blastocysts of *Perameles*. The formative area of the wall is perfectly distinct in the sections because of its greater thickness and the presence below it of the primitive entodermal cells. It attains its greatest thickness ($\cdot 027$ mm.) peripherally, whilst it is thinnest centrally ($\cdot 006$ mm.), so that, taken as a whole, it is not quite such a uniformly thickened layer as is that of the *Perameles* blastocysts. Primitive entodermal cells are present below it, but not in great abundance (figs. 82, 84, 85, *ent.*). In fig. 83, a formative cell is seen in division, the axis of the spindle being oblique to the surface. The trophoblastic ectoderm (figs. 82, 83, *tr. ect.*) is composed of the usual flattened cells, and varies in thickness from $\cdot 005$ to $\cdot 0067$ mm.

In the blastocyst cavity, adjacent to the trophoblastic

ectoderm on the left side of fig. 82, there is visible a small spherical cell similar to the degenerate cells met with in blastocysts of *Dasyurus*.

My last stage of *M. ruficollis* comprises an excellently preserved blastocyst, measuring $\cdot 8$ mm. in diameter, in which the embryonal ectoderm and the entoderm are definitely established. It thus corresponds to the 8, '01 stage of *Dasyurus* (blastocysts 5-5.5 mm. diameter). The embryonal area is circular and measures $\cdot 468$ mm. in diameter. Its constituent cells are cubical and from $\cdot 008$ to $\cdot 013$ mm. in thickness, whilst the trophoblastic ectoderm is formed of flattened cells, $\cdot 006$ mm. in thickness. The entoderm is present as a continuous layer of attenuated cells below the embryonal ectoderm, and it probably also forms a continuous layer below the trophoblastic ectoderm. Entodermal cells are certainly present over the lower polar region of the vesicle, but it is difficult to be certain from the sections whether or not they form a perfectly continuous layer. The shell membrane has a thickness of $\cdot 0026$ mm.

I have a corresponding blastocyst of *Petrogale penicillata* $\cdot 915$ mm. in diameter, with an oval, embryonal area $\cdot 525 \times \cdot 45$ mm. in diameter, and a later blastocyst of *M. ruficollis* 1.46 mm. in diameter, with a circular embryonal area $\cdot 57$ mm. in diameter.

CHAPTER VI.—GENERAL SUMMARY AND CONCLUSIONS.

The observations recorded in the preceding pages and the conclusions deducible therefrom may be summarised as follows :

(A) Ovum.—The uterine ovum of *Dasyurus* is characterised (1) by its large size relatively to those of Eutheria; (2) by the presence externally to the zona of a layer of albumen and a shell-membrane, both laid down in the Fallopian tube and homologous with the corresponding structures in the Monotreme ovum, the shell-membrane, like the shell of the latter, increasing in thickness in the uterus; (3) by its marked

polarity, its lower two thirds consisting of formative cytoplasm, dense and finely granular in appearance, owing to the presence of fairly uniformly distributed deutoplasmic material, and containing the two pronuclei, its upper third being relatively clear and transparent, consisting as it does of a delicate reticulum of non-formative cytoplasm, the meshes of which are occupied by a clear deutoplasmic fluid. Study of the process of vitellogenesis in ovarian ova demonstrates that this fluid represents surplus deutoplasmic material which has not been utilised in the upbuilding of the formative region of the ovum.

The fate of the clear non-formative portion of the ovum is a very remarkable one. Prior to the completion of the first cleavage, it is separated off from the formative remainder of the ovum as a spherical mass or yolk-body, which takes no direct part in development, though it becomes enclosed in the blastocyst cavity on completion of the blastocyst wall at the upper pole. Its contained deutoplasmic fluid is to be regarded as the product of an abortive attempt at the formation of a solid yolk-mass, such as is found in the Monotreme ovum. By its elimination the potentially yolk-laden telolecithal ovum becomes converted into a secondarily homolecithal, holoblastic one. All the evidence is held to support the conclusion that the Marsupials are descended from oviparous ancestors with meroblastic ova.

(B) Cleavage.—Cleavage begins in the uterus, is total, and at first equal and of the radial type. The first two cleavage planes are meridional and at right angles to each other. The resulting four equal-sized blastomeres lie disposed radially around the polar diameter like those of the Monotreme (not in pairs at right angles to each other as in Eutheria), and enclose a segmentation cavity open above and below, their upper ends partially surrounding the yolk-body. The third cleavage planes are again meridional, each of the four blastomeres becoming subdivided equally into two. The resulting eight cells form an equatorial ring in contact with the inner surface of the sphere formed by the egg-envelopes. They

contain deutoplasmic material, which is, however, located mainly in their lower halves. The ensuing fourth cleavages are equatorial, and in correlation with the just-mentioned disposition of the deutoplasm, are unequal and qualitative, each of the eight blastomeres becoming subdivided into an upper smaller and clearer cell, with relatively little deutoplasm fairly uniformly dispersed through the cytoplasm, and a lower larger, more opaque cell with much deutoplasm, mainly located in a broad zone in the outer portion of the cell-body. A 16-celled stage is thus produced in which the blastomeres are characteristically arranged in two superimposed rings, each of eight cells, an upper of smaller, clearer cells next the yolk-body, and a lower of larger, denser cells. The former is destined to give origin to the formative or embryonal region of the blastocyst wall, the latter to the non-formative or extra-embryonal region of the same.

(c) Formation of the Blastocyst.—There is in the Marsupial no morula stage as in Eutheria, the blastomeres proceeding directly to form the wall of the blastocyst. The cells of the two rings of the 16-celled stage divide at first meridionally and then also equatorially, the division planes being always vertical to the surface. The daughter-blastomeres so produced, continuing to divide in the same fashion, gradually spread towards opposite poles in contact with the inner surface of the firm sphere formed by the zona and the thickened shell-membrane. Eventually they form a complete cellular lining to the said sphere and it is this which constitutes the wall of the blastocyst. The latter is accordingly unilaminar at its first origin, and it remains so in *Dasyurus* until it has attained, as the result of active growth accompanied by the imbibition of fluid from the uterus, a diameter of 4–5 mm. It consists of two parts or regions, distinct in origin and in destiny, and clearly marked off from each other in later blastocysts by a definite junctional line approximately equatorial in position, viz. an upper, embryonal or formative region derived from the upper cell-ring of the 16-celled stage, and a lower, extra-embryonal or non-

formative region derived from the lower cell-ring of the same stage.

(D) Later History of the Two Regions of the Blastocyst Wall (for details see pp. 72-74).—From the embryonal region are derived the embryonal ectoderm and the entire entoderm of the vesicle. I conclude, therefore, that it is the homologue of the inner cell-mass or embryonal knot of the Eutherian blastocyst. The extra-embryonal region directly furnishes the outer extra-embryonal layer of the vesicle wall, i. e. the outer layer of the omphalopleure and chorion of later stages. Assuming, as the facts of comparative anatomy and palæontology entirely justify us in doing, that the Mammals are monophyletic and of reptilian origin, and further assuming that the foetal membranes are homologous structures throughout the Amniotan series (also in my view a perfectly justifiable assumption)¹, then the homologies of this extra-embryonal region of the Marsupial blastocyst are not far to seek. It is clearly the homologue of the extra-embryonal ectoderm of the Sauropsidan and Monotreme egg, and the homologue also of the outer enveloping layer of the Eutherian blastocyst, to which Hubrecht has given the special name of "trophoblast." In my view the trophoblast is none other than extra-embryonal ectoderm which in the viviparous mammals, in correlation with the intra-uterine mode of development, has acquired a special significance for the nutrition of the embryo.

These, then, are my conclusions, and to me they seem on general grounds perfectly obvious, viz.: (1) that the embryonal or formative region of the unilaminar Marsupial blastocyst is the homologue of the inner cell-mass or

¹ How Assheton can maintain ('09, p. 266) "that the amnion of the rabbit is not more homologous to the amnion of the Sauropsidan than the horny teeth of *Ornithorhynchus* are homologous to the true teeth of the mammal or reptile, which they have supplanted," how he can hold this view and yet proceed to utilise the presence of the amnion as one of the leading characters distinguishing the Amniota from the Anamnia, I fail to comprehend. Surely the presence of a series of purely analogous structures in a group is of no classificatory value.

Table of Comparison of the Early Ontogeny in the Three Mammalian Sub-classes.

	Prototheria.	Metatheria.	Eutheria.
Secondary egg- envelopes	Shell	Shell-membrane	No shell-membrane.
Ovum	Albumen	Albumen	Albumen (in some forms).
	Zona	Zona	Zona.
	Large for mammalia (3.5-4 mm. in diameter), telolecithal with discrete yolk-spheres, like those of <i>Sauropsida</i> .	Minute compared with that of Prototheria, but larger than average Eutherian ovum (.24 mm. in diameter in <i>Dasyurus</i>), telolecithal in type, but becoming secondarily homolecithal as result of elimination of surplus deutoplasmic material.	Minute (varying from .07 mm. in mouse to .2 mm. in man), with, in some forms, polar differentiation of its formative and deutoplasmic materials.
Cleavage	Meroblastic, at first radial, blastomeres of 4-celled stage being radially arranged around polar diameter; results in formation of a several-layered cleavage-disc which gives origin to a unilaminar blastodermic membrane (Semon), composed of embryonal and extra-embryonal regions, though no clear line of separation between them has as yet been recognised.	Secondarily holoblastic and of radial type; blastomeres of 4-celled stage radially arranged and enclosing segmentation cavity. Third cleavages meridional like the first two; in absence of yolk-mass the resulting eight blastomeres forming an open ring. Fourth cleavages equatorial, unequal and qualitative, with resulting formation of two superimposed cell-rings, respectively formative (embryonal) and non-formative (extra-embryonal) in significance.	Secondarily holoblastic; blastomeres of 4-celled stage, in absence of yolk-mass and shell, not arranged radially but in two pairs at right angles to each other, thus forming a cross-shaped group. Further divisions result in formation of solid morula, composed of two predetermined groups of cells, homologous with the formative and non-formative cell-rings of the Metatherian.

	Prototheria.	Metatheria.	Eutheria.
Blasto- cyst	Formed directly by the peripheral growth of the extra-embryonal region of the unilaminar blastodermic membrane, in contact with inner surface of zona. Blastocyst cavity represents subgerminal cavity extended by liquefaction so as to include entire yolk-mass.	Formed directly by the spreading towards opposite poles, of the products of division of the two cell-rings of the 16-celled stage in contact with inner surface of the sphere constituted by the egg envelopes. Blastocyst cavity represents the persistent segmentation cavity.	Formed indirectly as result of complete envelopment of the formative by the non-formative cells in the morula stage, and subsequent formation of blastocyst cavity by the confluence of inter- or intra-cellular vacuolar spaces, either or both.
—	Embryonal region of unilaminar blastodermic membrane freely exposed and superficial, there being no evidence of the existence at any period during early stages of development of a cellular layer externally to it. The entypic condition therefore does not occur.	Unilaminar wall of blastocyst formed conjointly by the formative (embryonal) and non-formative (extra-embryonal) regions; formative region from the first superficial. The entypic condition therefore does not occur.	Unilaminar wall of blastocyst formed exclusively by the non-formative (tropho-ectodermal) cells, the formative cells of the embryonal knot being completely enclosed, i.e. the entypic condition occurs.
—	Embryonal region of unilaminar blastodermic membrane probably gives origin to parent cells of entoderm by a process of proliferation. At an early period before blastocyst wall is completed (?). • Extra-embryonal region of same directly forms the extra-embryonal ectoderm.	Formative region of blastocyst wall furnishes the embryonal ectoderm and the entire entoderm of the vesicle, and is therefore homologous with the formative region of Marsupial blastocyst. The enveloping layer of non-formative cells forms the outer layer of the omphalopleure and chorion of later stages, and is therefore homologous with the extra-embryonal ectoderm of the Prototheria.	The embryonal knot furnishes the embryonal ectoderm and the entire entoderm of the vesicle, and is therefore homologous with the formative region of Marsupial blastocyst. The enveloping layer of non-formative cells forms the outer layer of the omphalopleure and chorion of later stages, and is therefore the homologue of the non-formative region of the Marsupial blastocyst and of the extra-embryonal ectoderm of the Monotreme.

embryonal knot of the Eutherian blastocyst; and (2) that the extra-embryonal or non-formative region of the same is the homologue of the extra-embryonal ectoderm of the Sauropsida and Monotremata and of the trophoblast of the Eutheria.

As regards conclusion (1) there is not likely to be much difference of opinion, but as regards (2), whilst perhaps the majority of embryologists support the obvious, not to say common-place view which I here advocate, it seems certain that it will prove neither obvious nor acceptable to those mammalian embryologists (I refer specifically to my friends Professor A. A. W. Hubrecht and Mr. R. Assheton) who, with only Selenka's account of early Marsupial ontogeny before them, have formulated other and quite divergent views as to the morphological nature of the outer enveloping layer of the Eutherian blastocyst. It is therefore necessary to discuss this question further, though I would fain express my conviction that had the observations recorded in this paper been earlier available, much vain speculation as to the phylogeny of the trophoblast might possibly have been avoided.

CHAPTER VII.—THE EARLY ONTOGENY OF THE MAMMALIA IN THE LIGHT OF THE FOREGOING OBSERVATIONS.

In entering on a discussion of the bearings of the results of my study of the early development of Marsupials on current interpretations of early Mammalian ontogeny, and especially of the homologies of the germ-layers, I desire at the outset to emphasise my conviction that, specialised though the Marsupials undoubtedly are in certain features of their anatomy, e. g. their dentition, genital ducts, and mammary apparatus, the observations recorded in the preceding pages of this paper afford not the slightest ground for the supposition that their early ontogeny is also of an aberrant type, devoid of significance from the point of view of that of other mammals. On the contrary, I hope to demonstrate that the Marsupial type of early development not only readily

falls into line with that of Eutheria, and with what we know of the early development of the Prototheria, but furnishes us with the key to the correct interpretation of that extraordinarily specialised developmental stage, the Eutherian blastocyst. In particular I hope to show that the description which I have been able to give of the mode of formation of the Marsupial blastocyst, bridges in the most satisfactory fashion the great gap which has till now existed in our knowledge of the way in which the transition from the Monotrematous to the Eutherian type of development has been effected.

1. The Early Development of the Monotremata.

Our knowledge of the early development of the oviparous mammals is admittedly still far from complete. Nevertheless it is not so absolutely fragmentary that it can be passed over in any general discussion of early mammalian ontogeny, and I certainly cannot agree with the opinion of Assheton ('08, p. 227) that from it "we gain very little help towards the elucidation of Eutherian development." On the contrary, I think that the combined observations of Semon ('94), and Wilson and Hill ('07) shed most valuable light on the early ontogenetic phenomena in both the Metatheria and Eutheria. I propose therefore to give here a very brief resumé of the chief results of these observers,¹ and at the same time to indicate how the knowledge of early Monotreme ontogeny we possess, limited though it be, does help us to a better understanding of the phenomena to which I have just referred.

The ovum, as is well known from the observations of Caldwell ('87), is Reptilian in its character in all but size. It is yolk-laden and telolecithal, the yolk consisting of discrete yolk-spheres, and it is enclosed outside the zona (vitelline membrane) by a layer of albumen and a definite shell.

¹ In so doing I have largely utilised the phraseology of Wilson and Hill's paper ('07).

At the moment of entering the oviduct it has a diameter of 3.5–4 mm. (2.5–3 mm. according to Caldwell), and is therefore small relatively to that of a reptile of the same size as the adult Monotreme, but large relatively to those of other mammals, being about twelve times larger than that of *Dasyurus*, and about eighteen times larger than that of the rabbit.

Cleavage is meroblastic. The first two cleavage planes are at right angles to each other, as in the Marsupial, and divide the germinal disc into four approximately equal-sized cells (Semon, Taf. ix, fig. 30). Each of these then becomes subdivided by a meridional furrow into two, so that an 8-celled stage is produced, the blastomeres being arranged symmetrically, or almost symmetrically, on either side of a median line, perhaps corresponding to the primary furrow (Wilson and Hill, p. 37, text-figs. 1 and 2). Imagine the yolk removed and the blastomeres arranged radially, and we have at once the open ring-shaped 8-celled stage of *Dasyurus*. The details of the succeeding cleavages are unknown. Semon has described a stage of about twenty-four cells (Semon, Taf. ix, fig. 31), in which the latter formed a one-layered circular plate with no evidence of bilateral symmetry, and this is succeeded by a stage also figured by Semon (figs. 32 and 33, cf. also Wilson and Hill, Pl. 2, fig. 2), in which the blastoderm has become several cells thick, though it has not yet increased in surface extent. It is bi-convex lens-shaped in section, its lower surface being sharply limited from the underlying white yolk. No nuclei are recognisable in the latter, either in this or any subsequent stage, nor is there ever any trace of a syncytial germ-wall, features in which the Monotreme egg differs from the Sauropsidan.

The next available stage, represented by an egg of *Ornithorhynchus*, described by Wilson and Hill ('07, p. 38, Pl. 2, fig. 4), and by an egg of *Echidna*, described by Semon ('94, p. 69, figs. 22 and 33), is separated by a considerable gap from the preceding, and most unfortunately so, since it belongs to the period of commencing formation of the germ-layers. The

cellular lens-shaped blastoderm of the preceding stage has now extended in the peripheral direction so as to enclose about the upper half of the yolk-mass, and in so doing it has assumed the form, almost exclusively, of a unilaminar thin cell-membrane, composed of flattened cells and closely applied to the inner surface of the zona. At the embryonic pole, however, in the region of the white yolk-bed, there are present in the *Ornithorhynchus* egg a few plump cells, immediately subjacent to the unilaminar blastoderm, but separate and distinct from it, whilst in the *Echidna* egg Semon's figure (fig. 33), which is perhaps somewhat schematic, shows a group of scattered cells, similar to those in the *Ornithorhynchus* egg but placed considerably deeper in the white yolk-bed. Unfortunately we have no definite evidence as to the significance of these internally situated cells. One of two possible interpretations may be assigned to them. Either they represent the last remaining deeply placed cells of the blastodisc of the preceding stage, which have not yet become intercalated in the unilaminar blastodermic membrane believed by Semon to be the condition attained in eggs of about this stage of development, or they are cells which have been proliferated off from this unilaminar blastoderm, to constitute the parent cells of the future yolk-entoderm. As regards *Echidna*, Semon expresses a definite enough opinion; he holds that these deeply placed cells actually arise by a somewhat diffuse proliferation or ingrowth from a localised depressed area of the blastoderm at the embryonic pole, and that they give origin to yolk-entoderm. This interpretation of Semon seems probable enough in view of the mode of origin of the entoderm in the *Metatheria* and *Eutheria*. Moreover in the next available stage, an egg of *Ornithorhynchus*, just over 6 mm. in diameter, described by Wilson and Hill, the blastoderm is already bilaminar throughout its extent, so that we might very well expect to find the beginnings of the entoderm in the somewhat younger eggs.

In the 6 mm. egg just referred to, the peripheral portion of the unilaminar blastoderm of the preceding stage has grown

so as to enclose the entire yolk-mass in a complete ectodermal envelope, whilst internally to that a complete lining of yolk-entoderm has become established. As the result of these changes, and of the imbibition of fluid from the uterus, the solid yolk-laden egg has become converted into a relatively thin-walled vesicle or blastocyst, possessed of a bilaminar wall surrounding the partly fluid vitelline contents of the egg. Throughout the greater part of its extent the structure of the vesicle wall is very simple. It consists externally of an extremely attenuated ectodermal cell-membrane closely adherent to the deep surface of the vitelline membrane (zona), and within that of a layer of yolk-entoderm, composed of large swollen cells, containing each a vesicular nucleus, and a number of yolk-spheres of varying size. Over a small area, overlying the white yolk-bed, however, the ectodermal layer of the wall presents a different character to that described above. Its constituent cells are here not flattened and attenuated, but irregularly cuboidal in form and much more closely packed together; moreover they stand in proliferative continuity with a subjacent mass of cells, also in process of division. The irregular superficial layer and this latter mass together form a thickened lenticular cake, .5 mm. in greatest diameter, projecting towards the white yolk-bed but separated from it by the yolk-entoderm, which retains its character as a continuous cell-membrane. This differentiated, thickened area of the wall, situated as it is at the upper pole of the egg, as marked by the white yolk-bed, must be held to represent a part of the future embryonal region. Wilson and Hill incline to regard it as in some degree the equivalent of the "primitive plate" of Reptiles and as the initial stage in the formation of the primitive knot of later eggs. This question, however, does not closely concern us here: the point I wish to emphasise is the relative inactivity of the cells composing the embryonal region of the blastoderm in the Monotreme as compared with the marked activity displayed by those constituting the peripheral (extra-embryonal) region of the same. It is these latter cells which by their

rapid growth complete the envelopment of the yolk-mass and so constitute the lower hemisphere of the blastocyst.

The bilaminar blastocyst of the Monotreme, formed in the manner indicated above, is entirely comparable with the Marsupial blastocyst of the same developmental stage. There are differences in detail certainly (e. g. in the characters, time of formation, and rate of spreading of the entoderm, in the mode of formation of the blastocyst cavity and in its contents, in the apparent absence in the Monotreme of any well-marked line of division between the embryonal and extra-embryonal regions of the ectoderm, in the relatively earlier appearance of differentiation in the embryonal region in the Monotreme as compared with the Marsupial), but the agreements are obvious and fundamental; in particular, I would emphasise the fact that in both the embryonal region is superficial and freely exposed, and forms part of the blastocyst wall just as that of the reptile forms part of the general blastoderm. Moreover, should future observations confirm the view of Semon that the primitive entodermal cells of the Monotreme are proliferated off from the embryonal region of the unilaminar blastoderm, then we should be justified in directly comparing the latter with the unilaminar wall of the Marsupial blastocyst, and in regarding it also as consisting of two differentiated regions, viz. a formative or embryonal region, overlying the white yolk-bed, and giving origin to the embryonal ectoderm and the yolk-entoderm, and a non-formative region which rapidly overgrows the yolk-mass so as to eventually completely enclose it, just as does the less rapidly growing extra-embryonal ectoderm of the Sauropsidan blastoderm.¹ Meantime I see no reason for doubting that this rapidly growing peripheral portion of the unilaminar blastoderm of the Monotreme is anything else than extra-embryonal ectoderm homogenous with that of the reptile. Indeed, I am not aware that any embryologist except Hubrecht thinks otherwise. Even Assheton is, I believe, content to

¹ We should further be justified in concluding that the entoderm is similar in its mode of origin in all three mammalian sub-classes.

regard the outer layer of the Monotreme blastocyst as ectodermal. Hubrecht's view is that the primitive entodermal cells of Semon give origin, not to yolk-entoderm, but to the equivalent of the embryonal knot of Eutheria, whilst the unilaminar blastodermic membrane itself is a larval layer—the trophoblast—that portion of it overlying the internally situated cells representing the covering layer (Rauber's layer) of the Eutherian blastocyst. "For this view," remarks Assheton [1909, p. 233], "I can see no reason derivable from actual specimens described and figured by those four authors" (Caldwell, Semon, Wilson and Hill), with which criticism I am in entire agreement, as also with the following statement, which, so far as the Metatheria are concerned, is based on my own results: "Neither in the Prototheria [n] or the Metatheria is there really any tangible evidence of a trophoblast occurring as a covering layer over the definitive epiblast as in Eutheria" (p. 234).

In connection with the peripheral growth of the unilaminar blastoderm in the Monotreme, it is of interest to observe that this takes place, not apparently in intimate contact with the surface of the solid yolk, as is the case with the growing margin of the extra-embryonal ectoderm in the Sauropsidan egg, but rather in contact with the inner surface of the thickened zona, perhaps as the result of the accumulation in the perivitelline space of fluid which has diffused into the latter from the uterus. In other words, the peripheral growth of the extra-embryonal ectoderm to enclose the yolk-mass appears to take place here in precisely the same way as the spreading of the non-formative cells in *Dasyurus* to complete the lower pole of the blastocyst. In my view the latter phenomenon is none other than a recapitulation of the former; on the other hand, I regard the spreading of the formative cells in *Dasyurus* towards the upper pole as a purely secondary feature, conditioned by the loss of the yolk-mass and the attainment of the holoblastic type of cleavage.

If it be admitted that the outer extra-embryonal layer of the Monotreme blastocyst is homogenous with the extra-

embryonal ectoderm of the Reptile, then it seems to me there is no escape from the conclusion that these layers are also homogenous with the non-formative region of the unilaminar Marsupial blastocyst. I need only point out here that the chief destiny of each of the mentioned layers, and I might also add that of the outer enveloping layer of the Eutherian blastocyst (the so-called trophoblast), is one and the same, viz. to form the outer layer of the chorion (false amnion, serous membrane) and omphalopleure (unsplit yolk-sac wall, Hill [97]),¹ and that to deny their homogeneity to each other implies the non-homogeneity of these membranes and the amnion in the Amniotan series, and consequently renders the group name Amniota void of all morphological meaning.

The rapidity with which the enclosure of the yolk-mass is effected, and the relative tardiness of differentiation in the embryonal region are features which sharply distinguish the early ontogeny of the Monotremes from that of the Sauropsida, and which, in my view, are of the very greatest importance, since they afford the key to a correct understanding of the peculiar cœnogenetic modifications observable in the early ontogeny of the Metatheria and Eutheria. To appreciate the significance of these features it is necessary to take account of the great difference which exists between the Sauropsidan and Monotreme ovum in regard to size, as well as of the very different conditions under which the early development goes on in the two groups. The Sauropsidan egg is large enough to contain within its own confines the amount of yolk necessary for the production of a young one complete in all its parts and capable of leading an independent existence immediately it leaves the shell. Furthermore, it is also large

¹ In certain Amniotes the layers in question appear also to participate in the formation of the inner lining of the amnion (amniotic ectoderm) (cf. Assheton [09], pp. 248-9), but this does not affect the statement in the text. In the Sauropsida and Monotremata I think I am correct in saying that no sharp distinction is recognisable between the embryonal and extra-embryonal regions of the ectoderm, hence it is difficult, if not impossible, to determine with certainty their relative participation in the formation of the amniotic ectoderm.

enough to provide room for the development of an embryo without any secondary growth in size after it leaves the ovary. Moreover we have to remember that after it has become enclosed in the shell, it remains but a short time in the oviduct and receives little or no additional nutrient material from the oviducal walls. The yolk-mass in any case retains its solid character; there is no necessity for its rapid enclosure, and so enclosure is effected slowly, contemporaneously with the differentiation of the embryo.

In the Monotreme the conditions are altogether different. The ripe ovarian ovum when it enters the oviduct has a diameter of about 3.5 to 4 mm., and is thus considerably smaller than that of a Reptile of the same size as the adult Monotreme. The amount of yolk which it is capable of containing is not anything like sufficient to last the embryo throughout the developmental period, and, moreover, it does not provide the space essential for the development of an embryo on the ancestral Reptilian lines. As Assheton ('98, p. 251) has pointed out, "the difference in size between the fertilised ovum of a reptile or bird or of a mammal is very great; but the difference in size between the embryo of, say, a bird with one pair of mesoblastic somites and of a mammal of the same age is comparatively small. This means that nearly the same space is required for the production of the mammalian embryo as of the Sauropsidan, and has to be provided." In the Monotreme not only is additional room necessary, but also additional nutrient material, sufficient with that already present in the egg to last the embryo throughout the period of incubation. Both are acquired contemporaneously during the sojourn of the egg in the uterine portion of the oviduct, wherein the egg increases greatly in size. When it enters the uterus, the Monotreme egg has a diameter, inclusive of its membranes, of about 4-5 mm.; when it is laid, it measures in *Ornithorhynchus*, in its greatest diameter, 16-19 mm., and somewhat less in the case of *Echidna*. Prior to the enclosure of the yolk the increase in diameter, due to the accumulation of fluid in

the perivitelline space and between the zona and shell, is but slight. But as soon as the yolk becomes surrounded by a complete cellular membrane, i. e. as soon as the egg has become converted into a thin-walled blastocyst, rapid growth sets in, accompanied by the active imbibition of the nutrient fluid, which is poured into the uterine lumen as the result of the secretory activity of the abundantly developed uterine glands. The fluid absorbed not only keeps the blastocyst turgid, but it brings about the more or less complete disintegration of the yolk-mass, its constituent spherules becoming disseminated in the fluid contents of the blastocyst cavity. Although a distinct and continuous subgerminal cavity, such as appears beneath the embryonal region of the Sauropsidan blastoderm, does not occur in the Monotreme egg, vacuolar spaces filled with fluid develop in the white yolk-bed underlying the site of the germinal disc and appear to represent it. As Wilson and Hill remark ('03, p. 317), "one can, without hesitation, homologise the interior of the vesicle with the subgerminal cavity of a Sauropsidan egg, extended so as to include by liquefaction the whole of the yolk itself." In the Marsupial the blastocyst cavity has a quite different origin, since it represents the persistent segmentation cavity, whilst in the Eutheria the same cavity is secondarily formed by the confluence of intra- or inter-cellular vacuolar spaces, but no one, so far as I know, has ever ventured to assert that, because of this difference in mode of origin, the blastocyst cavity in the series of the Mammalia is a non-homogenous formation.

To return to the matter under discussion, it appears to me that the necessity which has arisen, consequent on the reduction in size of the ovum, for rapid growth of the same in order to provide room for the development of an embryo and for the storage of nutrient material furnished by the maternal uterus, affords a satisfactory explanation of the much more marked activity of the extra-embryonal region of the blastoderm as compared with the embryonal, which is such a striking feature in the early ontogeny of the Monotremes, and not

only of them, but, as Assheton has pointed out ('98, p. 251), of the higher mammals as well (cf. the process of epiboly and the inertness at first displayed by the formative cells of the embryonal knot as compared with the activity of the non-formative or tropho-ectodermal cells), an activity which results in the rapid completion of that characteristically mammalian developmental stage—the blastocyst or blastodermic vesicle.

The necessity for the early formation of such a stage, capable of rapidly growing in a nutrient fluid medium provided by the mother, has profoundly influenced the early ontogeny in all three mammalian subclasses, and naturally most of all that of the Eutheria, in which reduction of the ovum, both as regards size and secondary envelopes, has reached the maximum. And I think there can be little doubt but that it is this necessity which has induced that early separation of the blastomeres into two categories, respectively formative and non-formative in significance, which has long been recognised as occurring in Eutheria, and which I have shown also occurs amongst the Metatheria. This early separation of the blastomeres into two distinct groups is not recognisable in the Sauropsida, and the idea that it is in some way connected with the loss of yolk which the mammalian ovum has suffered in the course of phylogeny, was first put forward, I believe, by Jenkinson. In his paper on the germinal layers of Vertebrata ('06, p. 51) he writes: "Segmentation therefore is followed in the Placentalia by the separation of the elements of the trophoblast from those destined to give rise to the embryo and the remainder of its foetal membranes, and this 'precocious segregation' seems to have occurred phylogenetically during the gradual loss of yolk which the egg of these mammals has undergone." Whether or not such a "precocious segregation" has already become fixed in the Monotremes, future investigation must decide (cf. ante, p. 90).

The loss of yolk, with resulting reduction in size which the Monotreme ovum has suffered in the course of phylogeny, we

must assume to have taken place gradually and in correlation with the longer retention of the egg in the oviduct, the elaboration of the uterine portion of the same as an actively secretory organ, and the evolution of the mammary apparatus. The Monotremes thus render concrete to us one of the first great steps in mammalian evolution so far as developmental processes are concerned, viz. the substitution for intra-ovular yolk of nutrient material furnished directly by the mother to the developing egg or embryo. We see in them the beginnings of that process of substitution of uterine for ovarian nutriment which reaches its culmination in the Eutheria with their microscopic yolk-poor ova and long intra-uterine period of development. The Marsupials show us in *Dasyurus* an interesting intervening stage so far as the ovum is concerned, in that this, though greatly reduced as compared with that of the Monotreme, still retains somewhat of its old tendencies and elaborates more yolk-material than it can conveniently utilise, with the result that it has to eliminate the surplus before cleavage begins. But as concerns their utilisation of intra-uterine nutriment, they have specialised along their own lines, and instead of exhausting the possibilities implied by the presence of that, they have extensively elaborated the mammary apparatus for the nutrition of the young, born in a relatively immature state, after a short period of intra-uterine life (cf. Wilson and Hill [’97, p. 580]).

In view of the fact that the young Monotreme enjoys three developmental periods, viz. intra-uterine, incubatory, and lactatory, the question might be worthy of consideration whether it may not be that the Marsupial has merged the incubatory period in the lactatory, the Eutherian the same in the intra-uterine.

2. The Early Development of the Metatheria and Eutheria.

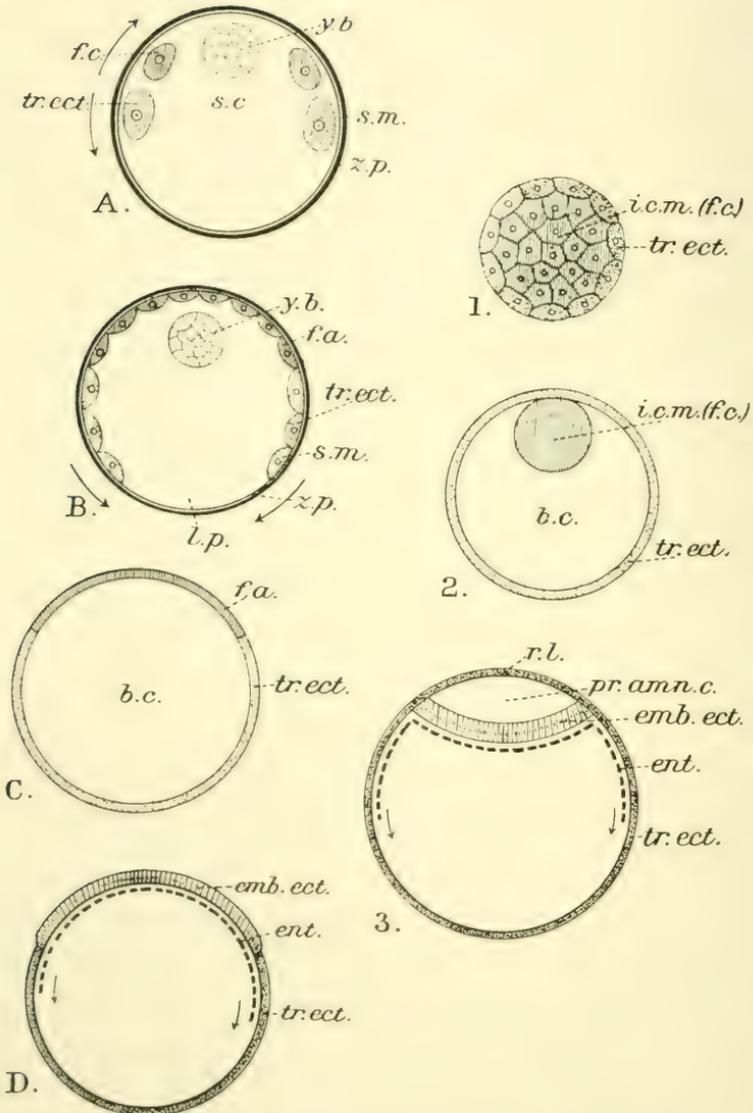
It will have become evident from the foregoing that the Metatherian mode of early development is to be regarded as

but a slightly modified version of the Prototherian, such differences as exist between them being interpretable as cœnogenetic modifications, induced in the Metatherian by the practically complete substitution of uterine nutriment for intra-ovular yolk, a substitution which has resulted in the attainment by the marsupial ovum of the holoblastic type of cleavage. In the present section I hope to demonstrate how the early ontogeny of the Metatheria enables us to interpret that of the Eutheria in terms of that of the Prototheria.

If we proceed to compare the early development in the Metatheria and Eutheria, we encounter, from the 4-celled stage onwards, such obvious and profound differences in the mode of formation of the blastocyst, and in the relations of its constituent parts, that the differences seem at first sight to far outweigh the resemblances. Nevertheless, apart from their common possession of the same holoblastic mode of cleavage, there exists one most striking and fundamental agreement between the two in the fact that in both there occurs, sooner or later during the cleavage process, a separation of the blastomeres into two distinct, pre-determined cell-groups, whose individual destinies are very different, but apparently identical in the two subclasses. In the Marsupial, as typified by *Dasyurus*, the fourth cleavages are, as we have seen, unequal and qualitative, and result in the separation of two differentiated groups of blastomeres, arranged in two superimposed rings, viz. an upper ring of eight smaller, less yolk-rich cells, and a lower of eight larger, more yolk-rich cells. The evidence justifies the conclusion that the former gives origin directly to the formative or embryonal region of the vesicle wall, the latter to the non-formative or extra-embryonal region.

Amongst the Eutheria the evidence is no less clear. It has been conclusively shown by various observers (Van Beneden, Duval, Assheton, Hubrecht, Heape, and others) that, sooner or later, there occurs a separation of the blastomeres into two distinct groups, one of which eventually encloses the other completely. The two groups may be clearly distinguishable

TEXT-FIG. 2.



Diagrams illustrating the mode of formation of the blastocyst in Metatheria (A-D) and Eutheria (1-3). *b.c.* Blastocyst cavity. *i.c.m.* Inner cell-mass. *pr.amn.c.* Primitive amniotic cavity. *r.l.* Rauber's layer. *s.c.* Segmentation cavity. For other reference letters see explanation of plates (p. 125).

in early cleavage stages, owing to differences in the characters and staining reactions of their cells, and in such cases there is definite evidence of the occurrence of a process of overgrowth or epiboly, whereby one group gradually grows round and completely envelops the other, so that in the completed morula a distinction may be drawn between a central cell-mass and a peripheral or enveloping layer (rabbit, Van Beneden; sheep, Assheton). In other cases, where it has been impossible to recognise the existence of these two distinct cell-groups in the cleavage stages, we nevertheless find, either in the completed morula or in the blastocyst, that a more or less sharp distinction may be drawn between an enveloping layer of cells and an internally situated cell-mass (inner cell-mass).

E. van Beneden, in his classical paper on the development of the rabbit, published in 1875, was the first to recognise definitely the existence of two categories of cells in the segmenting egg of the Eutherian mammal. In this form he showed how in the morula stage a cap of lighter blastomeres gradually grows round and envelops a mass of more opaque cells by a process of overgrowth or epiboly. In his more recent and extremely valuable paper on the development of *Vespertilio* ('99), he again demonstrated the existence of two groups of blastomeres as well in the segmenting egg as in the completed morula, but failed to find evidence of epiboly in all cases. Nevertheless he holds fast to the opinion which he expressed in 1875: "Que la segmentation s'accompagne, chez les Mammifères placentaires, d'un enveloppement progressif d'une partie des blastomères par une couche cellulaire, qui commence à se différencier dès le début du développement," and states that "dans tous les œufs arrivés à la fin de la segmentation et dans ceux qui montraient le début de la cavité Blastodermique j'ai constamment rencontré une couche périphérique complète, entourant de toutes parts un amas cellulaire interne, bien séparé de la couche enveloppante." The latter layer he regards as corresponding to the extra-embryonal ectoderm of the Sauropsida, and points out that

“chez tous les Chordés les premiers blastomères qui se différencient et qui avoisinent le pôle animal de l’œuf sont des éléments épiblastiques. C’est par la couche cellulaire qui résulte de la segmentation ultérieure de ces premiers blastomères épiblastiques que se fait, chez les Sauropsides, l’enveloppement du vitellus. Dans l’œuf réduit à n’être plus qu’une sphère microscopique, l’épibolie a pu s’achever dès la fin de la segmentation, voire même avant l’achèvement de ce phénomène.” The “amas cellulaire interne” (embryonal knot, inner cell mass), Van Beneden shows, differentiates secondarily into “un lécithophore et un bouton embryonnaire.” The former is the entoderm of other authors, the latter the formative or embryonal ectoderm. Hubrecht, in the forms studied by him (*Sorex*, *Tupaia*, *Tarsius*¹) finds a corresponding differentiation. In *Tupaia* he describes the morula stage as consisting of a single central lightly staining cell, which he regards as the parent cell of the inner cell-mass of later stages, and of a more darkly staining peripheral layer which forms the unilaminar wall of the blastocyst. Here, then, the parent cells of the two cell-groups would appear to be separated at the first cleavage. Hubrecht, like Van Beneden, holds that the inner cell-mass furnishes the embryonal ectoderm and the entire entoderm of the blastocyst. The peripheral layer he has termed the trophoblast ('88, p. 511), and in his paper on the placentation of the hedgehog ('89, p. 298) he defines the term as follows: “I propose to confer this name to the epiblast of the blastocyst as far as it has a direct nutritive significance, as indicated by proliferating processes, by immediate contact with maternal tissue, maternal blood, or secreted material. The epiblast of the germinal area—the formative epiblast—and that which will take part in the formation of the inner lining of the amnion cavity is, ipso facto, excluded from the definition.” Thus the name

¹ In *Erinaceus* the entoderm, from Hubrecht's observations, appears to be precociously differentiated, prior to the separation of the embryonal ectoderm from the overlying trophoblast, but the details of the early development in this form are as yet only incompletely known.

trophoblast was originally employed by Hubrecht as a convenient term designatory of what he at the time regarded as the extra-embryonal ectoderm of the mammalian blastocyst. In the course of his speculations on the origin of this layer, however, he has reached the conclusion that it is really of the nature of "a larval envelope, an Embryonalhülle" ('08, p. 15), inherited by the mammals, not from the reptiles (which have no direct phylogenetic relationship to the latter), but from their remote invertebrate ancestors ("vermiform predecessors of cœlenterate pedigree, provided with an ectodermal larval investment [Larvenhülle]").

Assheton, again, although he was unable to convince himself ('94) of the correctness of van Beneden's account of the occurrence of a process of epiboly in the segmenting eggs of the rabbit, finds in the sheep ('98) that a differentiation into two groups of cells is recognisable "perhaps as early as the eight segment stage," and that one of the groups gradually envelops the other. "Let it be noted," he writes ('98, p. 227), "that we have now to face the fact, based on actual sections, that there is in certain mammals a clear separation of segments at an early stage into two groups, one of which eventually completely surrounds the other," and instances Van Beneden's observations on the rabbit (of the correctness of which he, however, failed to satisfy himself, as noted above), Duval's observations on the bat, Hubrecht's on *Tupaia*, and his own on the sheep. Assheton thinks this phenomenon "must surely have some most profound significance," but finds himself unable to accept the interpretations of either Van Beneden or Hubrecht, and puts forward yet another view, "based on the appearance of some segmenting eggs of the sheep" ('08, p. 233), "that in cases where this differentiation does clearly occur, it is a division into epiblast and hypoblast, the latter being the external layer" ('98, p. 227). Assheton thus differs from all other observers in holding that the inner cell-mass or embryonal knot of the Eutherian blastocyst gives origin solely to the formative or embryonal ectoderm, and I believe I am correct in stating that he also

differs from all other observers in holding that the outer enveloping layer of the same is entodermal.¹

The fact, then, of the occurrence amongst Eutheria of a "precocious segregation" of the blastomeres into two distinct groups, one of which eventually surrounds the other completely, is not in dispute, though authorities differ widely in the interpretation they place upon it. In the Eutherian blastocyst stage, the enveloping layer forms the outer unilaminar wall of the vesicle, and encloses the blastocyst cavity as well as the other internally situated group. This latter typically appears as a rounded cell-mass, attached at one spot to the inner surface of the enveloping layer, but more or less distinctly marked off from it. It is generally termed the inner cell-mass or embryonal knot ("amas cellulaire interne" of Van Beneden). For the enveloping layer Hubrecht's name of "trophoblast" is now generally employed, even by those who refuse to adopt the speculative views with which its originator has most unfortunately, as I think, enshrouded this convenient term.

I have demonstrated the occurrence of an apparently comparable "precocious segregation" of the blastomeres into two distinct groups in one member of the Metatheria which there is no reason to regard as an aberrant type, and I have shown beyond all shadow of doubt that from the one group, which constitutes what I have termed the formative region of the unilaminar vesicle-wall, there arise the embryonal ectoderm and the entire entoderm of the vesicle, both embryonal and extra-embryonal, and that the other group, which constitutes the non-formative region of the vesicle-wall, directly furnishes the extra-embryonal ectoderm, i. e. the ectoderm of the omphalopleure and chorion.²

¹ Assheton states ('08, p. 233, cf. also '98, p. 220) that his interpretation "owes much also to the theoretical conclusions of Minot and Robinson." However that may be, both Minot and Robinson in their most recent writings continue to speak of the chorionic ectoderm.

² Whether or not it participates in the formation of the amniotic ectoderm future investigation must decide.

As regards Eutheria, we have seen that Van Beneden and Hubrecht, though their views in other respects are widely divergent, both agree that the inner cell-mass of the blastocyst furnishes the embryonal ectoderm (as well as the amniotic ectoderm wholly or in part) and the entire entoderm of the vesicle. That, in fact, is the view of Mammalian embryologists generally (Duval and Assheton excepted),¹ and if we may assume it to be correct, then it would appear that the later history of the formative region of the Marsupial blastocyst and that of the inner cell-mass of the Eutherian are identical. That being so, and bearing in mind that both have been shown, at all events in certain Mammals, to have an identical origin as a group of precociously segregated blastomeres,² I can come to no other conclusion than that they are homogenous formations. If that be accepted, then this fact by itself renders highly probable the view that the so-called trophoblast of the Eutherian blastocyst is homogenous with the non-formative region of the Metatherian vesicle, and when we reflect that both have precisely the same structural and topographical (not to mention functional) relations in later stages, inasmuch as they constitute the ectoderm of the chorion and omphalopleure (with or without participation in the formation of the amniotic ectoderm), and that both have a similar origin in those Mammals in which a precocious segregation of the blastomeres has been recognised, their exact

¹ The view of Duval ['95], based on the study of *Vespertilio*, that the inner cell-mass gives rise solely to entoderm, and that the enveloping layer furnishes not only the extra-embryonal but also the embryonal ectoderm, is shown by Van Beneden's observations on the same form to be devoid of any basis of fact. Assheton's views are referred to below (p. 110).

² The fact that the phenomenon of the "precocious segregation" of the blastomeres into two groups with determinate destinies has already become fixed in the Marsupial lends additional weight to the view of Van Beneden that such a segregation will eventually be recognised as occurring in all Eutheria without exception. Without it, it is difficult to understand how the entypic condition, characteristic of the blastocysts of all known Eutheria, is attained, unless by differentiation in situ, which seems to me highly improbable.

homology need no longer be doubted. In the preceding section of this paper (ante, pp. 91, 92) I have shown reason for the conclusion that the non-formative region of the Marsupial blastocyst is the homologue of the extra-embryonal ectoderm of the Monotreme and Reptile, and if that conclusion be accepted it follows that the outer enveloping layer of the Eutherian blastocyst, the so-called trophoblast of Hubrecht, is none other than extra-embryonal ectoderm, as maintained by Van Beneden, Keibel, Bonnet, Jenkinson, Lee, MacBride and others, the homologue of that of Reptilia.

I am therefore wholly unable to accept the highly speculative conclusions of Hubrecht, set forth with such brilliancy in a comparatively recent number of this Journal ('08), as to the significance and phylogeny of this layer. These conclusions, on the basis of which he has proceeded to formulate such far-reaching and, indeed, revolutionary ideas not only on questions embryological, but on those pertaining to the phylogeny and classification of vertebrates, have already been critically considered by Assheton ('09) and MacBride ('09), also in the pages of this Journal, and found wanting, and they are, to my mind, quite irreconcilable with the facts I have brought to light in regard to the early development of Marsupials. I yield to no one in my admiration for the epoch-making work of Hubrecht on the early ontogeny and placentation of the Mammalia, and I heartily associate myself with the eulogium thereanent so admirably expressed by Assheton in the critique just referred to (p. 274), but I am bound to confess that as concerns his views on the phylogeny of this layer, which he has termed the "trophoblast," he seems to me to have forsaken the fertile field of legitimate hypothesis for the barren waste of unprofitable speculation, and to have erected therein an imposing edifice on the very slenderest of foundations.

Before I proceed to justify this, my estimate of Hubrecht's views on the phylogeny of the trophoblast, let me first set forth his conception so far as I understand it. He starts with the assumption that the vertebrates (with the exception

of Amphioxus, the Cyclostomes, and the Elasmobranchs) are descended from "vermiform predecessors of cœlenterate pedigree" possessed of free-swimming larvæ, in which there was present a complete larval membrane of ectodermal derivation, and of the same order of differentiation "as the outer larval layer which in certain Nemertines, Gephyreans, and other worms often serves as a temporary envelope that is stripped off when the animal attains to a certain stage of development." When, for oviparity and larval development, viviparity and embryonic development became established in the Protetrapodous successors of the ancestral vermiform stock, the larval membrane did not disappear. On the contrary, it is assumed that it merely changed "its protective or locomotor function into an adhesive one," and so, development now taking place in utero, it is quite easy to understand how the larval membrane could gradually become transformed into a trophic vesicle, containing the embryo as before, and functional in the reception of nutriment from the walls of the maternal uterus. The final stages in the evolution of this trophic vesicle constituted by the old larval membrane are met with amongst the mammals, since in them it became vascularised so as to constitute a "yet more thorough system of nourishment at the expense of the maternal circulatory system." Such, then, is the phylogeny of the trophoblast according to Hubrecht. The Eutherian mammals, which it is held trace their descent straight back to some very early Protetrapodous stock, viviparous in habit and with small yolk-poor, holoblastic eggs, exhibit the trophoblast in its most perfect condition. Hubrecht therefore starts with them, and attempts to demonstrate the existence of a larval membrane, or remnants of such, externally to the embryonal ectoderm in all vertebrates with the exceptions already mentioned. There is no question of its existence in the Meta- and Eutherian mammals. "We may," writes Hubrecht ('08, p. 12), . . . "insist upon the fact that . . . all Didelphia and Monodelphia hitherto investigated show at a very early moment the didermic stage out of

which the embryo will be built up enclosed in a cellular vesicle (the trophoblast), of which no part ever enters into the embryonic organisation." The common possession by the Metatheria and Eutheria of a larval membrane is after all only what might be expected, "since after Hill's ('97) investigations, we must assume that the didelphian mammals are not descended from Ornithodelphia but from monodelphian placental ancestors." As concerns the Prototheria, although they cannot in any sense be regarded as directly ancestral to the other mammals, we nevertheless find the trophoblastic vesicle "comparatively distinct." "In many reptiles and birds," however, it is "distinguished with great difficulty from the embryonic shield," and this is explained by the fact that the Sauropsida which are assumed to have taken their origin from the same Protetrapodous stock as the mammals but along an entirely independent line, have secondarily acquired, like the Prototheria, the oviparous habit, with its concomitants, a yolk-laden egg and a shell, and this latter acquisition has naturally tended "to relegate any outer larval layer to the pension list" ('09, p. 5). "Concerning the yolk accumulation in the Sauropsidan egg, there is no trouble at all to suppose that the vesicular blastocyst of an early viviparous ancestor had gradually become yolk-laden. The contrary assumption, found in the handbooks, that the mammalian egg, while totally losing its yolk, has yet preserved the identical developmental features as the Sauropsid, is in reality much more difficult to reconcile with sound evolutionary principles" ('09, p. 5).

Amongst the lower Vertebrates the larval membrane is clearly enough recognisable in the so-called Deckschicht of the Teleostomes, Dipnoans, and Amphibians. It is frankly admitted that Amphioxus, the Cyclostomes, and the Elasmobranchs "show in their early development no traces of a Deckschicht" (larval layer, trophoblast), but there is no difficulty about this, since it is easy enough to suppose, in view of other characters, that "the Selachians may very well have descended from ancestors without any outer larval layer"

('08, p. 151), and "for Cyclostomes the same reasoning holds good" (p. 152).

The trophoblast, then, is conceived of by Hubrecht as a larval membrane of ectodermal derivation, which invests the embryonal anlage in all Vertebrates with the exceptions mentioned, which is subject to secondary reduction, and which is homologous throughout the series. As I understand the conception, what is ordinarily called extra-embryonal ectoderm in the Sauropsida is not trophoblast, otherwise Hubrecht could hardly write—"in reptiles and birds traces of the larval layer have in late years been unmistakably noticed" ('09, p. 5); nevertheless what other writers have termed embryonal and extra-embryonal ectoderm in the Prototheria is claimed by Hubrecht as trophoblast (at all events that is my interpretation of his statement that a trophoblastic vesicle is present in these forms), and yet some years ago Hubrecht ('04, p. 10) found it difficult "to understand that the name has been misunderstood both by embryologists and gynæcologists." My own feeling is that the more recent developments in his views have tended to obscure rather than to clarify our ideas as to the trophoblast, especially if we must now hold that the chorion or serosa of the Sauropsida is not homologous with that of the Prototheria, which necessarily follows if the extra-embryonal ectoderm of the Sauropsidan is not the same thing as that of the Monotreme.

Assuming that we have formed a correct conception of the trophoblast as a larval membrane, and bearing in mind that it is best developed in the Metatheria and Eutheria, since these alone amongst higher Vertebrates have retained unaltered the viviparous habits of their Protetrapodous ancestors, let us see what basis in fact there is for the statement of Hubrecht ('08, p. 68) that "before the ectoderm and the entoderm have become differentiated from each other there is in mammals a distinct larval cell-layer surrounding (as soon as cleavage of the egg has attained the morula stage) the mother-cells of the embryonic tissues." Now that statement as it stands, I have no hesitation in characterising as entirely

misleading, inasmuch as it is applicable not to the Mammalia as a whole, but, so far as it refers to matters of undisputed fact, to one only of the three mammalian subclasses, viz. the Eutheria. So far as the latter are concerned, practically all observers, as we have seen, are agreed that there is present during at least the early stages of development a complete outer layer of cells which encloses the embryonal anlage or inner cell-mass (that portion of it immediately overlying the latter being termed the "Deckschicht" or "Rauber's layer"). It is, of course, this enveloping layer or trophoblast which Hubrecht interprets as a larval membrane. It fulfils the conditions, and were the Eutheria the only Vertebrates known to us, the idea might be plausible enough.

Turning now to the Metatheria, and remembering that these, according to Hubrecht, are descended from the Eutheria, we should naturally expect to find the supposed larval membrane fully developed, with all its ancestral relations; and so we do if we are content to accept Hubrecht's interpretation of Selenka's results and figures in the case of *Didelphys*. The "urentodermzelle" of Selenka is for Hubrecht "undoubtedly the mother-cell of the embryonic knob," the ectoderm of Selenka is manifestly the trophoblast—a complete larval layer. It is no doubt unfortunate that Hubrecht had to rely on the work of Selenka as his source of information on the early development of Marsupials, but it must be remembered that he reads his own views into Selenka's figures. On the basis of my own observations on the early ontogeny of Marsupials, I have no hesitation in affirming that a larval membrane, in the sense of Hubrecht, does not exist in any of the forms (*Dasyurus*, *Perameles*, *Macropus*) studied by me. The observations recorded in the preceding pages of this paper demonstrate, in the case of *Dasyurus* without the possibility of doubt, the entire absence of any cellular layer external to the formative region of the blastocyst, i.e. in a position corresponding to that occupied by Rauber's layer in Eutheria, whilst in the case of *Perameles* and *Macropus*, they yield not

the slightest evidence for the existence of any such layer. The formative region of the Marsupial blastocyst, which is undoubtedly the homologue of the inner cell mass of the Eutheria, forms from the first part of the unilaminar blastocyst wall, and is freely exposed. The remainder of the latter is constituted by a layer of non-formative cells, the destiny of which is the same as that of the so-called trophoblast of the Eutheria. I have therefore ventured to suggest that they are one and the same. If, then, the trophoblast is really a larval membrane, we must assume, in the case of the Marsupial, either that its "Deckschicht" portion has been completely suppressed (but why it should have been I fail to understand, unless, perhaps, it is a result of the secondary acquisition by the Marsupials of a shell-membrane, these mammals being even now on the way to secondarily assume the oviparous habit!), or that the non-formative region of the Marsupials is not the homologue of the trophoblast, in which case the Marsupials must be held to have entirely lost the larval membrane, since there is no other layer present which could possibly represent it. These considerations may well give us pause before we calmly accept Hubrecht's conception of the trophoblast as a larval membrane present in all mammals without exception.

Coming now to the Prototheria, we find, according to Hubrecht, "the trophoblastic vesicle . . . yet comparatively distinct," and so it is if we accept the interpretation of Hubrecht of the observations and figures of Semon, Wilson and Hill. The unilaminar blastoderm of these authors is unmistakably the trophoblast. The cells situated internally to that in the region of the white yolk-bed are not entodermal, as suggested by Semon, but constitute for Hubrecht "the mother cells of the embryonic knob." I need only quote again the opinion of Assheton thereanent and express my agreement therewith; he writes ('09, p. 233): "For this view I can see no reason derivable from actual specimens described and figured by those four authors" (Caldwell, Semon, Wilson and Hill). It would appear, then, that the assumption of

Hubrecht of the presence of a larval membrane of the nature postulated in the Prototheria and Metatheria is devoid of foundation in fact, so that there but remains the question of the significance of the outer enveloping layer of the Eutherian blastocyst. As regards that, I venture to think that the alternative interpretation of E. van Beneden and other investigators, which I have attempted to develop in the pages of this paper, affords a simpler and more satisfying explanation of its significance and phylogeny than that advocated by Prof. Hubrecht, an interpretation, moreover, which is more in accordance, not only with all the known facts, but "with sound evolutionary principles" and with the conclusions arrived at by the great majority of comparative anatomists and palæontologists as to the origin and inter-relationships of the Mammalia.

And I also venture to think that what has just been said holds true with reference to the views advocated by Mr. Assheton. These views owed their origin to certain appearances which he found in some segmenting ova of the sheep (but, be it noted, not in all those he examined), and he has attempted to re-interpret not only his own earlier observations, but those of other workers on the early ontogeny of the Eutheria in the light of his newer faith, and not only so, he holds that it is also possible to apply that in the interpretation of the early ontogeny of Marsupials (v. '08, p. 235, and '09, p. 229). He maintains that the inner cell-mass of Eutheria is purely ectodermal, and that the enveloping trophoblast layer of the blastocyst arises in common with the entodermal lining of the same and is therefore also entodermal. "On the theory I advocate," he writes ('09, p. 235), "the trophoblast is of Eutherian mammalian origin only and is not homologous to any form of envelope outside the group of Eutherian mammals." These views of Assheton are not only at variance with those of all other investigators who have worked at the early ontogeny of Eutheria, but they are quite irreconcilable with my observations on the development of *Dasyurus* herein recorded. I claim to have shown in that Marsupial that the formative region, the

homologue of the inner cell-mass, gives origin not only to the embryonal ectoderm, but to the entire entoderm, whilst the non-formative region, whose homology to the trophoblast of Eutheria is admitted by Assheton, arises quite independently of the entoderm and a long time before the latter makes its appearance. There is, then, in *Dasyurus* no question of a common origin of the entoderm and the non-formative or trophoblastic region of the blastocyst wall. And exception may be taken to Assheton's views on quite other grounds (e. g. the question of the homologies of the fœtal membranes in the series of the Amniota), as he himself is well aware, and as Jenkinson ('00) has also emphasised. I feel, however, I can leave further discussion of Assheton's views until such time as my observations on *Dasyurus* are shown to be erroneous or inapplicable to other Marsupials.

3. The Entypic Condition of the Eutherian Blastocyst.

If, now, on the basis of the homologies I have ventured to advocate in the preceding pages, we proceed to compare the Metatherian with the Eutherian blastocyst, we have to note that, whereas in the latter the extra-embryonal or trophoblastic ectoderm alone forms the blastocyst wall in early stages and completely encloses the embryonal knot, in the former, the homologous parts, viz. the non-formative or extra-embryonal and the formative or embryonal regions, both enter into the constitution of the unilaminar blastocyst wall, there being no such enclosure of the one by the other as occurs in the Eutherian blastocyst (Text-fig. 2, p. 98). It is characteristic of the Marsupial as of the Monotreme that the embryonal region is from the first superficial and freely exposed. It is spread out as a cellular layer and simply forms part of the blastocyst wall or blastoderm. It is equally characteristic of the Eutherian that the homologous part, the embryonal knot, has at first the form of a compact mass, which is completely enclosed by the trophoblastic ectoderm.

The latter alone constitutes the unilaminar wall of the blastocyst and has the embryonal knot adherent at one spot to its inner surface. The formative cells which compose the knot thus take at first no part in the constitution of the outer wall of the blastocyst, and may or may not do so in later stages according as the covering layer of the trophoblast (the Deckschicht or Raubër's layer) is transitory or permanent. This peculiar developmental condition, characterised by the internal position of the formative or embryonal cells within the blastocyst cavity, has been termed by Selenka ('00) "entypy" (Entypie des Keimfeldes).¹ It is a phenomenon exclusively found in the Eutheria and characteristic of them alone, amongst the mammals. In the Marsupial, as in the Monotreme, the formative cells are freely exposed, and constitute from the first part of the blastocyst wall just as those of the Sauropsida form a part of the general blastoderm. Limited as entypy thus appears to be to the higher mammals, the probability is that we have to do here with a purely secondary, adaptive feature.

If we proceed to inquire what is the significance of this remarkable difference in the early developmental phenomena of the lower and higher mammals, it seems to me that we have to take account, in the first place, of the differences in the structure of their respective eggs, and especially we have to bear in mind that the Eutherian ovum is considerably more specialised than even the Metatherian. It is on the average smaller than the latter, i.e. it has suffered in the course of phylogeny still further reduction in size, and has lost, to an even greater extent than the Marsupial ovum, the store of food-yolk ancestrally present in it. Moreover, it has suffered a still further reduction in respect of its secondary egg-membranes. The Metatherian ovum still retains in its shell-membrane a

¹ "Unter Entypie des Keimfeldes möchte ich daher verstanden wissen: Die nicht durch Bildung typischer Amnionfalten geschehende, sondern durch eine schon während der Gastrulation erfolgende Abschñürung des Keimfeldes ins Innere der Eiblasenhñlle (Chorion)" ('00, p. 203).

vestigial representative of the shell of the presumed oviparous common ancestor of the Metatheria and Eutheria. The Eutherian ovum, on the other hand, has lost all trace of the shell in correlation with its more complete adaptation to the conditions of intra-uterine development. The albumen layer is variable in its occurrence, being present in some (e.g. rabbit) and absent in others (e.g. pig, Assheton), whilst the zona itself, though always present, is variable both as to its thickness and the length of time it persists.

Strangely enough, although the prevailing opinion amongst mammalian embryologists is that the Eutherian ovum has been derived phylogenetically from an egg of the same telolecithal and shell-bearing type as is found in the Monotremes, no one, so far as I am aware, has ever taken the shell into account, and ventured to consider in what way its total disappearance from an ovum already greatly reduced in size, might affect the course of the early developmental phenomena. That is what I propose to do here, for in my view it is just in the complete loss of the shell by the Eutherian ovum that we find the key to the explanation of those remarkable differences which are observable between the early ontogeny of the Eutheria and Metatheria, and which culminate in the entypic condition so distinctive of the former. The acquisition of a shell by the Proamniota conditioned the appearance of the amnion. The loss of the shell in the Eutheria conditioned the occurrence in their ontogeny of entypy.

As we have seen, the mammalian ovum, already in the Monotremes greatly reduced in size as compared with that of reptiles, and quite minute in the Metatheria and Eutheria, contains within itself neither the cubic capacity nor the food material necessary for the production of an embryo on the ancestral reptilian lines. We accordingly find that the primary object of the first developmental processes in the mammals has come to be the formation of a vesicle with a complete cellular wall, capable of absorbing nutrient fluid from the maternal uterus and of growing rapidly, so as to provide the space necessary for embryonal differentiation.

In the Monotremes this vesicular stage is rapidly and directly attained as the result, firstly, of the rearrangement of the blastomeres of the cleavage-disc to form a unilaminar blastodermic membrane overlying the solid yolk, and, secondly, of the rapid extension of the peripheral (extra-embryonal) region of the same, in contact with the inner surface of the firm sphere furnished by the egg-envelopes. During the completion of the blastocyst embryonal differentiation remains in abeyance, and practically does not start until after growth of the blastocyst is well initiated.

In the Marsupial, notwithstanding the fact that the ovum has become secondarily holoblastic, the mode of formation of the blastocyst is essentially that of the Monotreme. Cleavage is of the radial type, and owing to the persistence of the shell, which with the zona forms a firm resistant sphere enclosing the egg, the radially arranged blastomeres are able to assume the form of an open ring and to proceed directly to the formation of the unilaminar wall of the blastocyst. The enclosing sphere provides the necessary firm surface over which the products of division of the upper and lower cell-rings of the 16-celled stage can respectively spread towards opposite poles, so as to directly constitute the formative and non-formative regions of the blastocyst wall. In my opinion it is the persistence of the resistant shell-membrane round the ovum which conditions the occurrence in the Marsupial of this direct method of blastocyst formation. As in the Monotreme, so here also embryonal differentiation commences only after the blastocyst has grown considerably in size.

In the Eutheria, on the other hand, in the absence of the shell-membrane, not only is the mode of formation of the blastocyst quite different to that in the Marsupial, but the relations of the constituent parts of the completed structure also differ markedly from those of the homogeneous parts in the latter. The cleavage process here leads only indirectly to the formation of the blastocyst, and must be held to be cœnogenetically modified as compared with that of

lower mammals. In the cross-shaped arrangement of the blastomeres in the 4-celled stage, in the occurrence of a definite morula-stage and of the entypic condition, we have features in which the early ontogeny of the Eutheria differs fundamentally from that of the Metatheria. They are intimately correlated the one with the other, and are met with in all Eutheria, so far as known, but do not occur either in the Prototheria or the Metatheria, so that we must regard them as secondary features which were acquired by the primitive Eutheria under the influence of some common causal factor or factors, subsequent to their divergence from the ancestral stock common to them and to the Metatheria. Now the cross-shaped 4-celled stage and the morula-stage are undoubtedly to be looked upon simply as cleavage adaptations of prospective significance in regard to the entypic condition, so that the problem reduces itself to this—how came these adaptations to be induced in the first instance? In view of the facts that in the Metatheria, in the presence of the shell-membrane, the formation of the blastocyst is the direct outcome of the cleavage process, and is effected along the old ancestral lines without any enclosure of the formative cells by the non-formative, whilst in the Eutheria, in the absence of the shell-membrane, blastocyst formation results only indirectly from the cleavage-process, is effected in a way quite different from that characteristic of the Metatheria, and involves the complete enclosure of the formative by the non-formative cells, I venture to suggest that the cleavage adaptations which result in the entypic condition were acquired in the first instance as the direct outcome of the total loss by the already greatly reduced Eutherian ovum of the shell-membrane.¹ This view necessarily implies that the presence of a thick zona such as occurs round the ovum in certain Eutheria is secondary, and what we know of this membrane in existing Eutheria is at all events not adverse to that conclusion.

¹ This suggestion I first put forward in a course of lectures on the early ontogeny and placentation of the Mammalia delivered at the University of Sydney in 1904.

Amongst the Marsupials the zona is quite thin (about $\cdot 0016$ mm. in *Dasyurus*), presumptive evidence that it was also thin in the ancestral stock from which the Meta- and Eutheria diverged, whilst amongst the Eutheria themselves the zona, as Robinson ('03) has pointed out, is not only of very varying thickness, but persists round the ovum for a very varying period in different species. It appears to be thinnest in the mouse ($\cdot 001$ mm.), in most Eutheria it is considerably thicker ($\cdot 01$ mm., bat, dog, rabbit, deer), whilst in *Cavia* it reaches a thickness of as much as $\cdot 02$ mm. In those forms in which the blastocyst early becomes embedded in, or attached to, the mucosa, the zona naturally disappears early. In the rat, mouse and guinea-pig it disappears before the blastocyst is formed. Hubrecht failed to find it in the 2-celled egg of *Tupaia*, and it was already absent in the 4-celled stage of *Macacus nemestrinus*, discovered by Selenka and described by Hubrecht. On the other hand, it may persist for a much longer period, up to the time of appearance of the primitive streak (rabbit, dog, ferret). These facts sufficiently demonstrate the variability of the zona in the Eutherian series, and its early disappearance in certain forms before the completion of the blastocyst stage shows that it can have no supporting function in regard to that.

Postulating, then, the disappearance of the shell-membrane and the presence of a relatively thin, non-resistant zona (with perhaps a layer of albumen) round the minute yolk-poor ovum of the primitive Eutherian, and remembering that the ovum starts with certain inherited tendencies, the most immediate and pressing of which is to produce a blastocyst comprising two differentiated groups of cells, the problem is how, in the absence of the old supporting sphere constituted by the egg-envelopes, can such a vesicular stage be most easily and most expeditiously attained? The Eutherian solution as we see it in operation to-day is really a very simple one, and withal a noteworthy instance of adaptation in cleavage (Lillie, '99). In the absence of any firm supporting membrane round the egg, and the consequent impossibility of the blastomeres pro-

ceeding at once to form the blastocyst wall, they are under the necessity of keeping together, and to this end cleavage has become adapted. For the ancestral radial arrangement of the blastomeres in the 4-celled stage, characteristic of the Monotreme and Marsupial, there has been substituted a cross-shaped grouping into two pairs, and, as the outcome of this adaptive alteration in the cleavage planes, there results from the subsequent divisions, not an open cell-ring, as in the Marsupial, but a compact cell-group or morula. In this we again encounter precisely the same differentiation of the blastomeres into two categories, respectively formative (embryonal) and non-formative (trophoblastic) in significance, as is found in the 16-celled stage of the Marsupial, but, since the two groups of cells are here massed together, and in the absence of any firm enclosing sphere, cannot spread independently so as to form directly the wall of the blastocyst, there has arisen the necessity for yet other adaptive modifications. Attention has already been directed to the tardiness of differentiation in the embryonal region of the Monotreme and Marsupial blastocyst, and here in the minute Eutherian morula we find what is, perhaps, to be looked upon as a further adaptive exaggeration of this same feature in the inertness which is at first displayed by the formative cells, and which is in marked contrast with the activity shown by the non-formative ectodermal cells.¹ It is these latter, it

¹ The inertness of the formative cell-mass is accounted for by Assheton ('98, p. 251) as follows: "Now, as the epiblast plays the more prominent part in the formation of the bulk of the embryo during the earliest stages, it clearly would be useless for the embryonic part to exhibit much energy of growth until the old conditions [in particular sufficient room for embryonal differentiation] were to a certain extent regained; hence the lethargy exhibited by the embryonic epiblast in mammals during the first week of development. No feature of the early stages of the mammalian embryo is more striking than this inertness of the embryonic epiblast—or, as I should now prefer to call it, simply epiblast—during the first few days." Assheton, it should be remembered, holds that the inner cell-mass of Eutheria furnishes only the embryonal ectoderm.

should be recollected, which exhibit the greatest growth-energy during the formation of the blastocyst in the Monotreme and Marsupial, and so their greater activity in the Eutherian morula is only what might be expected. Dividing more rapidly than the formative cells, they gradually grow round the latter, and eventually form a complete outer layer enveloping the inert formative cell-group. This process of overgrowth or epiboly is entirely comparable in its effect with the spreading of the extra-embryonal region of the unilaminar blastodermic membrane in the Monotreme to enclose the yolk-mass, and with that of the non-formative cells in the Marsupial to complete the lower hemisphere of the blastocyst, growth round an inert central cell-mass being here substituted for growth over the inner surface of a resistant sphere constituted by the egg-envelopes, such as occurs during the formation of the blastocyst in the Monotreme and Marsupial. Just as the first objective of the cleavage process in the latter is to effect the completion of the cellular wall of the blastocyst, so here the same objective recurs, and is attained in the simplest possible way in the new circumstances, viz. by the rapid envelopment of the formative by the non-formative cells. Thus at the end of the cleavage process in the Eutherian we have formed a solid entypic morula in which an inner mass of formative cells is completely surrounded by an outer enveloping layer of non-formative or tropho-ectodermal cells, homogenous with the extra-embryonal ectoderm of the Sauropsidan and Monotreme and the non-formative region of the unilaminar blastocyst of the Marsupial. Conversion of the solid morula into a hollow blastocyst capable of imbibing fluid from the uterus and of growing rapidly now follows. Intra- or intercellular vacuoles appear below the inner cell-mass, by the confluence of which the blastocyst cavity is established, and the inner cell-mass becomes separated from the enveloping layer of tropho-ectoderm, except over a small area where the two remain in contact.

The complete enclosure of the formative cells of the inner cell-mass by the non-formative ectodermal cells of the

enveloping layer which produces this peculiar entypic condition in the Eutherian blastocyst, I would interpret, then, as a purely adaptive phenomenon, which in the given circumstances effects in the simplest possible way the early completion of the blastocyst wall, and whose origin is to be traced to that reduction in size and in its envelopes which the Eutherian ovum has suffered in the course of phylogeny, in adaptation to the conditions of intra-uterine development. In particular, starting with a shell-bearing ovum, already minute and undergoing its development in utero, I see in the loss of the shell such as has occurred in the Eutheria an intelligible explanation of the first origin of those adaptations which culminate in the condition of entypy. I am therefore wholly unable to accept the view of Hubrecht ('08, p. 78), that "what Selenka has designated by the name of Entypie is—from our point of view—no secondary phenomenon, but one which repeats very primitive features of separation between embryonic ectoderm and larval envelope in invertebrate ancestors."

I see no reason for supposing that the intimate relationship which is early established in many Eutheria between the trophoblastic ectoderm and the uterine mucosa has had anything to do with the origination of the entypic condition. In my view such intimate relationship involving the complete enclosure of the blastocyst in the mucosa only came to be established secondarily, after entypy had become the rule. On the other hand, the peculiar modifications of the entypic condition met with in rodents with "inversion" (e.g. rat, mouse, guinea-pig) are undoubtedly to be correlated, as Van Beneden also believed ('99, p. 332), with the remarkably early and complete enclosure or implantation of the germ in the mucosa such as occurs in these and other Eutheria. Similar views are expressed by Selenka in one of his last contributions to mammalian embryology. He writes ('00, p. 205)—"Dass die Entypie des Keimfeldes und die Blattinversion begünstigt wird durch die frühzeitige Verwachsung der Eiblase mit dem Uterus, ist nicht in Abrede zu stellen. Aber da dieser

Prozess auch in solchen Eiblasen der Säugetiere vorkommen kann, die überhaupt nicht, oder erst später mit dem Uterus verwachsen, so kann die Keimfeld-Entypie zwar durch die frühe Verwachsung veranlasst, aber nicht ausschliesslich hervorgerufen werden." He goes on to remark that—"Die Vorbedingungen zur Entypie müssen in der Struktur der verwachsenden Eibläse gesucht werden," and expresses his agreement with the views of Van Beneden as to the significance to be attributed to the early cleavage phenomena in Eutheria.

The attitude of the illustrious Belgian embryologist whose loss we have so recently to deplore, towards this problem is clearly set forth in the last memoir which issued from his hand. "Je suis de ceux," he wrote ('99, p. 332), "qui pensent que toute l'embryologie des Mammifères placentaires témoigne qu'ils dérivent d'animaux qui, comme les Sauropsides et les Monotrèmes, produisaient des œufs méroblastiques. Je ne puis à aucun point de vue me rallier aux idées contraires formulées et défendues par Hubrecht. L'hypothèse de Hubrecht se heurte à des difficultés morphologiques et physiologiques insurmontables: elle laisse inexpiquée l'existence, chez les Mammifères placentaires, d'une vésicule ombilicale et d'une foule de caractères communs à tous les Amniotes et distinctifs de ces animaux." Holding this view of the origin of the Eutheria, Van Beneden based his interpretation of their early ontogenetic phenomena on the belief that "la réduction progressive du volume de l'œuf d'une part, le fait de son développement intra-utérin de l'autre ont dû avoir une influence prépondérante sur les premiers processus évolutifs."

Balfour, in his classical treatise, had already some eighteen years earlier expressed precisely the same view. "The features of the development of the placental Mammalia," he wrote ('Mem. Edu.,' vol. iii, p. 289), "receive their most satisfactory explanation on the hypothesis that their ancestors were provided with a large-yolked ovum like that of Sauropsida. The food-yolk must be supposed to have ceased to be developed on the establishment of a maternal nutrition through

the uterus. . . . The embryonic evidence of the common origin of Mammalia and Sauropsida, both as concerns the formation of the layers and of the embryonic membranes is as clear as it can be."

That view of the derivation of the Mammalia receives, I venture to think, striking confirmation from the observations and conclusions set forth in the preceding pages of this memoir, and from it as a basis all attempts at a phylogenetic interpretation of the early ontogenetic phenomena in the Mammalia must, I am convinced, take their origin. Such an attempt I have essayed in the foregoing pages, with what success the reader must judge.

ADDENDUM.

The memoir of Prof. O. Van der Stricht, entitled "La structure de l'œuf des Mammifères (Chauve-souris, *Vesperugo noctula*): Troisième Partie" ('Mem. de l'Acad. roy. de Belgique,' 2nd ser., t. ii, 1909), came into my hands only after my own paper had reached its final form, and therefore too late for notice in the body of the text. In this extremely valuable contribution, Van der Stricht gives a detailed account of the growth, maturation, fertilisation, and early cleavage-stages of the ovum of *Vesperugo*, illustrated by a superb series of drawings and photo-micrographs. All I can do here, however, is to direct attention to that section of the paper entitled "Phénomènes de deutoplasmolyse au pôle végétatif de l'œuf" (pp. 92-96), in which the author describes the occurrence in the bat's ovum of just such a process of elimination of surplus deutoplasmic material as I have recorded for *Dasyurus*. Van der Stricht's interpretation of this phenomenon agrees, I am glad to find, with my own. He writes (pp. 92-93): "Ce deutoplasme rudimentaire, à peine ébauché dans l'ovule des Mammifères, paraît être encore trop abondant dans l'œuf de Chauve-souris, car ces matériaux de réserve, en partie inutiles, sont partiellement éliminés, expulsés de la cellule."

To this process of elimination of surplus deutoplasm he applies the name "deutoplasmolyse," and states that "Ce phénomène consiste dans l'apparition de lobules vitellins multiples, en nombre très variable, à la surface du vitellus au niveau du pôle végétatif. Ces bourgeons à peu près tous de même grandeur, les uns étant cependant un peu plus volumineux que les autres, apparaissent dans le voisinage des globules polaires et présentent la structure du deutoplasme. Ils sont formés de vacuoles claires, à l'intérieur desquelles on aperçoit parfois de petits grains vitellins, dont il a été question plus haut. . . . Ce processus de deutoplasmolyse devient manifeste surtout après l'expulsion du second globule polaire, pendant la période de la fécondation. Il peut être très accentué, au stade du premier fuseau de segmentation et au début de la segmentation de l'œuf, notamment sur des ovules divisés en deux et en quatre (figs. 59, 61, 62, d)." It would therefore appear that, whilst in *Dasyurus* the surplus deutoplasm is eliminated always prior to the completion of the first cleavage and in the form of a single relatively large spherical mass, in *Vesperugo* it is cast off generally, though not invariably, before cleavage begins, and in the form of a number of small separate lobules.

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EXPLANATION OF PLATES 1-9,

Illustrating Prof. J. P. Hill's paper on “The Early Development of the Marsupialia, with Special Reference to the Native Cat (*Dasyurus viverrinus*).”

[All figures are from specimens of *Dasyurus*, unless otherwise indicated. Drawings were executed with the aid of Zeiss's camera lucida, except figs. 61-63, which were drawn from photographs.]

LIST OF COMMON REFERENCE LETTERS.

Abn. Abnormal blastomere, fig. 37. *alb.* Albumen. *cg.* Coagulum. *d. p.* Discus proligerus. *d. z.* Deutoplasmic zone. *emb. a.* Embryonal area. *emb. ect.* Embryonal ectoderm. *ent.* Entoderm. *f. ep.* Follicular epithelium. *f. a.* Formative area of blastocyst wall. *f. c.* Formative cell. *f. z.* Formative zone. *i. c.* Internal cell, fig. 34. *l. ent.* Limit of extension of entoderm. *l. p.* Incomplete area of blastocyst wall at lower pole. *p. b¹.* First polar body. *p. b¹. s.* First polar spindle. *p. b². s.* Second polar spindle. *p. s.* Perivitelline space. *s. m.* Shell-membrane. *sp.* Sperm in albumen. *tr. ect.* Non-formative or trophoblastic ectoderm (tropho-ectoderm). *y. b.* Yolk-body. *z. p.* Zona.

PLATE 1.

Fig. 1.—Photo-micrograph ($\times 150$ diameters) of the full-grown ovarian ovum, $.27 \times .26$ mm. diameter. The central deutoplasmic zone (*d. z.*) and the peripheral formative zone (*f. z.*), in which the

vesicular nucleus (0.05×0.03 mm. diameter) is situated, are clearly distinguishable. The zona (*z. p.*) measures $0.0021-0.0025$ mm. in thickness. Outside it are the follicular epithelial cells of the discus proligerus (*d. p.*), which is thickened on the upper side of the figure, where it becomes continuous with the membrana granulosa. (D. v i v., 21. vii. '04, $\frac{10}{2}$. Hermann's fluid and iron-hæmatoxylin.)

Fig. 2.—Photo-micrograph ($\times 150$) of ripe ovarian ovum (in which first polar body is separated and second polar spindle is present, though neither is visible in figure), 0.29×0.23 mm. maximum diameter. Follicle 1.4×1.1 mm. diameter. The ovum exhibits an obvious polarity. Deutoplasmic zone (*d. z.*) in upper hemisphere; formative zone (*f. z.*) forming lower. (D. v i v., 14, 26. vii. '02, $\frac{8}{2-0}$. Flemming's fluid and iron-hæmatoxylin.)

Fig. 3.—Photo-micrograph ($\times 150$) of ripe ovarian ovum (0.28×0.24 mm. diameter) with first polar body (*p. b.*) and second polar spindle. First polar body, $0.026-0.03 \times 0.01$ mm. Second polar spindle, 0.013 mm. in length. (D. v i v., 14, 26. vii. '02, $\frac{10}{4-3}$. Flemming's fluid and iron-hæmatoxylin.)

Fig. 4.—Photo-micrograph ($\times 256$) of ovarian ovum in process of growth ("pseudo-alveolar" stage). Ovum, 0.26×0.20 mm. diameter. Zona, $0.0017-0.002$ mm. in thickness. (D. v i v., 14, 26. vii. '02, $\frac{2}{3-1}$. Hermann, iron-hæmatoxylin.)

Fig. 5.—Photo-micrograph ($\times 1250$) of peripheral region of ripe ovarian ovum (0.28×0.126 mm. diameter) with first polar spindle (0.015×0.013 mm.). (D. v i v., 23. vii. '02, $\frac{7}{2-2}$. Ohlmacher's fluid, iron-hæmatoxylin.)

Fig. 6.—Photo-micrograph ($\times 1250$) of peripheral region of ripe ovarian ovum (0.26×0.18 mm.), showing first polar body (*p. b.*) (0.03×0.006 mm.). (D. v i v., 14, 26. vii. '02, $\frac{4}{1-1}$. Flemming, iron-hæmatoxylin.)

Fig. 7.—Photomicrograph ($\times 1250$) of peripheral region of ovum, fig. 3, showing portion of first polar body (*p. b.*), and the second polar spindle. The dark body lying between *p. b.* and the surface of the ovum is a displaced red blood-corpuscle.

Figs. 8 and 9.—Photo-micrographs (\times about 84) of unsegmented ova, respectively 0.33 mm. and 0.35 mm. in diameter, from the uterus, taken immediately after their transference to the fixing fluid (picro-nitro-osmic acid), showing the shell-membrane (*s. m.*), laminated albumen (*alb.*), with sperms (*sp.*), the zona (*z. p.*), perivitelline space (*p. s.*), and the body of the ovum, with its formative (*f. z.*), and deutoplasmic (*d. z.*) zones. (D. v i v., 15, 19. vii. '01.)

Fig. 10.—Photo-micrograph ($\times 150$) of section of unsegmented ovum almost immediately after its passage into the uterus, showing the very

thin shell-membrane externally (*s. m.*) (about $\cdot 0016$ mm. in thickness), the albumen (*alb.*), zona (*z. p.*), and the dentoplasmic (*d. z.*) and formative (*f. z.*) zones of its cytoplasmic body. The male pronucleus is visible in the formative zone. Diameter of entire egg about $\cdot 29$ mm. (D. viv., 15, 19. vii. '01, $\frac{5}{8}$. Picro-nitro-osmic and iron-hæmatoxylin.)

Fig. 11.—Photo-micrograph ($\times 150$) of section of unsegmented ovum from the uterus, slightly older than that of fig. 10. Diameter of entire egg in fresh state $\cdot 34$ – $\cdot 35$ mm., of the ovum proper $\cdot 3 \times \cdot 28$ mm.; thickness of shell, $\cdot 0024$ mm. In the figure the female pronucleus is visible near the centre of the formative zone (*f. z.*), and the male pronucleus lies a little above it and to the right. The perivitelline space (*p. s.*) is partially occupied by coagulum. (D. viv., 21. v. '03, $\frac{7}{2}$. Hermann, iron-hæmatoxylin.)

PLATE 2.

Fig. 12.—Photo-micrograph ($\times 150$) of an unsegmented ovum from the uterus, of the same batch as that of fig. 11, and $\cdot 34$ mm. in diameter. The two pronuclei are visible in the central region of the formative zone.

Fig. 13.—Photo-micrograph ($\times 330$) of uterine ovum. Stage of first cleavage spindle. Diameter, $\cdot 315$ mm. (D. viv., 1, 15. vii. '01, $\frac{8}{2}$. Picro-nitro-osmic, iron-hæmatoxylin.)

Fig. 14.—Photo-micrograph (\times about 78) of egg in the 2-celled stage, taken immediately after its transference to the fixing fluid. Lateral view. *y. b.* Yolk body. Diameter of entire egg about $\cdot 34$ mm. (D. viv., 1, 15. vii. '01. Picro-nitro-osmic.)

Fig. 15.—Photo-micrograph (\times about 78) of another 2-celled egg, seen from lower pole. Diameter, $\cdot 35$ mm. (D. viv., 4 B, 23. vi. '02. Perenyi's fluid.)

Fig. 16.—Photo-micrograph (\times about 78) of another 2-celled egg, of the same batch as preceding. End view, showing one of the two blastomeres and the yolk-body (*y. b.*).

Fig. 17.—Photo-micrograph ($\times 150$) of vertical section of 2-celled egg, $\cdot 34$ mm. in diameter, showing the shell-membrane ($\cdot 0064$ mm. thick), traces only of the albumen, the zona (*z. p.*), and the two blastomeres (the left one measuring, from the sections, $\cdot 16 \times \cdot 18 \times \cdot 10$ mm., its nucleus $\cdot 031 \times \cdot 027$ mm.; the right one, $\cdot 16 \times \cdot 19 \times \cdot 09$ mm., its nucleus, $\cdot 03 \times \cdot 028$ mm.). Note the differentiation in their cytoplasmic bodies. (D. viv., 6, 21. vii. '01, $\frac{6}{2}$. Picro-nitro-osmic and iron-hæmatoxylin.)

Fig. 18.—Photo-micrograph ($\times 150$) of vertical section of 2-celled egg, $\cdot 32$ mm. in diameter, with shell-membrane $\cdot 005$ mm. thick, showing the two blastomeres, and enclosed between their upper ends the yolk-

body (*y. b.*). (D. viv., 1, 15 . vii . '01, $\frac{2}{3}$. Picro-nitro-ösmic, iron-hæmatoxylin.)

Figs. 19 and 20.—Photo-micrographs (\times about 70) of 4-celled eggs taken immediately after transference to Perenyi's fluid. Fig. 19, side view, showing yolk-body (*y. b.*); fig. 20, polar view. Diameter of entire egg about .35 mm. (D. viv., 14 B, 18 . vi . '02. Perenyi.)

Fig. 21.—Photo-micrograph (\times about 70) of another 4-celled egg, from the same batch as the preceding, seen from lower pole.

Fig. 22.—Photo-micrograph (\times 150) of section of 4-celled egg of same batch as those of figs. 19 and 20. The two right and the two left blastomeres respectively form pairs, so that the plane of the first cleavage is parallel with the sides of the plate, that of the second with the top and bottom of the same. The two left blastomeres are still connected by a narrow cytoplasmic bridge. Thickness of shell, .0072 mm.

Fig. 23.—Photo-micrograph (\times 150) of a vertical section through a 4-celled egg, .35 mm. in diameter, showing two of the blastomeres and a small portion of the yolk-body (*y. b.*). Note, as in fig. 22, the marked differentiation in the cytoplasm of the blastomeres. (D. viv., 4, 27 . vi . '01. Picro-nitro-ösmic, iron-hæmatoxylin.)

Figs. 24 and 25.—Photo-micrographs (\times 140) of horizontal sections through a 16-celled egg, .38 mm. diameter, fig. 24 showing the eight larger, more yolk-rich cells of the lower (non-formative) ring, and fig. 25 the eight smaller, less yolk-rich cells of the upper (formative) ring. Shell .0075 mm. in thickness, yolk-body (not included in the figures) .11 \times .10 mm. in diameter. (D. viv., 3 B, 26 . vi . '01; 15, $\frac{2}{3}$ and $\frac{1}{2}$. Picro-nitro-ösmic and iron-hæmatoxylin.)

Fig. 26.—Photo-micrograph (\times 140) of a vertical section of an egg of the same batch and size as that represented in figs. 24 and 25, but with seventeen cells—formative = 9 ($6 + [1 \times 2] + 1$) in division; non-formative = 8. Two of the formative cells (*f. c.*) of the upper ring are seen enclosing between them the faintly marked yolk-body (*y. b.*), and below them two of the much more opaque non-formative cells (*tr. ect.*) of the lower ring.

PLATE 3.

Fig. 27.—Photo-micrograph (\times about 76) of the just completed blastocyst, .39 mm. in diameter. From a spirit specimen. The dark spherical mass (*eg.*) in the blastocyst cavity is simply coagulum, produced by the action of the fixative (picro-nitro-ösmic acid) on the albuminous fluid which fills the blastocyst cavity. (D. viv., 2 B, 16 . vii . '01.)

Fig. 28.—Photo-micrograph (\times about 76) of a blastocyst of the same batch as the preceding, $\cdot 45$ mm. in diameter. From a spirit specimen. *cg.* Coagulum.

Fig. 29.—Photo-micrograph (\times about 75) of another blastocyst, $\cdot 45$ mm. diameter, of the same batch as the preceding, but taken immediately after transference to the fixative. Viewed from the upper pole. *y. b.* Yolk-body seen through the unilaminar wall.

Fig. 30.—Photo-micrograph (\times about 75) of a blastocyst of the same batch as the preceding, about $\cdot 39$ mm. in diameter, in which the cellular wall has not yet been completed over the lower polar region.

Fig. 31.—Photo-micrograph ($\times 140$) of a section of a blastocyst, $\cdot 39$ mm. diameter, of the same batch as the preceding and at precisely the same developmental stage, the cellular wall having yet to be completed over the lower polar region (*l. p.*). In the blastocyst cavity is seen the yolk-body (*y. b.*) partially surrounded by a mass of coagulum (*cg.*). (D. viv., 2 B, 16. vii. '01, m. = $\cdot 39, \frac{7}{8}$. Picro-nitro-osmic and iron-haematoxylin.)

Fig. 32.—Photo-micrograph ($\times 140$) of another blastocyst, $\cdot 41$ mm. in diameter, of the same batch as the preceding, also with the cellular wall still absent over the lower polar region. Shell-membrane $\cdot 0075$ mm. in thickness. *y. b.* Yolk-body. *c. g.* Coagulum. The cellular wall comprises about 130 cells.

Fig. 33.—Photo-micrograph ($\times 140$) of a blastocyst of the same batch as the preceding, with a complete unilaminar cellular wall. *y. b.* Yolk-body, in contact with inner surface of wall, in the region of the upper pole.

Fig. 34.—Photo-micrograph ($\times 100$) of a section of a blastocyst $\cdot 57$ mm. in diameter. *i. c.* Internal cell. (D. viv., 29. vi. '04, $\frac{1.5}{2}$. Picro-nitro-osmic.)

Fig. 35.—Photo-micrograph ($\times 100$) of a section of a blastocyst, $\cdot 73$ mm. diameter, of the same batch as the preceding, shell, $\cdot 0045$ mm. thick.

Fig. 36.—Photo-micrograph ($\times 100$) of a section of a blastocyst $\cdot 66$ mm. diameter, of the same batch as the preceding. Lower hemisphere opposite yolk-body (*y. b.*) formed of larger cells than upper. Hermann fixation.

Fig. 37.—Photo-micrograph ($\times 140$) of section of an abnormal vesicle, $\cdot 397$ mm. diameter of the same batch as the normal vesicles represented in figs. 27-33. *abn.* large binucleate cell, regarded as a blastomere of the lower hemisphere which has failed to divide in normal fashion, cf. text, p. 42.

PLATE 4.

Fig. 38.—Photo-micrograph ($\times 10$) of entire blastocyst 4.5 mm. diameter to show the junctional line (*j. l.*) between formative and non-formative regions. From a spirit specimen. (D. viv., β , 25. vii. '01. Picro-nitro-osmic.)

Fig. 39.—Photo-micrograph (\times about 10) of an entire blastocyst, 4.5 mm. diameter with distinct embryonal area (*emb. a.*). (D. viv., 5, 18. vii. '01.)

Fig. 40.—Photo-micrograph ($\times 10$) of entire blastocyst about 5 mm. diameter showing embryonal area (*emb. a.*), peripheral limit of entoderm (*l. ent.*), and the still unilaminar region of the wall (*tr. ect.*). (D. viv., 8. vi. '01.)

Fig. 41.—Photo-micrograph ($\times 150$) of an *in toto* preparation of the wall of a blastocyst of 3.5 mm. diameter. (D. viv., 16, 21. vii. '01.)

Fig. 42.—Photo-micrograph ($\times 150$) of an *in toto* preparation of the wall of a blastocyst of 3.25 mm. diameter. *j. l.* Junctional line between the formative (*f. a.*) and non-formative (*tr. ect.*) regions of the wall. (D. viv., 24. vii. '01.)

Figs. 43 and 44.—Photo-micrographs ($\times 150$) of *in toto* preparations of the wall of 4.5 mm. blastocyst showing the junctional line between the formative (*f. a.*) and non-formative (*tr. ect.*) regions. (D. viv., β , 25. vii. '01. Picro-nitro-osmic and Ehrlich's hæmatoxylin.)

Fig. 45.—Photo-micrograph ($\times 150$) of a corresponding preparation of the wall of a more advanced 4.5 mm. blastocyst ('99 stage), in which the two regions of the wall are now clearly distinguishable. (D. viv., 8. 7. '99. Picro-nitro-osmic, Ehrlich's hæmatoxylin.)

Fig. 46.—Photo-micrograph ($\times 150$) of a corresponding preparation of a slightly more advanced blastocyst ('04 stage). (D. viv., 6. 7. '04. Picro-nitro-osmic, Ehrlich's hæmatoxylin.)

PLATE 5.

Fig. 47.—Photo-micrograph ($\times 150$) of an *in toto* preparation of the formative region of a 6. 7. '04 blastocyst, showing the proliferation of spherical internal cells referred to in the text, p. 53.

Fig. 48.—Photo-micrograph ($\times 150$) of an *in toto* preparation of the wall of a vesicle of the same batch as that represented in fig. 39, in which a small part of the junctional line between the embryonal ectoderm and the extra-embryonal (*tr. ect.*) is visible, the free edge of the entoderm (*ent.*) not having reached it. (D. viv., 5, 18. vii. '01. Picro-nitro-osmic, Ehrlich's hæmatoxylin.)

Fig. 49.—Photo-micrograph ($\times 150$) of a corresponding preparation of a vesicle of the same batch as the preceding, in which the wavy and irregularly thickened free edge of the entoderm (*ent.*) practically coincides with the junctional line and so conceals it from view.

Fig. 50.—Photo-micrograph ($\times 150$) of an *in toto* preparation of a vesicle (S. vi. '01 batch) viewed from the inner surface as in the corresponding preceding figures. The entoderm in the region of the embryonal area has been removed, so that one sees the inner surface of the embryonal ectoderm (*emb. ect.*); it is still *in situ*, though not in a quite intact condition over the adjoining portion of extra-embryonal ectoderm. The entoderm has not yet extended over the region indicated by the reference line to *tr. ect.*, so that here the extra-embryonal ectoderm is clearly visible. The junctional line is apparent. (D. viv., S. vi. '01. Picro-nitro-osmic. Ehrlich's hæmatoxylin.)

Fig. 51 (Plate 3).—Photo-micrograph ($\times 310$) of a section of a 30-celled egg of *Perameles obesula*; egg *b*, 24×23 mm. diameter, showing the unilaminar layer formed by the blastomeres.

Fig. 52 (Plate 3).—Photo-micrograph ($\times 240$) of a section of a blastocyst of *P. nasuta* 29×26 mm. diameter, showing the shell-membrane (*s.m.*), zona (*z.p.*), and the unilaminar cellular wall. The portion of the latter adjacent to the reference lines is composed of smaller but thicker cells than the remainder.

PLATE 6.

Figs. 53 and 54.—Drawings ($\times 84$) of a 6-celled egg 34 mm. diameter, fig. 53 showing a side view and fig. 54 a view from the lower pole. Observe the characteristic ring-shaped arrangement of the blastomeres. *y. b.* Yolk-body, the shell-membrane, albumen layer with sperms included, and the zona are readily distinguishable. Outlines drawn with the aid of the camera lucida immediately after transference of the egg to the fixing fluid. (D. viv., 22, 16. vii. '01.)

Figs. 55 and 56.—Drawings (\times about 88) of a 16-celled egg (about 37 mm. diameter) as seen from the side and lower pole respectively, from the same batch as the eggs represented in figs. 24, 25, and 26. The characteristic arrangement of the blastomeres in two superimposed, open rings (each of eight cells) and the difference in size between the cells of the two rings are evident. The irregular body (*c.g.*) seen in the cleavage cavity in fig. 56 is a mass of coagulum. Drawn from a spirit specimen. The albumen layer as represented in fig. 56 is too thick. (D. viv., 3 B, 26. vi. '01.)

Figs. 57 and 58.—Drawings (\times about 85) of a 12-celled egg (38 mm. diameter) as seen from the side and lower pole respectively. Four of

the blastomeres of the 8-celled stage have already divided ($4 + 4 \times 2 = 12$). From a spirit specimen and from same batch as preceding.

Fig. 59.—Drawing (\times about 88) of a 31-celled egg (.375 mm. diameter) as seen from the lower pole. From a spirit specimen and from the same batch as the preceding. The irregular body in the blastocyst cavity is formed by coagulum. Formative cells = 16; non-formative = 14 + 1 in division.

Fig. 60.—Drawing (\times about 88) of another 31-celled egg (.375 diameter) from the same batch as the preceding. Side view.

Fig. 61.—Drawing (\times 100) of an entire blastocyst (.39 mm. diameter) from the same batch as those shown in figs. 27-29.

Fig. 62.—Drawing (\times about 80) of an entire blastocyst (.4 mm. diameter) from the same batch as the preceding.

Fig. 63.—Drawing (\times 80) of an entire blastocyst (.6 mm. diameter) made from a photograph taken directly after transference of the specimen to the fixing fluid. Cells of lower hemisphere with much more marked perinuclear areas of dense cytoplasm than those of the upper. (D. viv., 2, 11, vii. '01.)

Fig. 64.—Section of the wall of a blastocyst, 2.4 mm. diameter (\times 630). (D. viv., 7, vi. '01.)

Figs. 65, 66, 67.—Drawings (\times 630) of small portions of in toto preparations of the formative region of 6.7. '04 blastocysts to demonstrate the mode of origin of the primitive entodermal cells (*ent.*, fig. 67). Fig. 65 shows a dividing entodermal mother-cell in position in the unilaminar wall, surrounded by larger lighter staining cells (prospective embryonal ectodermal cells). In fig. 66 is seen a corresponding cell, a portion of whose cell-body has extended inwards so as to underlie (overlie in figure) one of the ectodermal cells of the wall. In fig. 67 are seen two entodermal cells, evidently sister-cells, the products of the division of such a cell as is seen in figs. 65 or 66. One of them (the upper) is still a constituent of the unilaminar wall, the other (*ent.*) is a primitive entodermal cell, definitely internal. (D. viv., 6, 7. '04. Picro-nitro-osmic, Ehrlich's hæmatoxylin.)

PLATE 7.

Figs. 68, 69, 70.—Drawings (\times 630) of portions of preparations similar to the above. For description see text. (D. viv., 6, 7, '04.)

Fig. 71.—Drawing (\times about 630) of a portion of an in toto preparation of the formative region of an '01 blastocyst showing two primitive entodermal cells, one of them in division. (D. viv., β , 25, vii. '01. Picro-nitro-osmic and Ehrlich.)

Fig. 72.—Drawing ($\times 630$) corresponding to the above, from the formative region of a 6.7. '04 blastocyst, also showing two primitive entodermal cells, evidently sister-cells.

PLATE 8.

Figs. 73, 74, 76.—Sections of the formative region of 6.7. '04 blastocysts, showing the attenuated shell-membrane, the unilaminar wall, and in close contact with the inner surface of the latter, the primitive entodermal cells (*ent.*) ($\times 630$).

Fig. 75.—Section corresponding to the above, showing an entodermal mother-cell (*ent.*), part of whose cell-body underlies the adjacent ectodermal cell of the wall. The spheroidal inwardly projecting cell on the left is probably also an entodermal mother-cell ($\times 630$).

Fig. 77.—Section ($\times 630$) of the non-formative region of a 6.7. '04 blastocyst.

Fig. 78.—Section ($\times 630$) of the embryonal area, and the adjoining portion of the still unilaminar extra-embryonal region of a blastocyst of the 5. '01 stage. *emb. ect.* Embryonal ectoderm. *ent.* Entoderm. *tr. ect.* Extra-embryonal ectoderm (tropho-ectoderm). The position of the junctional line is readily recognisable. (D. *viv.*, 5, 18. *vii.* '01. Picro-nitro-osmic and Delafield's hæmatoxylin.)

Fig. 79.—Section ($\times 630$) through the corresponding regions in an 8. *vi.* '01 blastocyst. Note the thickening of the embryonal ectoderm (*emb. ect.*), and the peripheral extension of the entoderm (*ent.*) below the tropho-ectoderm. (D. *viv.*, 8. *vi.* '01. Picro-nitro-osmic and Delafield.)

Fig. 80.—Section ($\times 600$) through the formative (embryonal) region of a blastocyst of *P. nasuta*, 1.3 mm. in diameter. It is thicker than that of the *Dasyure* blastocyst at the corresponding stage of development; the primitive entodermal cells are well marked.

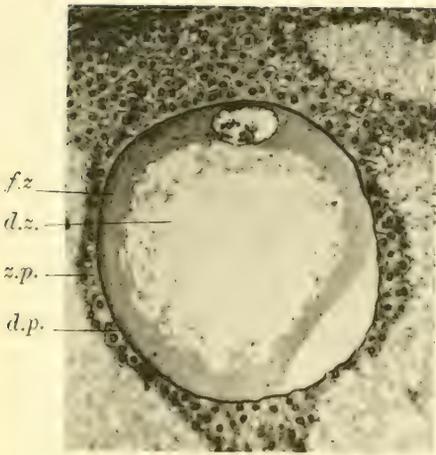
Fig. 81.—Section ($\times 600$) corresponding to the above from another 1.3 mm. blastocyst of *P. nasuta*, of the same batch as the preceding, but apparently very slightly earlier, the entodermal cells being still in process of separating from the unilaminar wall. *ent.* Entoderm. *tr. ect.* Tropho-ectoderm.

PLATE 9.

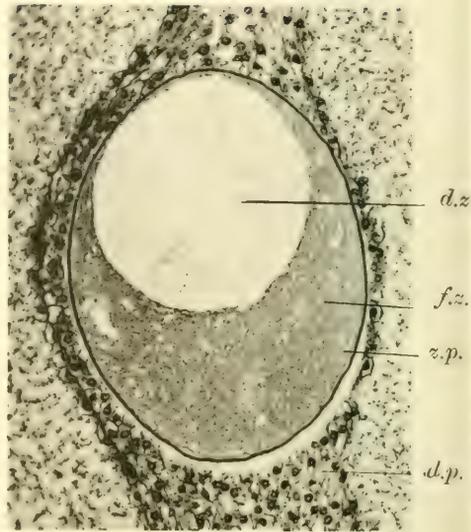
Fig. 82.—Section (\times about 430) of a section of a blastocyst of *M. ruficollis* .35 mm. in diameter, showing the major portion of the formative region (*f. a.*) and a small portion of the non-formative (*tr. ect.*).

The shell-membrane varies in thickness in the sections from .005 mm. over the former region to .003 mm. over the latter.

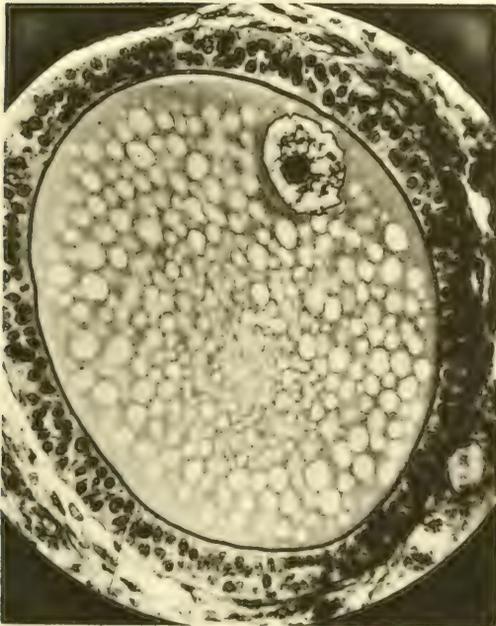
Figs. 83, 84, 85.—Drawings ($\times 630$) of small portions of the formative (and in fig. 83 of the adjoining portion of the non-formative) region of the above blastocyst of *M. ruficollis* more highly magnified. *ent.* Primitive entodermal cells. Note in fig. 83 a cell of the wall in division, the axis of the spindle being oblique to the surface.



1

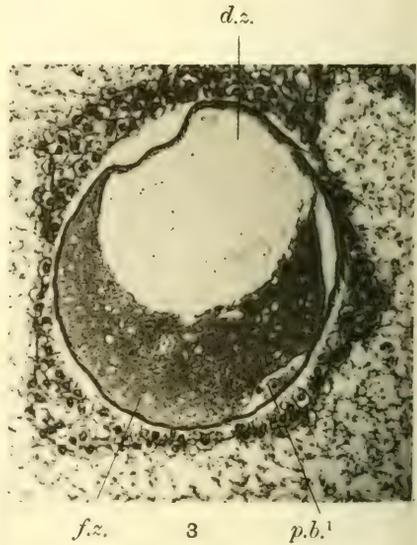


2



f.ep.

4

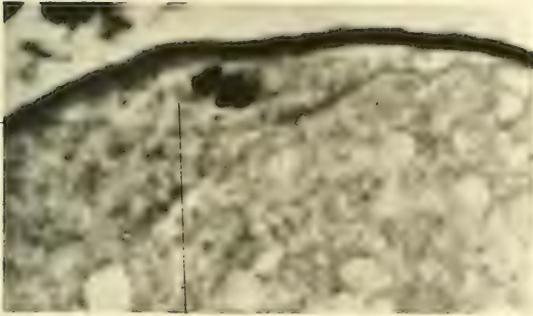


f.z.

3

p.b.¹

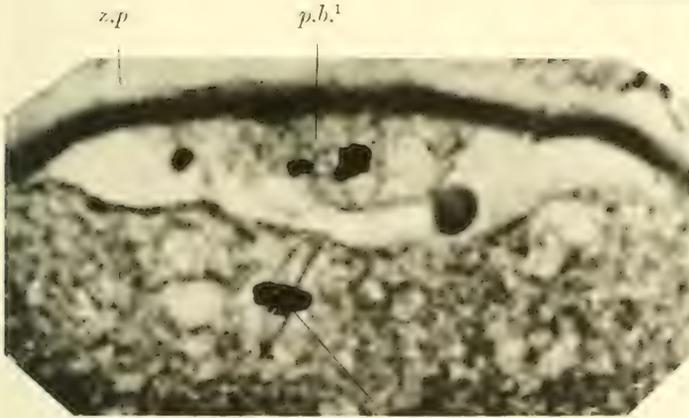
J. P. HILL, *photo.*
 WATERLOW & SONS LIMITED, *Collotype.*



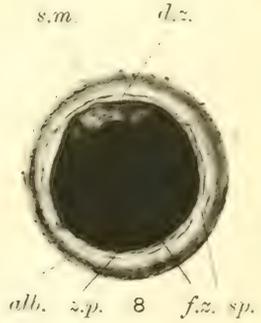
*p.b.*¹ 6



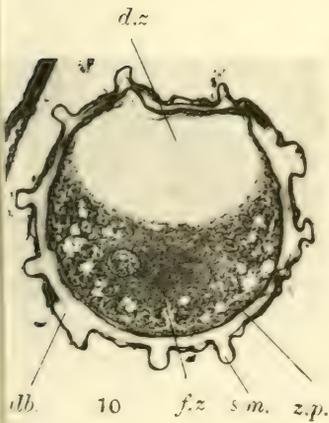
*p.b.*¹s. 5



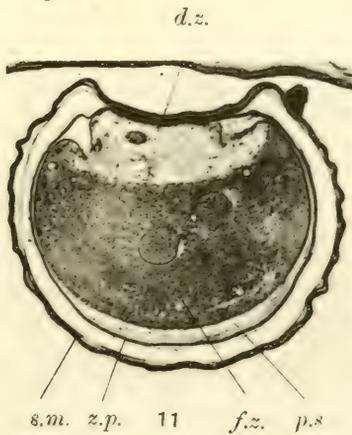
7 *p.b.*²s.



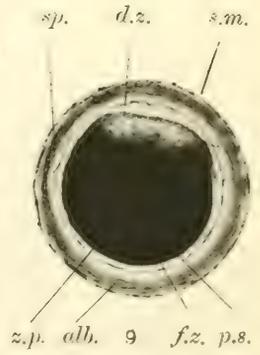
s.m. *d.z.*
8 *alb.* *z.p.* *f.z.* *sp.*



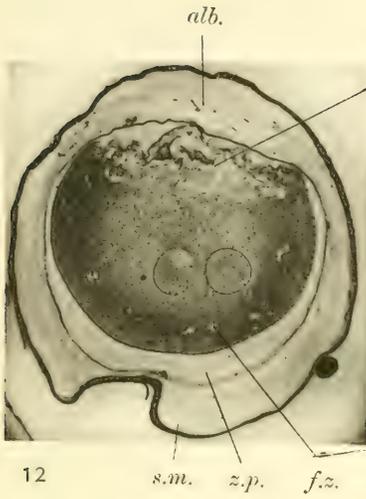
d.z.
10 *alb.* *f.z.* *s.m.* *z.p.*



d.z.
11 *s.m.* *z.p.* *f.z.* *p.s.*

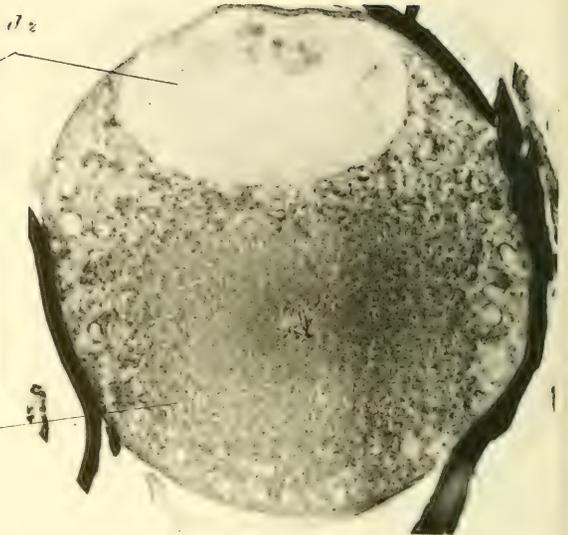


sp. *d.z.* *s.m.*
9 *z.p.* *alb.* *f.z.* *p.s.*

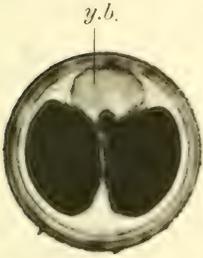


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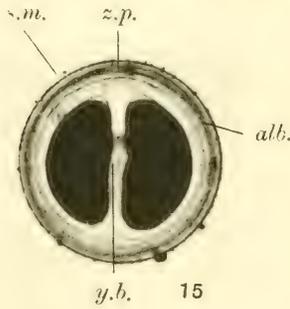
s.m. z.p. f.z.



13



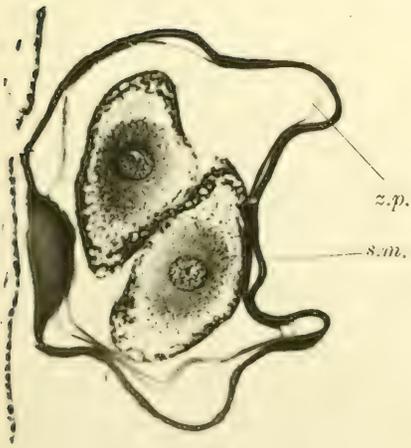
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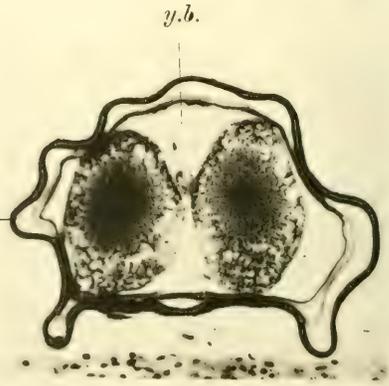
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16

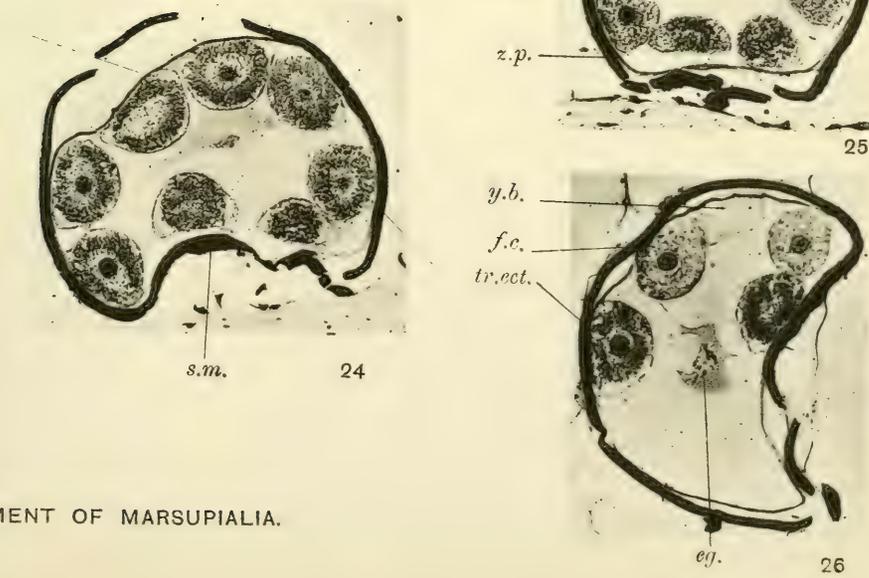
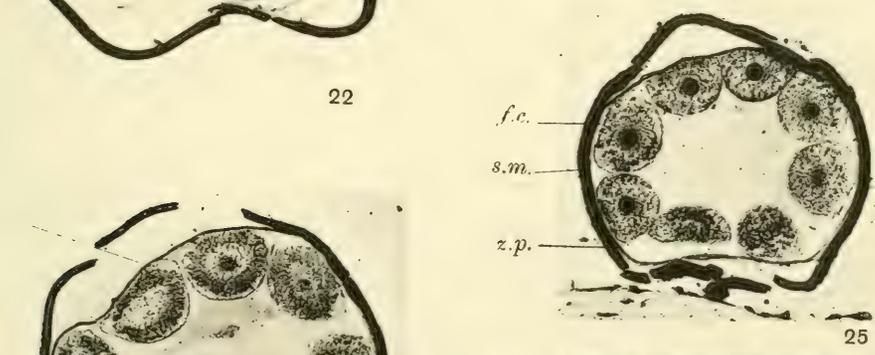
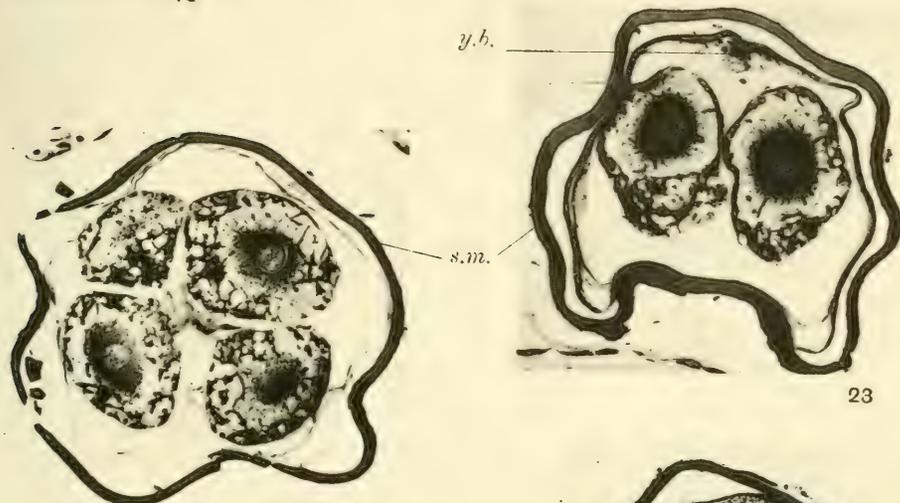
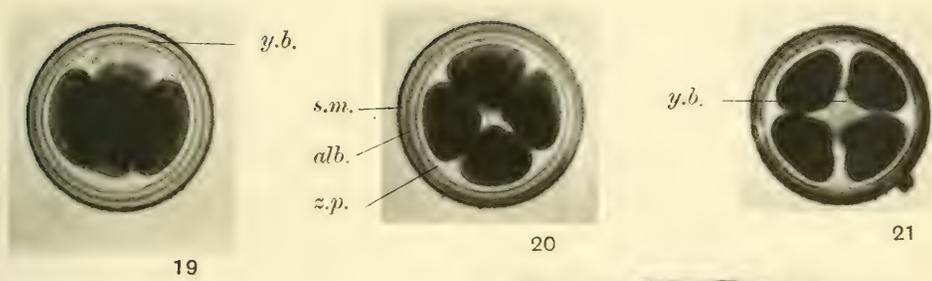


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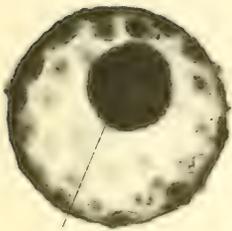


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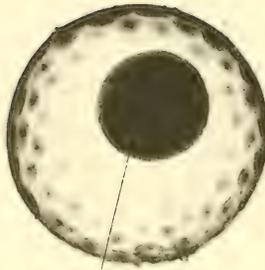
J. P. HILL, Photo.
WATERLOW & SONS LIMITED, Collotype.



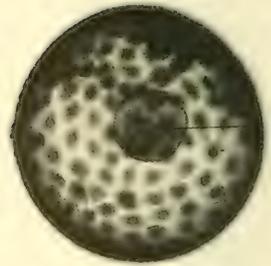




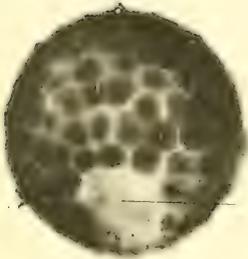
cg. 27



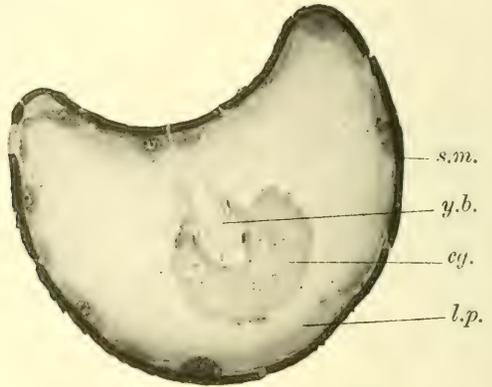
cg. 28



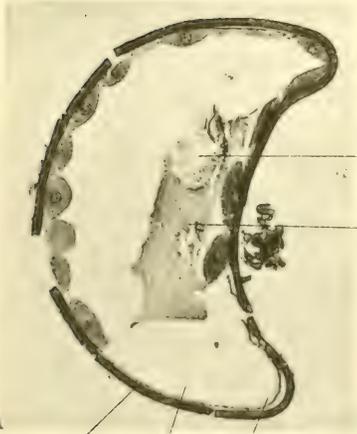
y.b. 29



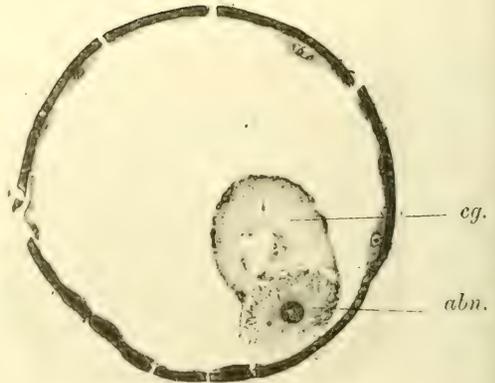
l.p. 30



s.m. y.b. cg. l.p. 32



y.b. cg. s.m. l.p. z.p. 31



cg. abn. 37

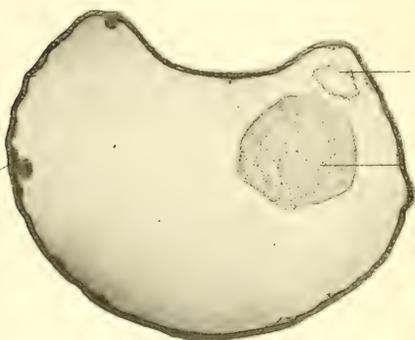
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WATERLOW & SONS LIMITED, Collotype.



cg.

y.b.

33

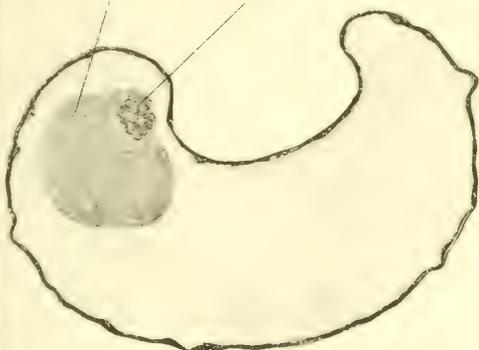


y.b.

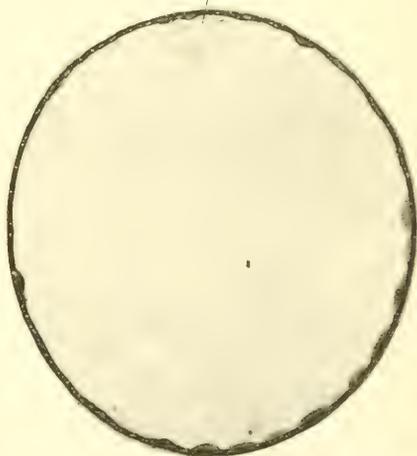
cg.

y.b.

34



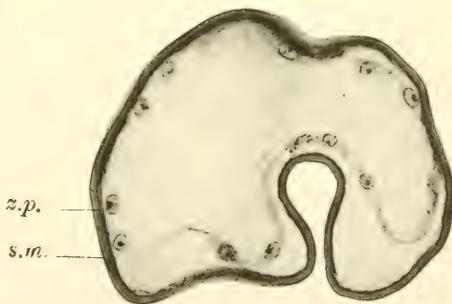
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36



51



z.p.

s.m.

52



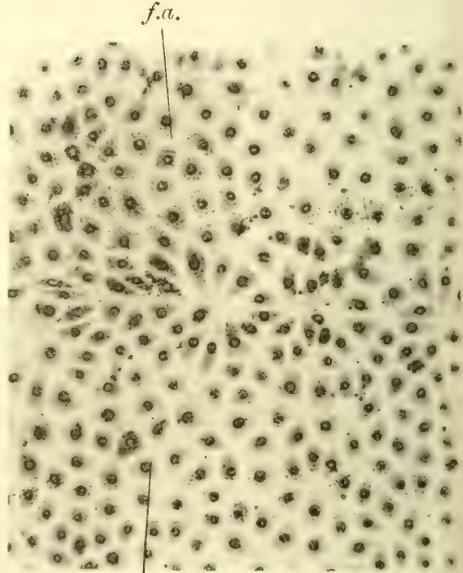
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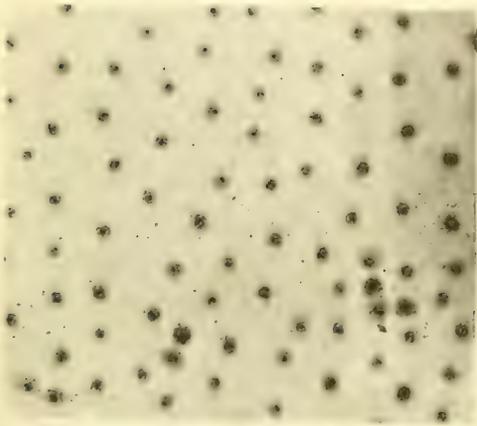
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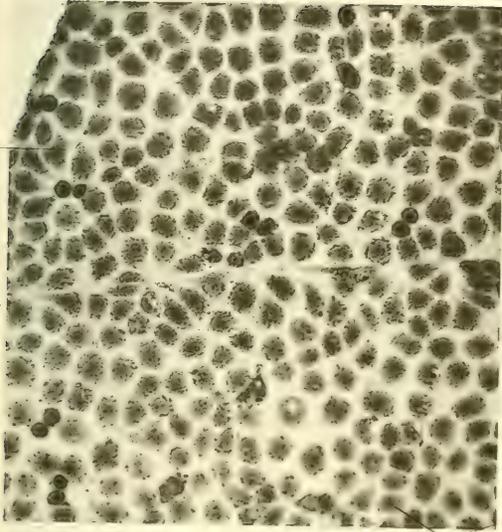


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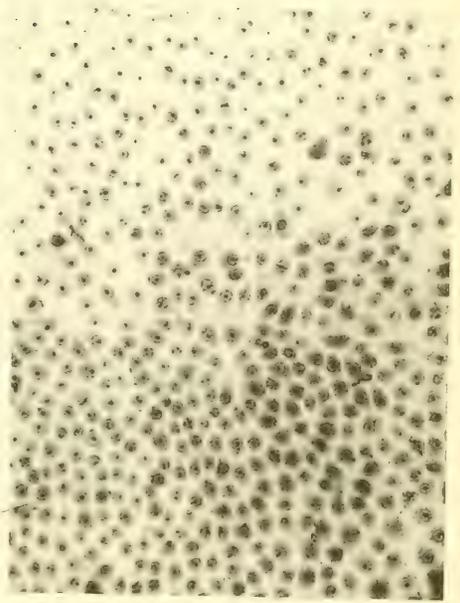
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J. P. HILL, Photo.
WATERLOW & SONS LIMITED, Collotype.



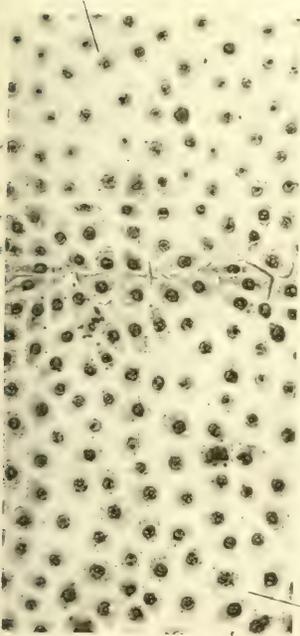
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tr.eci

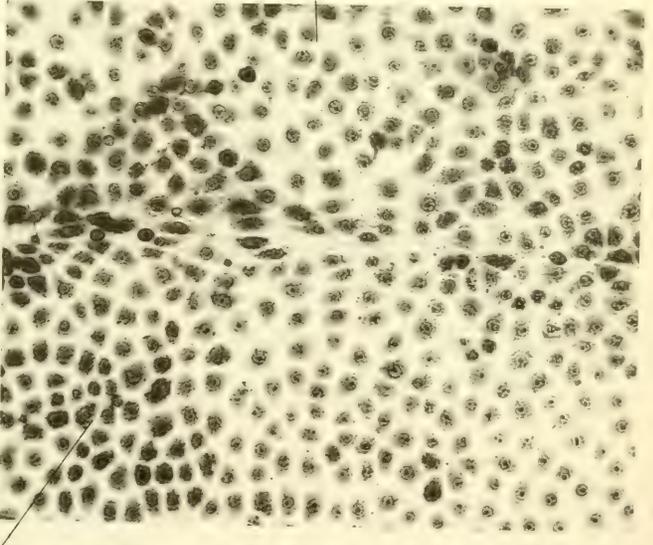


45

f.a.

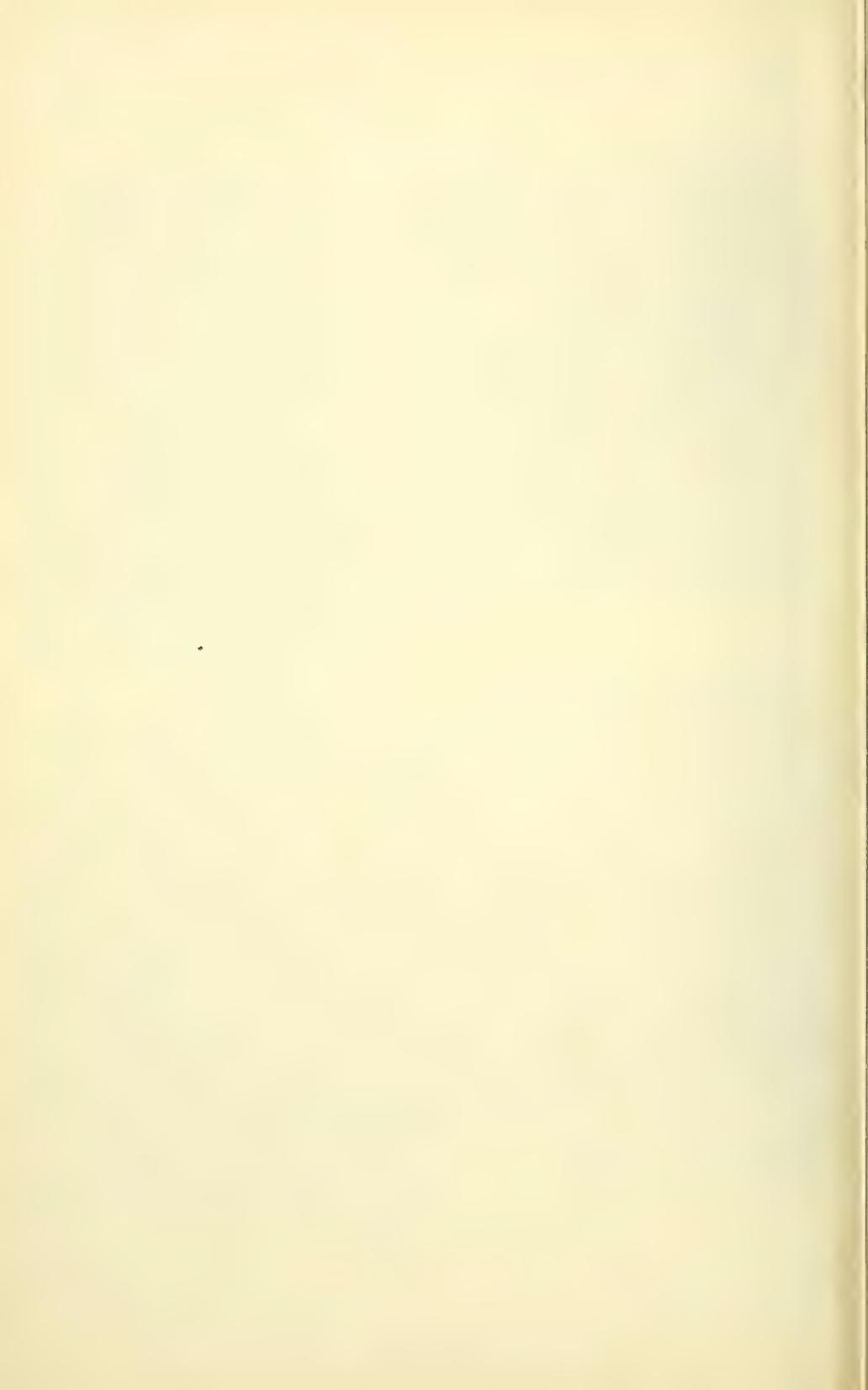


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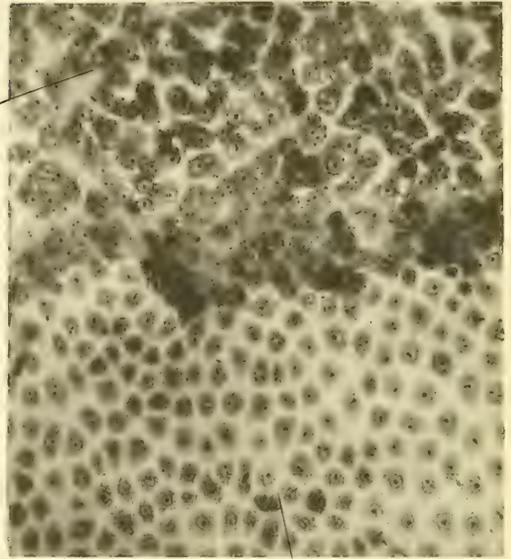
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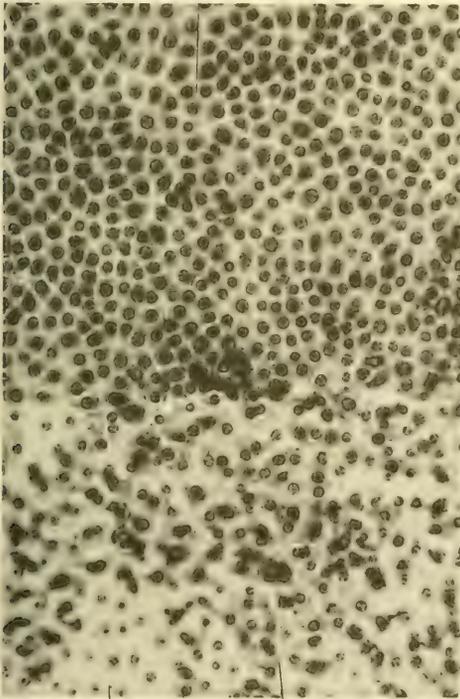
emb.ect.

48



tr.ect.

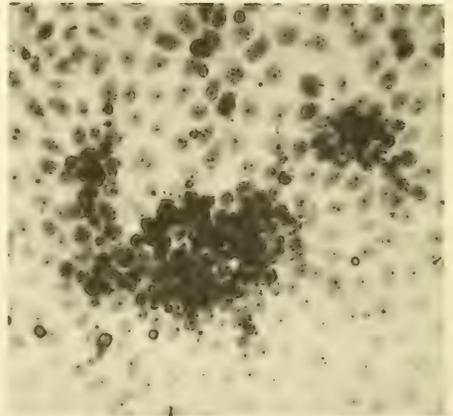
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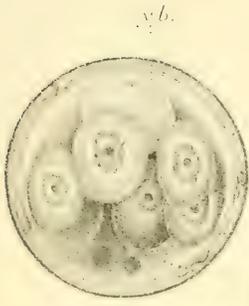
ent.

50

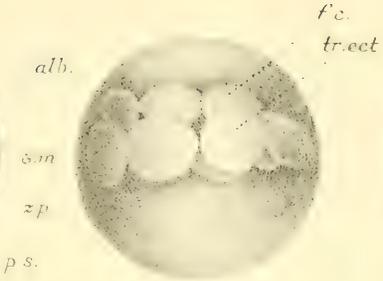


47

J. P. HILL, Photo.
WATERLOW & SONS LIMITED, Collotype.



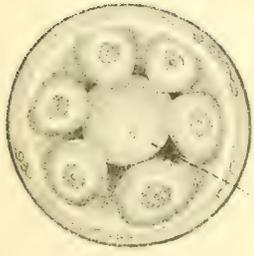
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55.



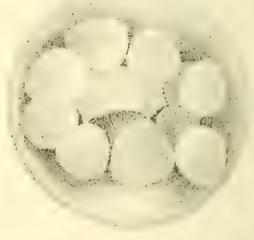
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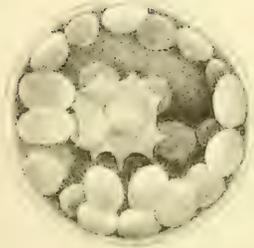
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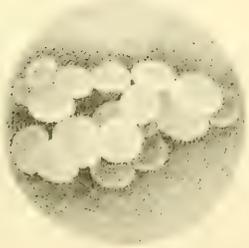
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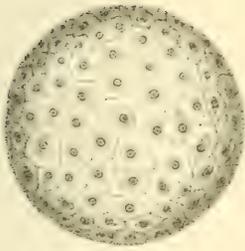
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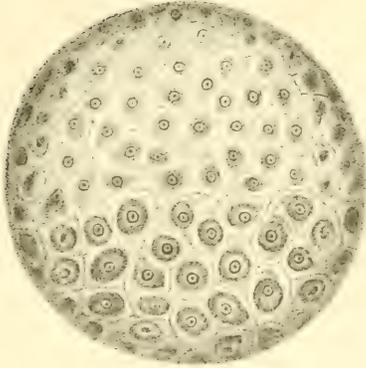
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61.



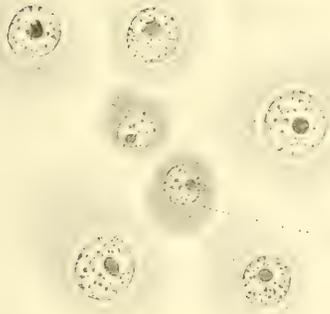
62.



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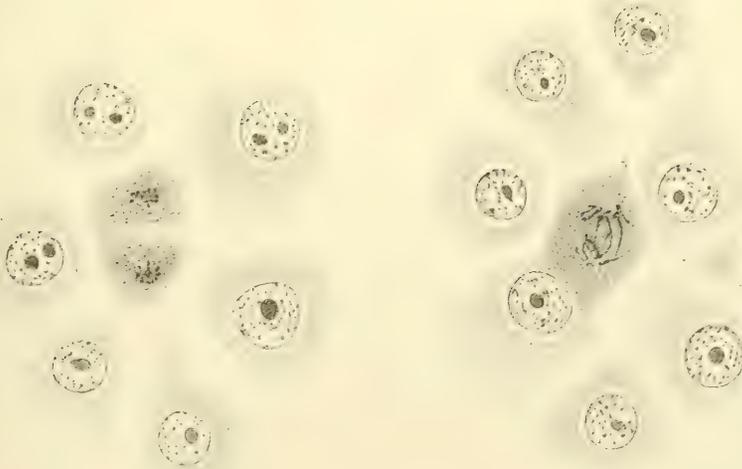
64.



67.

ent.

5 m.

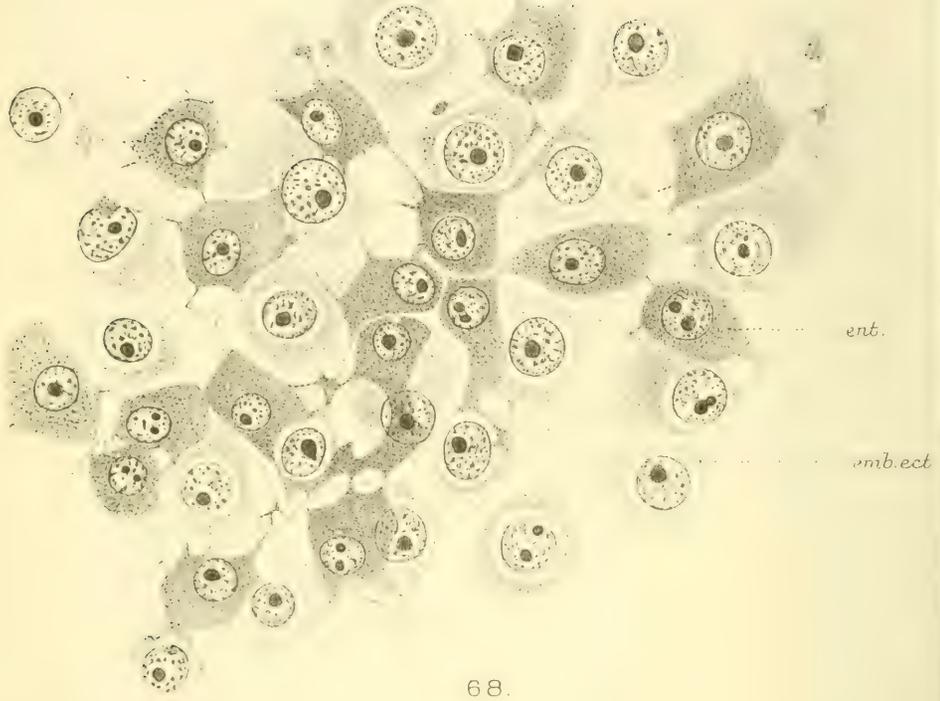


65.

66.







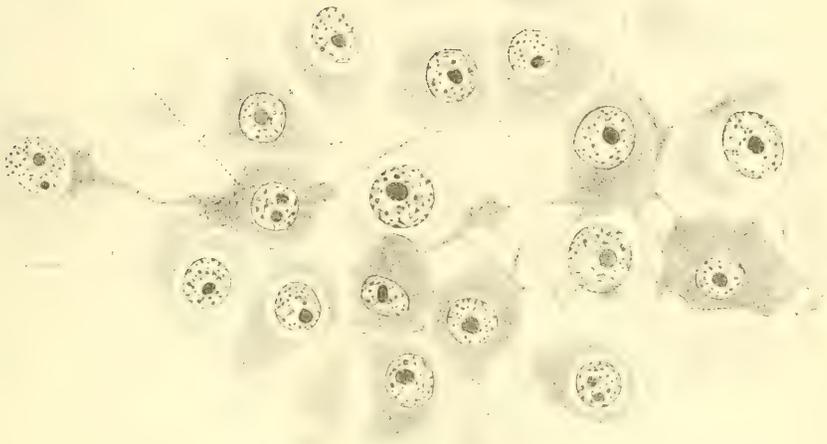
68.



69.

emb.ect

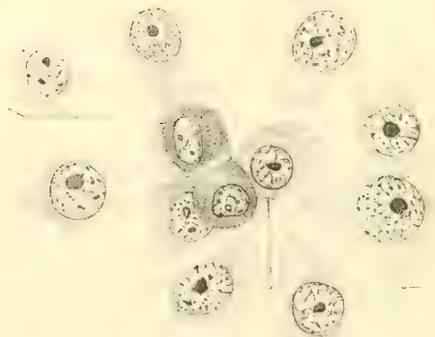
275.



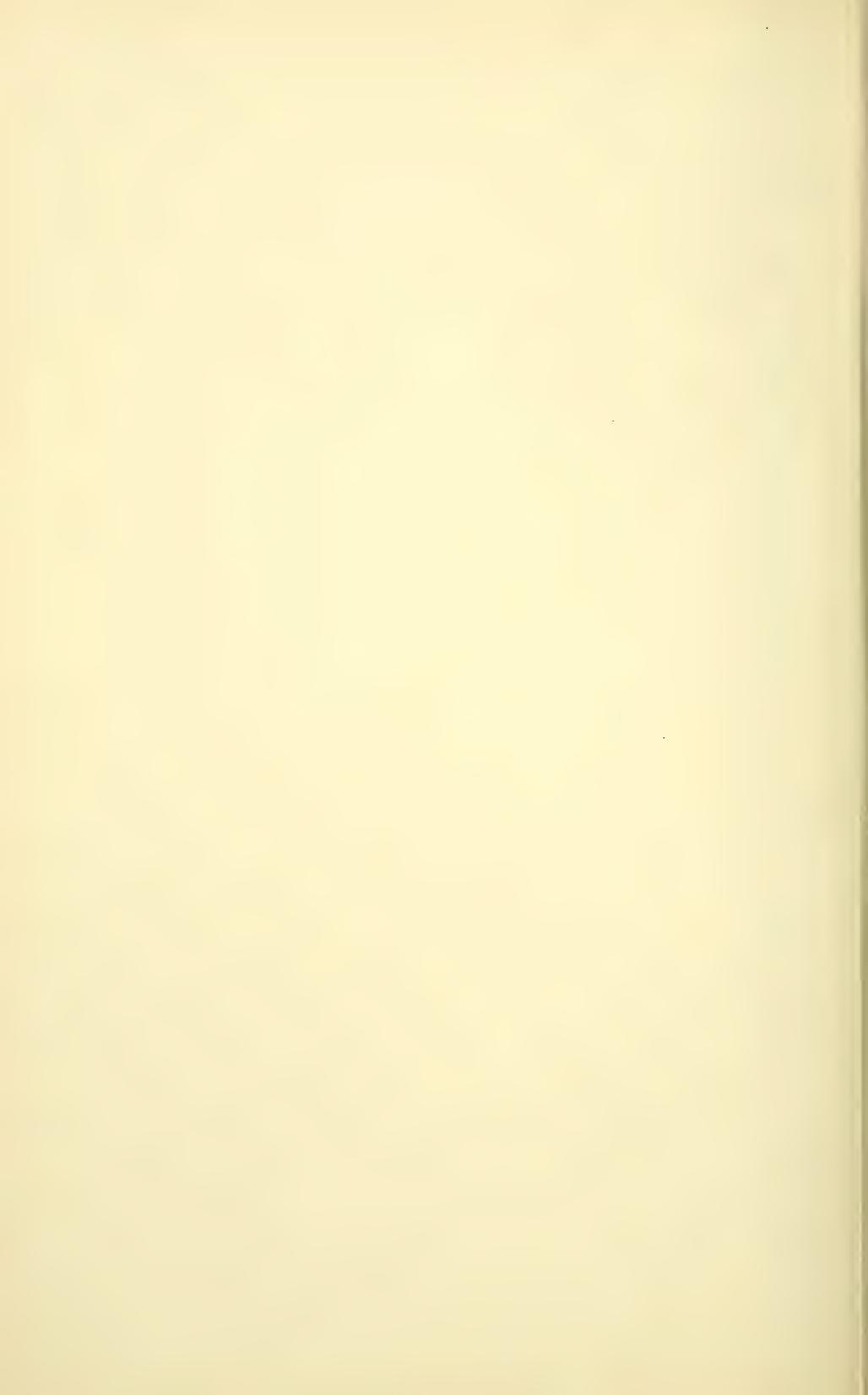
70.



71.



72.





73.



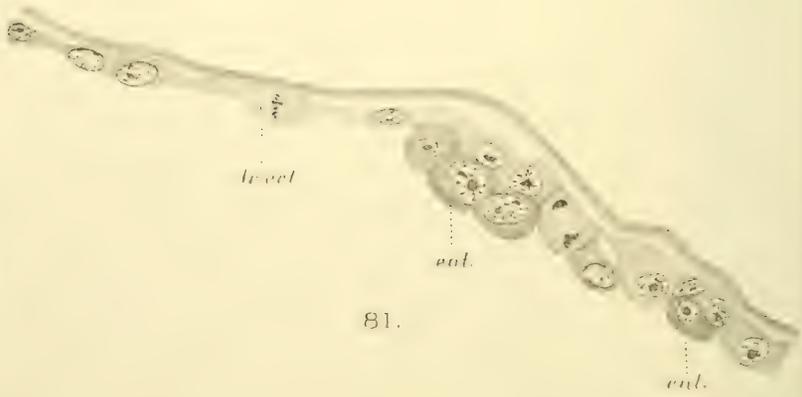
74.



75.

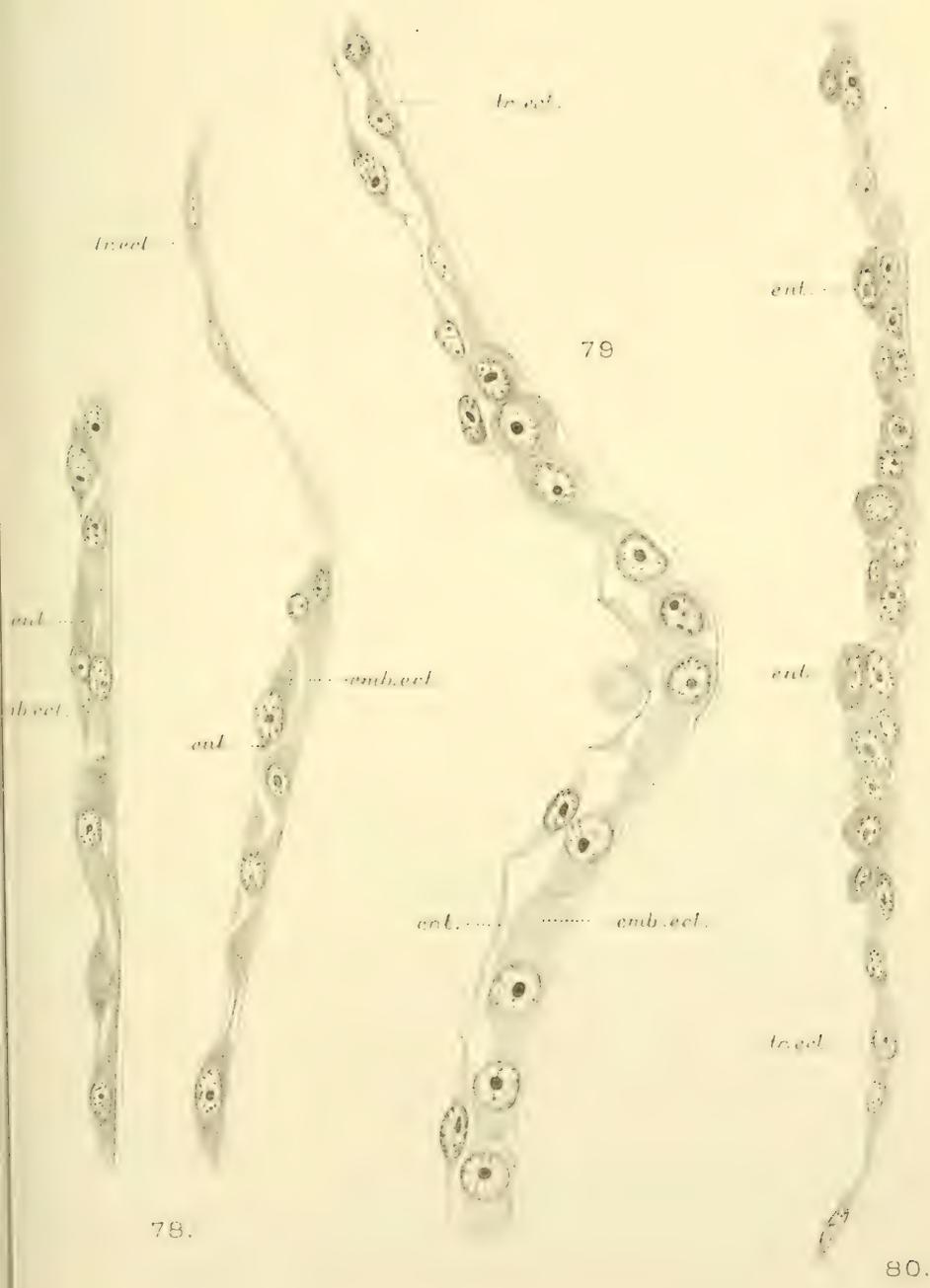


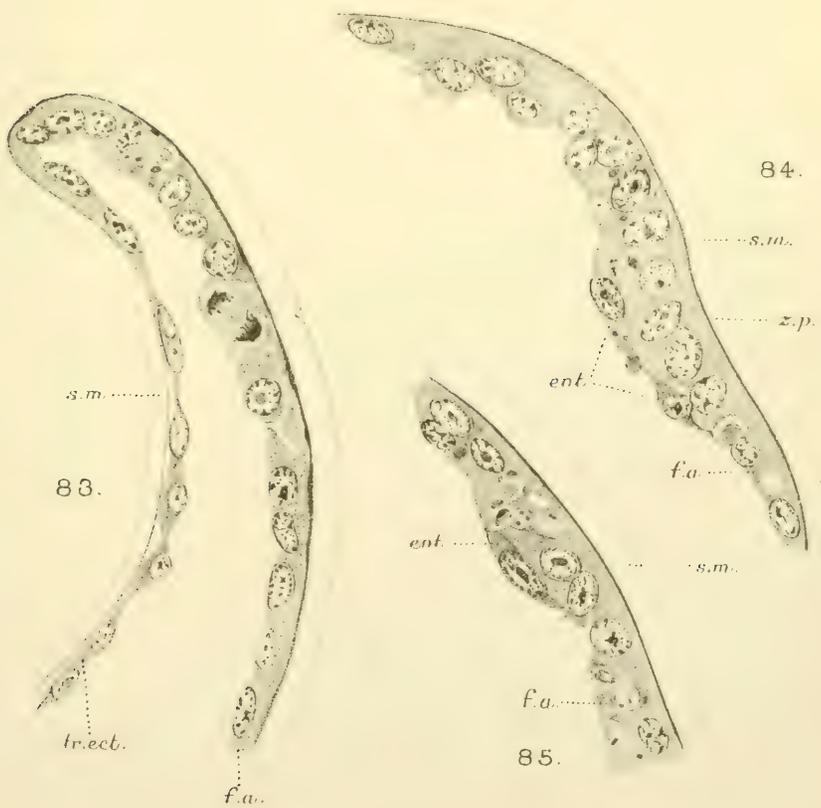
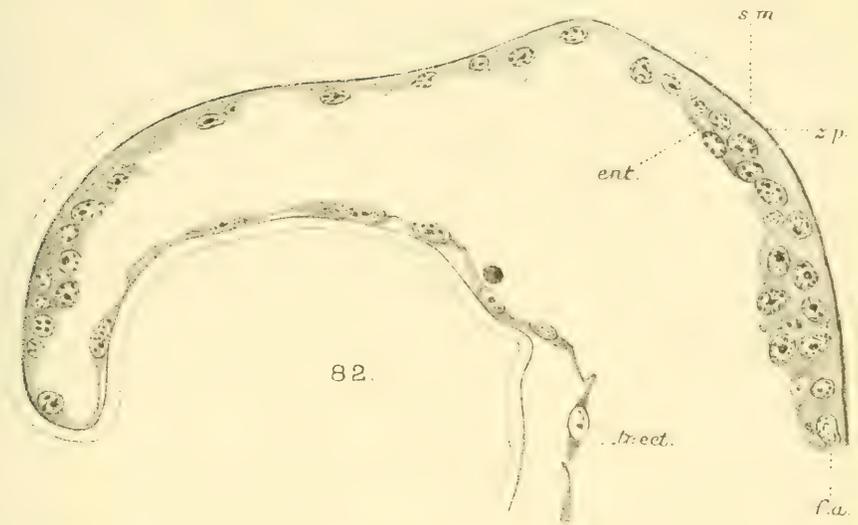
76.



81.

77.





Notes on a Deep-sea Echiuroid, *Acantho-
hamingia shiplei* (n. g. et n. sp.), with Re-
marks on the Species *Hamingia ijimai*,
Ikeda.

By
Dr. Iwaji Ikeda.

With Plate 10.

IN July, 1909, I obtained an Echiuroid animal through the kindness of Mr. Aoki, of the Marine Biological Station at Misaki, Japan. As at that time the date of my departure from Japan was near at hand I was compelled to bring with me the specimen, which remained untouched until the end of October, when I entered the Zoological Laboratory of the University of Cambridge. After some weeks' work in that laboratory I discovered that this single specimen was a very curious and undescribed species belonging to the Bonellidæ, and apparently to the genus *Hamingia*. Doubt was, however, thrown upon relationship of the new form to *Hamingia* by two remarkable features, the presence (i) of the abnormally numerous ventral hooks and (ii) of the skin-papillæ. The absence of the two structures just mentioned represents the most essential generic characteristics of *Hamingia*, distinguishing the genus from the rest of the Bonellidæ, *Bonellia* and *Protobonellia*.¹ Seeing many close relationships between the present species and *Hamingia ijimai*,²

¹ Ikeda, I., "Notes on a New Deep-sea Echiuroid, *Protobonellia mitsukurii*," 'Annot. Zool. Jap.,' vol. vi, Part IV, 1908.

² Ikeda, I., "On a New Echiuroid (*Hamingia ijimai*) from the Sagami Bay," 'Annot. Zool. Jap.,' vol vii, Part I, 1908.

both in its general "facies" and its anatomy, I suspected that the type-specimen of *H. ijimai* might possess the two structures mentioned above, and I asked Mr. S. Takahashi, my colleague in the College of Hiroshima, to re-examine the type-specimen. His reply was that *H. ijimai* has hooks similar in nature to those of the present species, but no skin-papillæ at all. Thus informed, I have decided to propose the new genus *Acanthohamingia* for the reception of both the present species and that which I formerly described as *Hamingia ijimai*. The question of classification will be more fully discussed later. The following contribution, though brief and incomplete in many points, is produced with the hope of adding something to our present knowledge of the group of Bonellidæ. Before going further, I wish to express my deep gratitude to Professor J. Stanley Gardiner, F.R.S., and Mr. A. E. Shipley, F.R.S., through whose great kindness and generosity I have been enabled to produce the present study, being supplied with every necessary convenience of the laboratory. I also feel greatly indebted to Mr. S. Takahashi for his kindness in supplying me with the prompt information for which I troubled him.

ACANTHOHAMINGIA SHIPLEI (n.g. et n. sp.).

This unique specimen was taken, in January of 1909, from the basin (the Okinosé) of the Sagami Bay at a depth of 400 fathoms, the same locality from which *Protobonellia mitsukurii* and *Hamingia ijimai* had been procured. Fig. 1, Plate 10, represents the animal in the preserved state and the natural size, the ventral side being turned upwards. As is shown in the figure, the skin of the body proper was seriously broken, on the left side, by a hook of the long line with which the animal was caught. Through the wound thus caused some loops, mostly torn, of the coiled intestine protruded. The fact last mentioned has made it difficult or almost impossible rightly to trace and identify different parts of the entangled intestinal loops. The general measurements of the animal are as follows:

Length of proboscis (measured as fully stretched)	85 mm.
Maximum breadth of proboscis	5 mm.
Length of body proper	62 mm.
Maximum breadth of body proper	22 mm.

As regards the colour of the animal in the fresh state, nothing could be ascertained beyond Mr. Aoki's statement that it was of a pale yellow colour; a faint tinge of this remained after preservation.

To refer more fully to the external morphology of the animal, the proboscis is of the usual shape, resembling that of *Hamingia ijimai*. It is deeply and widely grooved on the ventral side along its whole length. Near the anterior end it becomes abruptly narrowed and ends rather pointedly (fig. 2). Towards the mouth the free margins come together, partly overlapping each other, so as to form a funnel-shaped passage directly leading to the mouth. The whole surface of the organ is smooth, but with many minute wrinkles.

Both extremities of the body proper are rounded. The integument, which is rather thin and somewhat transparent in the most swollen part of the body, is rather rough owing to the presence partly of dense accumulation of skin-folds in both terminal regions and partly of scattered papillary bodies over the remaining parts. The skin-folds, which are about 0.1 mm. thick, are arranged in irregular transverse rows something like the leaves of a book. In fig. 1 some conspicuous folds (or more properly, grooves separating the folds) are represented. Arising close to the mouth there is a light and narrow mid-ventral groove about 10 mm. long, which begins anteriorly with a narrow, streak-like fissure and terminates posteriorly in a comparatively broad depression. This groove is in my opinion of the same nature as that formerly described by me in *Hamingia ijimai*. It might be named the genital groove, since in both species it is practically concurrent with the genital aperture, which is situated at the posterior angle of the groove.

No special papillæ around the genital aperture, such as

occur in *Hamingia arctica*,¹ are found in the genital groove.

The papillary bodies are scattered almost uniformly over the non-wrinkled surface, but they are more densely crowded at places where the skin is more contracted. When viewed from the surface they appear as small opaque patches of irregular shape and various size, which are separated from each other mostly by numerous light, transverse furrows, varying somewhat in length and in their course (fig. 3). Most of them are nearly flat, but some of larger size (measuring 0.3–0.4 mm. in diameter) are slightly elevated above the skin-surface. Each structure (fig. 4) looks, if seen by transmitted light, like a thick accumulation of very small refringent granules heaped up into several smaller irregular-shaped masses. Some of the granules are seen to be of the same nature as those found dispersed throughout the general surface of the skin. In the wrinkled regions, also, the papillary bodies are constantly present, and are crowded on to the very ridges of the transverse skin-folds. The only difference which exists between them and the dispersed papillæ is that they are a little smaller in size and more irregular in shape.

The structure of the skin resembles that described by Horst² in *Hamingia arctica* (= *gracilis*), that is, the skin is composed of six distinct layers—the epidermis, the cutis, the outer circular, the middle longitudinal, the inner oblique muscles, and the peritoneal epithelium. But the present species shows some points of peculiarity with regard to the histology of the cutis and the epidermis. The cutis (fig. 5, *cu.*), which is the thickest of all the dermal layers, consists essentially of a gelatinous matrix, which is traversed by very numerous and highly delicate fibrils (*fb.*) running

¹ Danielsen, D. C., and Koren, J., 'The Norwegian North Expedition, 1876–1878; *Gephyrea* (Zoology), 1881, Christiania.'

² Horst, R., "Die *Gephyrea* gesammelt während der zwei ersten Fahrten des 'Willem Barent,'" 'Niederland. Arch. für Zool.,' suppl., Bd. 42, 1881.

radially across the whole thickness. Besides these fibrils, the cutis contains small, mostly round, and rather sparsely dispersed spaces (*lac.*), each of which encloses a very small cell (*c.*). Although owing to the imperfect preservation I have been unable to make out exactly the minute structure of these corpuscle-like cells, yet, as far as my preparations show, the latter are indubitably nucleated, and are provided with a few slender processes reaching the sides of the enclosing capsule. These cells are no doubt similar to the cutis-corpuscles known in the cutis of *Hamingia arctica* (Danielsen and Koren, and Horst); in this case, however, the cells are not embedded in any special cavity. I am not able at present to decide whether these lacuna-spaces are or are not largely artefacts due to strong contraction of the corpuscles themselves.

The epidermis is comparatively thin, consisting of a single layer of cells, cubical or subcylindrical in shape, and of unicellular glands of various sizes. Rather curiously, the cuticle, which is clearly differentiated in *Hamingia arctica*, is not found as a distinct layer or, at least, cannot be distinguished from the epidermal epithelium. Whereas in smooth parts of the skin the gland-cells are in general small and only sparsely distributed, in the papillæ they (*p. gl.*) are so conspicuously large and so closely aggregated that the real state of their arrangement is often hardly perceptible. The papillary glands are very variable in size and shape and show different structures, probably according to states of the secretory activity of the cells; hence the cell contents, which stain deeply with hæmatoxylin, may be seen as a compact mass, or as an aggregation of minute granules, or as a more lightly stained mass showing a reticular structure.

No pigment-granules are recognisable in any of dermal tissues.

In connection with the skin, the ventral hooks may be described. They are unusually numerous, that is, eight instead of two. As in *Bonellia miyajimai*,¹ hitherto the

¹ Ikeda, I., "On Three New and Remarkable Species of Echiuroids

only case in which this multiplicity of the ventral hooks has been recorded in the Echiuroids, the hooks are comparatively small (about 0.1 mm. in height), and are crowded together, without showing any regular arrangement, at a spot nearly in the middle of the genital groove (fig. 6). They are almost transparent, light brown in colour, and have a slightly curved sharp apex. Another point of peculiarity is shown by the fact that the hooks, when examined from the inside of the skin, are not borne on the usual muscular cushion provided with radiating muscles, as in the case with all known Echiuroids. They are simply rooted in the thickness of the epidermis, not even a slight bulge of skin being present. It was the discovery of these hooks that made me feel the necessity of a re-examination of the type specimen of *Hamingia ijimai*, which has many points of agreement with the present species. As I remarked in the introductory paragraphs, Mr. Takahashi examined the type specimen for me and found out ten small hooks in the middle of the genital groove. Fig. 7 is a sketch of the hooks of *Hamingia ijimai* drawn by Mr. Takahashi. Here we see the ten hooks directed posteriorly and crowded irregularly. Judging from this figure, which was drawn by means of Abbe's Zeichenapparat, it will be seen that these hooks of *Hamingia ijimai* are straighter and larger than those of the present species. As these hooks in both species are minute and embedded in the narrow streak-like groove, I unfortunately overlooked them when I was examining the type specimen of *Hamingia ijimai*.

Referring to the internal anatomy, the animal shows some noteworthy characteristics.

As some loops of the alimentary canal were, as before stated, severed off, the real state of connection of these torn loops with others still remaining in the natural position, and further, the actual and relative lengths of the three tracts (fore-, mid-, and hind-guts) of the whole canal, could not be made out. As far as the general characters of the canal are (*Bonellia miyajimai*, *Thalassema tanioides*, and *T. elegans*)," 'Journ. Coll. Sci. Imp. Univ., Tokyo, Japan,' vol. xxi, art. 8, 1907.

concerned, the present species shows no striking characteristic points as compared with the known allied forms of the Bonellidæ. This similarity is especially remarkable if the present species is compared with *Hamingia ijimai*, the one interesting point of difference being the reversed or posterior (in this species) instead of anterior position of the junction between the fore and the hind guts. This posterior shifting of the junction of the two parts of the gut causes the extraordinary elongation of the neuro-intestinal vessel.

On both sides of the posterior terminal part of the rectum there are two bushy groups of anal glands. With regard to the manner of branching of the organs, the present species presents a remarkable point of identity to *Hamingia ijimai*, for in both species the main tube or stem is multiplied in number. Fig. 8 represents the organ on the right side cut short of all branches in order to bring forth more clearly the relation which the organ bears to the rectum. There four larger and smaller main stems are seen clustered together at their roots, which arise from the side walls of the rectum almost independently from one another. That stem standing out most dorsally (hindmost in the figure) is the stoutest, and gives off several secondary branches. The organ on the left side is essentially of the same nature as that just described, the only difference being that the dorsal largest stem arises more decidedly apart from the others. Each of these main stems gives rise to numerous branches (from primary to tertiary), to which comparatively large funnels are attached, about three to eight to each terminal branchlet. All of the primary as well as many of the larger secondary branches are attached in their mid-way respectively by a thick fixing muscle arising from the inside of the skin.

The vascular system of the body proper consists, as in many other species of Echiuroids, of the ventral vessel, the dorsal vessel, and the neuro-intestinal vessel. The ventral vessel (fig. 9, *v. v.*) is supported by a conspicuously wide mesentery (*m. s.*), arising from, and running along, the whole length of the nerve-cord (*v. n.*). Reaching the posterior end

of the body, the ventral vessel and the mesentery do not end with the nerve-cord, but run a little further over the ventral surface of the rectum, very near the anus (see fig. 8), so that they recall a feature somewhat resembling the rectal mesentery known in some forms of the genus *Thalassema*.

About 15 mm. behind the external aperture of the oviduct (*o. d.*, fig. 9) the ventral vessel gives off the neuro-intestinal vessel (*n. i. v.*), which is remarkably long, being 0.70 mm. The extraordinary length of the vessel is, as referred to before, correlated to the reversed posterior position of the junction of the fore-gut with the mid-gut, to which the vessel under consideration finds its first attachment. A short way off (about 5 mm.) from this attachment the vessel becomes split into two long branches 20 mm. long, which run parallel to, but entirely apart from, the intestine. They are, however, connected to the collateral intestine (*c. i.*) by means of a series of numerous delicate muscle-fibres, which frequently branch towards the vessels and end mostly with a small nodule-like swelling firmly adhering to the surface of the vessels (see fig. 9). It is greatly to be regretted that these two vessels could not be traced completely owing to the destruction of part of the mid-gut.

It is not less interesting to note that the dorsal vessel does not arise, in the usual way, from the mid-gut, where the neuro-intestinal branches are attached, but it originates from a part of the fore-gut about 50 mm. anterior to the beginning of the collateral intestine (see *d. v.*, fig. 9). Under these conditions, and since the hinder portion of the fore-gut passes close to the pharynx (*ph.*), the dorsal vessel has to run a very short way (about 10 mm.) to reach the pharynx. At the point where it reaches the gut, the dorsal vessel is seen to pass over to two villi-like ridges lying side by side and directed posteriorly. No doubt these structures are a part of the so-called heart, which in the present case is not seen as such. Very probably the heart may be present as a diffuse sinus-like space in the gut-walls,

extending between the roots both of the dorsal and the neuro-intestinal vessels.

The single oviduct (*od.*, fig. 9) is situated on the right side of, and very close to, the ventral nerve-cord (*v. n.*) It is a comparatively small tubular sac, measuring about 16 mm. in length, and consists of four parts; the narrow and highly muscular neck, the swollen glandular part, the thin-walled reservoir, and the stalked funnel. As is the case with *Hamingia ijimai*, the funnel, which is fimbriated in its margin, springs from the very beginning of the reservoir. There were no egg-cells, either in the interior of the reservoir or in the glandular part. The female gonad also was not found either on the ventral vessel or at any other place.

This concludes the account of the anatomy of the female. Lastly, a few words must be devoted to the parasitic males, four of which were picked out of the glandular part of the oviduct of the female. With regard to the three whole males, one was broken to pieces while being removed. They are 3.8-4.2 mm. long and about 0.15 mm. thick (at the broadest part). The anterior end is slightly broader than the posterior. The whole surface is uniformly covered with cilia. There are no ventral hooks or any other sort of spines. Thus it is found that these males possess very nearly the same external characters as those of the males of *Hamingia ijimai*. So also in their internal anatomy both males of the two species seem to be almost identical. Only points of slight difference can be mentioned; these are:

(1) In the present species the body-cavity of the tail-region extends a little further posteriorly than it does in *Hamingia ijimai*.

(2) The alimentary canal is complete in the present species, while in *H. ijimai* it consists of many discontinuous pieces.

(3) The sperm-reservoir in the present species is a little longer than that of *H. ijimai*.

But none of these features seem to be of decisive specific characters. We may naturally expect a close morphological similarity between two such forms as *H. ijimai* and *A.*

shiplei, for here are two causes promoting similarity, namely, the close specific relationship on one hand, and the similar degenerative processes due to parasitism on the other.

The description so far given sufficiently indicates that the present species is a member of the family Bonellidæ and is more closely related to the genus *Hamingia* than to *Bonellia* or *Protobonellia*. It also plainly indicates that in the present species several important generic characteristics of *Hamingia* as diagnosed by Danielsen and Koren¹ are absent. Thus in the female of *Hamingia* the ventral hooks and the skin-papillæ are absent, the anal glands are of the ordinary number, or two, and, in the male,² the ventral hooks are present. The rest of the generic characteristics of *Hamingia*, for instance, the shape of the proboscis, the texture of the skin, the number of the oviducts, and the sexual dimorphism, are not peculiar to the genus, since some or nearly all of them may be recognised in the genera *Bonellia* and *Protobonellia*. Thus compared, it becomes obvious that the present species does not belong to the genus *Hamingia*. The multiplied condition of the anal glands, and the fact that the ventral hooks lack a muscular sheath as well as radial muscles, are certainly two interesting characteristics which accurately distinguish the present species from every known Bonellian Echiuroid except *Hamingia ijimai*. The latter species is that with which I made the erroneous generic identification, chiefly owing to having overlooked the presence of the small hooks in the female form. The hooks which are now found in the two species in the same condition seem to be strikingly different from those known in other Echiuroids in one important point, that is, they are in an extremely abnormal, and, very probably, degenerative state of existence. In *Bonellia miyajimai*, which has hitherto represented the single case known of the acanthous abnormality, the abnor-

¹ Vide note on p. 138.

² The male was not known to Danielsen and Koren, but was discovered and described by Sir Ray Lankester, 'Ann. and Mag. Nat. Hist.,' 1883, xi, pp. 37-43.

mality seems hardly to imply a degeneration, as it causes no essential change in the structure of the whole hook-apparatus, except in the number of hooks. But it seems to me very probable that even this kind of abnormality actually indicates a certain phase antecedent to the total degeneration of the hooks as known in *Bonellia misakiensis*¹ or in the genus *Hamingia*, if we take into consideration the present case in which the abnormality in number is coupled with the entire absence of the muscular apparatus. If considered from the point of view of the ventral hooks only, the two species, which are similarly characterised, may be looked upon as if they were an intermediate form between *Protobonellia* and *Hamingia*, both of which have the ordinary proboscis. But such a view as the above cannot be maintained if we take into consideration the anomalous anal glands possessed by the two species, because such a multiplied condition of the organs could not be regarded as an intermediate characteristic. Besides, we see in the embryology of the group Echiuroids that the anal glands arise at first as two paired in-growths of the ectoderm near the larval anus.

The facts and considerations stated above point to the conclusion that the present species and that which I described formerly as *Hamingia ijimai* are to be classed as a genus distinct from any already existing in the Echiuroids. I propose to call the new genus *Acanthohamingia* and the two allied species respectively as *Ac. ijimai* and *Ac. shiplei*, the new species being named in honour of Mr. A. E. Shipley, F.R.S.

The new genus may be diagnosed as follows :

ACANTHOHAMINGIA.

A sexually dimorphic Echiuroid.

Female.—The shape of the proboscis is much like that of *Thalassema*; the skin is thin and delicate in texture, with

¹ Ikeda, I., "The Gephyrea of Japan," 'Journ. Coll. Sci. Imp. Univ. Tokyo, Japan,' vol. xx, art. 4, 1904.

or without papillæ, which are but poorly developed; the genital opening lies in a narrow longitudinal groove of the skin (the genital groove), in which also lie crowded numerous ventral hooks wanting the muscular sheath and radial muscles; the anal glands are more than two in number and branch off several times before ending in funnels; the oviduct is one and unpaired, with a stalked funnel.

Male.—The body is long and slender, the whole surface being uniformly ciliated. No ventral hook is present; the spermatic duct is long and wide, with a single funnel opening to the body-cavity.

The two species are briefly described as follows:

ACANTHOHAMINGIA SHIPLEI, n. s. p.

A deep-sea *Hamingia*-like Echiuroid. The proboscis is long and narrow, and ends with a rather abruptly pointed tip. The skin is thin and partly semi-transparent, and is covered with small papillæ poorly developed. In the middle part of the genital groove are rooted and crowded the small and numerous hooks with a curved and pointed apex. The neuro-intestinal vessel is disproportionately long; the dorsal and neuro-intestinal vessels arise from the gut at two widely separated places. The anal glands consist of four main stems on one side. The single oviduct and the parasitic males are of the same form and structure as those of *Ac. ijimai*.

ACANTHOHAMINGIA IJIMAI, IKEDA.

Synonym: *Hamingia ijimai*, Ikeda.

A deep-sea Echiuroid having nearly the same external feature as that of the preceding species. The proboscis ends with a rounded margin. The skin is thin, semi-transparent, and devoid of any sort of papillæ. The ventral hooks, which are crowded in the middle of the genital groove, are less curved and larger than in *Ac. shiplei*. The anal glands

consist of three main stems on one side, arising widely apart from each other and from the rectum. The single oviduct is of the same shape and position as that of *Ac. shiplei*. The males have a Nematode-like shape. The whole surface is uniformly ciliated. No ventral hook is present. The spermatic duct is long and wide, ending with a single funnel.

ZOOLOGICAL LABORATORY,
UNIVERSITY OF CAMBRIDGE;
May, 1910.

EXPLANATION OF PLATE 10,

Illustrating Dr. Iwaji Ikeda's "Notes on a Deep-sea Echiuroid, *Acanthohamingia shiplei* (n. g. et n. sp.), with Remarks on the Species *Hamingia ijimai*, Ikeda."

Fig. 1.—Ventral view of the animal; natural size.

Fig. 2.—Ventral view of the proboscis tip; magnified about five times.

Fig. 3.—Surface view of the skin in the middle part of the body, to show the arrangement of the papillæ; magnified about fifteen times.

Fig. 4.—A magnified view of a papilla of a larger size.

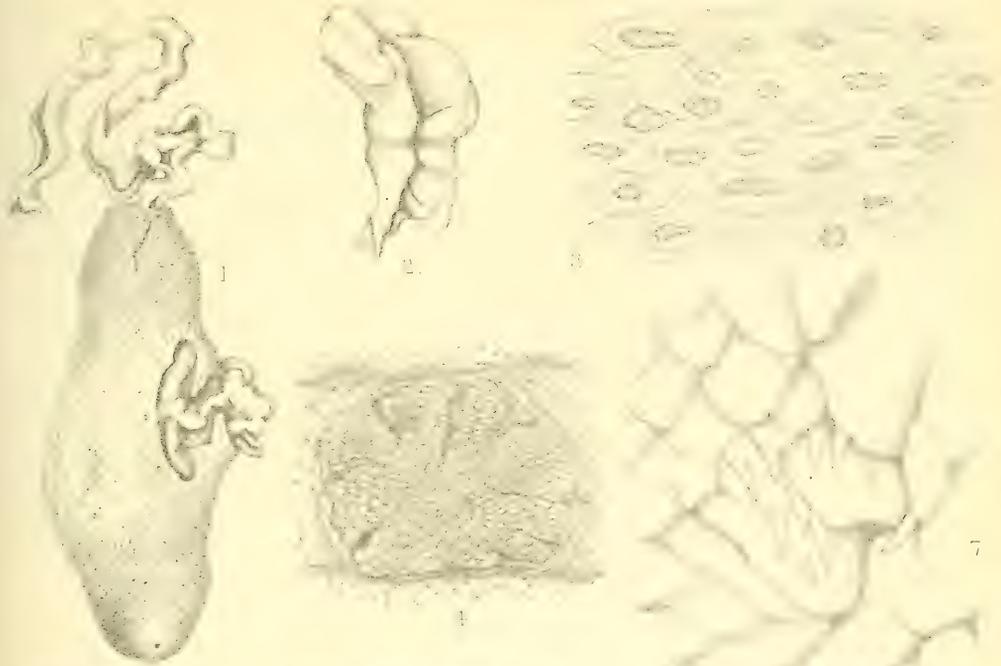
Fig. 5.—Transverse, slightly obliquely cut section of the skin, passing through a papilla: seen with oc. 2 and ob. D (Zeiss); *c.*, corpuscular cell in a lacunar space (*lac.*); *c. m.*, circular muscles; *cu.*, cutis; *ep.*, epidermal epithelium; *fb.*, fibrils in the cutis; *p. gl.*, papillary gland-cells.

Fig. 6.—Ventral hooks in the genital groove; seen with oc. 2 and ob. A (Zeiss).

Fig. 7.—Ventral hooks of *Ac. ijimai*; seen with oc. 1 and ob. AA (Zeiss).

Fig. 8.—Rectum near the anus with the roots of the anal glands (on the right-hand side) and the nerve-cord (*n.*) and ventral vessel (*v. v.*); magnified about ten times.

Fig. 9.—A sketch showing the oviduct (*od.*) and the blood-vessels in situ; very slightly enlarged; *c. i.*, collateral intestine; *d. v.*, dorsal vessel; *f. g.*, fore-gut; *m. g.*, mid-gut; *m. s.*, mesentery supporting the ventral vessel; *n. i. v.*, neuro-intestinal vessel; *ph.*, pharynx; *v. n.*, nerve-cord; *v. v.*, ventral vessel.



**A Study of the Blood of certain Coleoptera:
Dytiscus marginalis and Hydrophilus
piceus.**

By

J. O. Wakelin Barratt, M.D., D.Sc.,

and

George Arnold, M.Sc.,

From the Cancer Research Laboratory (Mrs. Sutton Timmis Memorial),
University of Liverpool.

With Plate II.

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INTRODUCTION.

THE present investigation had its origin in a study of the cell changes occurring in malignant growths, during the course of which attention was directed towards the presence of wandering cells in such growths. It appeared likely that light would be thrown upon the morphology and life-history of the wandering cells of the higher vertebrates by comparison with the free cells of the blood of various invertebrates. To this end the present investigation, which is confined to Coleoptera, was undertaken. The work, however, extended beyond the limits originally assigned, for it became necessary,

partly in order to prepare an isotonic fluid for the blood-cells studied, and partly in order to determine the nature of the medium in which they lived, to examine the fluid part of the blood also.

The literature of the subject is scattered and appears to be very scanty, so that further research in the light of the more recent development of methods of investigation seemed very desirable.

As early as 1864 Landois (1) studied the blood of insects, noting the colour, smell, and reaction, and ascertaining the presence of iron in the serum. He did not, however, give a definite classification of the blood-cells, though he states that division takes place by the nucleus usually splitting into two parts.

The morphology of the formed elements in Molluscs and Arthropods was further studied by Cattaneo (2, 1889) and Wagner (3, 1890).

Cuénot (4, 1891) gave a voluminous but not very illuminating contribution to the literature of the blood of invertebrates. This author observed that the blood of *Hydrophilus piceus* is at first pale yellow, and when exposed to the air becomes altered resembling caramel; neither uranidin, lutein nor fibrin is present; the albuminoid present, which coagulates at 60°-61°, is called hæmopheine. The blood of Blaps, which is also pale yellow, and on oxidation becomes quickly ochreous yellow, contains an albuminoid which is regarded as identical with hæmopheine.

An important observation in respect of the Coleoptera was made by Durham (5, 1892), who ascertained that the blood-cells of *Dytiscus* exhibited phagocytosis, readily ingesting particles of Indian ink.

Reference may here be made to a much more exhaustive examination of the cœlomic fluid of *Lumbricus* by Lim Boon Keng (6, 1896). This author found that the cœlomic fluid had a specific gravity of 1.007 to 1.009, and was of alkaline reaction; it also contained crystals, pigment and microbes, and held cells in suspension, some of which exhibited phagocy-

toxis. The latter were divided into—small non-granular, large hyaline, small granular, large granular and chloragogen cells, and also spindle cells.

Some interesting observations were made by Benham (7, 1901) on the cœlomic fluid of Acanthodrilids, which was found to undergo a sort of coagulation on standing, becoming white, sticky, and slimy. The cellular elements of the cœlomic fluid are divisible according to Benham into four groups; amœbocytes (granular cells), eleocytes (containing fatty globules), lamprocytes (containing granules), and lino-cytes (containing threads).

Hollande (8, 1909) divides the cellular elements of Coleoptera into three groups: lymphocytes, granular leucocytes, and leucocytes with spherules.

The Coleoptera selected for the present investigation have been *Hydrophilus piceus* (Linn.) and *Dytiscus margin. alis* (Linn.).

MODE OF COLLECTING BLOOD.

In order to obtain blood from *Hydrophilus* and *Dytiscus* the following procedure was adopted. The wing cases were lifted up and pinned aside in a paraffin dish. The wings were then divided with scissors, so as to display the dorsal segments of the abdomen. One of the dorsal segments was next opened at the side and a flap of chitin cut off after being previously freed from adherent connective tissue. The blood which was seen lying in the body cavity between the viscera was then removed drop by drop by means of a capillary tube. If this is carefully done it should be possible to withdraw blood without damaging any organ or setting free any cells derived from the body tissues.

GENERAL CHARACTERS OF THE BLOOD OF *DYTISCUS* AND *HYDROPHILUS*.

The average amount of blood obtainable from *Hydrophilus piceus* was .32 c.c. The amount of blood obtained

from five specimens (in April) was found to measure 1·6 c.c. (average amount ·32 c.c. from each); later in the same month three specimens yielded ·43 c.c. (average amount ·14 c.c. from each); on another occasion (in July) ·26 c.c. per specimen was obtained.

From *Dytiscus* the average amount obtainable was ·10 c.c. As affording some idea of the range observable the following data may be given: ·42 c.c. obtained from three specimens in February (average amount ·14 c.c. from each); ·375 c.c. obtained from six specimens also in February (average amount ·063 c.c. from each); 1·3 c.c. obtained from twelve specimens in March (average amount ·108 c.c. from each); 1·65 c.c. obtained from seventeen specimens in April (average amount ·10 c.c. from each).

The blood was found on centrifugalisation to consist partly of fluid and partly of suspended material. The latter was variable in different animals, but was relatively small both in *Hydrophilus* and in *Dytiscus*, amounting in the observations made to about 1 per cent. (by volume) of the blood.

The suspended material consisted partly of cells, partly of free granules. The latter are described in detail below in connection with the blood-plasma; the former are taken in the succeeding section. The cells formed a relatively small amount of the precipitate obtained on centrifugalisation, but owing to the circumstance that the two constituents of the precipitate cannot be separated, no quantitative comparison of the two could be made.

CHARACTERS OF THE BLOOD-PLASMA OF *DYTISCUS MARGINALIS*.

Colour and Spectroscopic Appearance.—The blood-plasma immediately after removal was, in a layer four millimetres thick, of a deep amber colour, subsequently changing at the surface of contact with the air to dark brown, almost black (well seen when the blood was kept in a narrow pipette, the upper layer of liquid becoming deeply coloured, while that below, where access of air was prevented, remained

unchanged). Since the blood darkened on exposure to air, or rather to oxygen, it follows that it contained exceedingly little dissolved oxygen in the straw-yellow condition which it exhibited in the living body. On spectroscopic examination of a layer six millimetres thick the portion of the spectrum lying to the blue end of the green was completely cut off and the green itself in part absorbed, but the red of the spectrum was little altered. When the blood had become darkened the spectrum became dim but no absorption bands were seen. The brownish-black colour which the blood assumed on exposure to air could not be removed by adding ammonium sulphide.

Odour.—The blood immediately after collection had a sweet smell somewhat resembling malt extract, but was also distinctly offensive. A faint odour of free ammonia was recognisable. On adding sodium hydrate and boiling, the issuing vapour readily turned neutral litmus paper blue, thus affording additional evidence of the presence of ammonia or an ammonium salt (it will be seen below that carbon dioxide was present in the blood-plasma).

Specific Gravity.—This ranged, in the specimens examined, from 1.025 to 1.027.

Reaction.—The blood when examined immediately after collection was always found to be alkaline to litmus paper.

Basicity and Acidity.—Observations were made immediately after removal of the blood from beneath thoracic or abdominal tergites, great care being taken to avoid injury to viscera. In every case it was found that the blood, which was strongly alkaline to litmus on removal, still remained alkaline on adding an equal volume of $\frac{N}{30}$ HCl; on adding an equal volume of $\frac{N}{20}$ HCl it became faintly alkaline; on adding an equal volume of $\frac{N}{10}$ HCl it became acid to litmus paper. The basicity of the blood-plasma (which is in part due to ammonium carbonate) is therefore slightly greater than is

represented by a $\frac{N}{20}$ solution of hydrochloric acid. As the blood-plasma is alkaline to litmus its basicity cannot be determined by adding potassium hydrate.

Coagulation.—No spontaneous coagulation of the blood of *Dytiscus marginalis* occurred on standing.

Composition.—The blood-plasma was found to contain 6.6 per cent. to 10.4 per cent. of solids, dried at 110° C. (1 c.c. of blood was taken for estimation of total solids).

On rendering the blood slightly acid with acetic acid and then heating, it became solid. When the blood was diluted with three parts of distilled water or .85 per cent. solution of sodium chloride, made slightly acid with a 1 per cent. solution of acetic acid and boiled, a brown precipitate formed. After centrifugalisation the supernatant liquid was found to remain turbid (this being apparently due to the presence of ammonium salt in the plasma), so that complete separation of the proteid from the non-proteid solids of the plasma could not by this means be effected. By weighing the brown precipitate after drying at 110° C. it was found that the former was not less than 1.3 per cent.; this figure has little value, however, since the supernatant liquid contained proteid forming a gelatinous mass as evaporation proceeded.

On adding blood to a large excess of distilled water turbidity appeared, followed by the formation of a white precipitate, showing the presence of globulin.

The dried solids of the plasma contained about 9 per cent. of ash, which was of a brownish-white earthy aspect. Owing to the small amount available further analysis of the ash was not possible.

Osmotic Pressure.—The freezing-point of the blood-plasma determined by Beckmann's method (1.3 c.c. of blood-plasma were employed) was $- .77^{\circ}$ C., corresponding to an undissociated $\frac{M}{23}$ solution.

Granular Material.—This consisted of granules 1μ to $.2 \mu$ in diameter, exhibiting Brownian movement and in part

precipitated on centrifugalisation. In addition numerous ultra-microscopic particles of much smaller size were recognisable on strong illumination against a dark background. The former granules when a film of blood was prepared by Leishman's method (alcohol fixation, staining with methylene-blue-eosin) stained blue.

Gases Dissolved in Blood.—1·65 c.c. of blood-plasma (obtained from seventeen *Dytisci*) were placed in connection with a Toepler pump and ·14 c.c. of gases extracted. On exposing this to the action of a 10 per cent. solution of caustic potash the volume was reduced by ·11 c.c. On adding a 50 per cent. solution of caustic potash containing 2·3 per cent. of pyrogallol a very slight diminution of volume, too small to determine, occurred, and ·03 c.c. of gas remained behind, representing nitrogen and argon. The percentage amounts of dissolved gases were therefore :

Carbon dioxide	6·7 per cent.
Nitrogen	1·8 „
	—
Total	8·5 „

CHARACTERS OF THE BLOOD-PLASMA OF *HYDROPHILUS PICEUS*.

Colour and Spectroscopic Appearance.—Immediately after collection the blood was, in a layer four millimetres thick, of a straw-yellow colour. Subsequently it became dark brown, the change first appearing at the upper surface, in contact with the air. When kept in hydrogen the blood remained for several hours of a pale yellow colour, whence it follows that the darkening is due to absorption of oxygen. The tension of dissolved oxygen in the blood must, therefore, be very low. On spectroscopic examination of the blood in a layer 18 mm. thick a general darkening of the spectrum was observed, the extent of the spectrum diminishing towards both the red and the blue, but no absorption bands were visible. When darkening of the blood occurred on standing

still further obscuration of the spectrum took place, but no absorption bands appeared.

Odour.—The blood had a faint offensive odour resembling decaying grass. No distinct odour of free ammonia could be detected, but on adding the blood (collected from the living insect a few minutes before use) to a solution of caustic potash (previously ascertained to be free from ammonia) and boiling, the issuing steam readily turned neutral litmus paper blue, showing the presence of an ammonium salt.

Specific Gravity.—This was found to be 1.012 (only one specimen was examined).

Reaction.—The blood examined immediately after collection was alkaline to litmus paper.

Basicity and Acidity.—The blood was tested immediately after collection, great care being taken to avoid injury to viscera during collection. The reaction remained slightly alkaline to litmus when mixed with an equal volume of $\frac{N}{50}$

HCl; when an equal volume of $\frac{N}{40}$ HCl was added the reaction

became neutral to litmus; when an equal volume of $\frac{N}{30}$ HCl

was added the reaction became acid. The basicity of the blood-plasma is therefore represented by a $\frac{N}{40}$ solution of

hydrochloric acid. Since the blood had an alkaline reaction its acidity could not be determined by the addition of caustic potash. It is obvious that the basicity was, as in the case of the blood-plasma of *Dytiscus*, in part due to the presence of ammonium carbonate, already referred to.

Coagulation.—No spontaneous coagulation of the blood occurred on standing.

Composition.—The blood-plasma contained 11.6 per cent. of solid matter (.43 c.c. of plasma was taken for the estimation of total solids).

The plasma contained proteid coagulable on acidifying with acetic acid and boiling, but as was the case with that of

Dytiscus, complete precipitation did not occur, so that a quantitative estimation of the amount of coagulable proteid was not possible.

On diluting the blood with ten times its volume of distilled water a copious white precipitate formed, showing the presence of globulin.

The dried solids of the plasma contained about 3 per cent. of ash of a white, porous, earthy aspect. Owing to the small amount of ash obtainable no determination of its composition could be made.

Osmotic Pressure.—The freezing-point of the blood-plasma, determined by Beckmann's method (1 c.c. of fluid was employed), was -0.647° C., corresponding to an undissociated $\frac{M}{28}$ solution.

Granular Material.—This consisted of small particles, exhibiting Brownian movement, 0.2μ to 2μ , in diameter, the former being the more numerous. In addition ultra-microscopic particles less than 0.2μ in diameter could be seen on strong illumination on a dark background. The granules increased in number on standing; some of the larger granules may have been derived from the disintegration of the blood-cells. The granules, in films fixed by Flemming's solution, stained by basic dyes.

Gases Dissolved in Blood-plasma.—By means of a Toepler pump the dissolved gases contained in 1.6 c.c. of blood-plasma (obtained from five *Hydrophili*) were collected and were found to measure 0.09 c.c. After the absorption of carbon dioxide by caustic potash the volume of gas was reduced to 0.03 c.c. Very little further reduction could be obtained by the action of pyrogallol in strongly alkaline solution. The percentage of dissolved gases was therefore:

Carbon dioxide	3.8 per cent.
Nitrogen	1.9 „
	—
Total	5.7 „

It will be noticed that no dissolved or loosely combined oxygen was obtained from the blood-plasma of *Dytiscus* and *Hydrophilus*. When oxygen was absorbed in vitro the blood-plasma became darkly coloured. It follows, therefore, that as long as the blood-plasma remains straw-yellow coloured the absence of dissolved oxygen may be inferred. No data are, however, available to indicate the means by which darkening of the circulating fluid is avoided in the living insect. The blood appears to serve solely as a nutritive medium. The tissue-cells, it may be observed, are in direct relationship to the finest ramifications of the tracheal vessels (9), which penetrate to all parts of the body of these insects. From the tracheal vessels the tissue-cells appear to derive their supply of oxygen directly, not being dependent on the mediation of the blood-plasma as in mammals and in animals living exclusively in water.

THE CHARACTERS OF THE BLOOD-CELLS.

The blood-cells were studied in films fixed in Flemming's strong solution, without previous drying, and also in dry films. In addition, Flemming's solution was added to the blood, and the formed elements, after centrifugalisation, embedded and cut in paraffin.

The stains chiefly used were Heidenhain's iron-alum hæmatoxylin, Breinl's methylenblue-saffranin-orange G. triple stain and basic fuchsin-methylenblue-orange G. triple stain. Intra vitam methylenblue staining was also employed.

In *Dytiscus marginalis* and *Hydrophilus piceus* the blood consists of flocculent suspended material made up of fine granules, about $1\ \mu$ to $2\ \mu$ in diameter, and of cells. These latter are of two kinds—(1) phagocytes, and (2) small round-cells.¹ The number of cells counted varied from 120 to 500 per cubic millimetre in *Dytiscus*, and from 1030 to 4440 per cubic millimetre in *Hydrophilus*.

The phagocytic cells are usually spindle-shaped when seen

¹ We have not observed blood-platelets in the plasma.

on edge, and round, with two polar prolongations, when viewed from above. They measure in both *Dytiscus* and *Hydrophilus* from $17\ \mu$ by $19\ \mu$ to $15\ \mu$ by $30\ \mu$. In *Dytiscus* the cytoplasm of these cells is coarser and more largely vacuolated than in *Hydrophilus*. The nucleus in *Dytiscus* has a definite membrane and the chromatin is diffusely and irregularly distributed. Faint strands of linin connect together the chromatin masses. Generally only one nucleolus is present (see figs. 1-4). In *Hydrophilus* a well-defined nuclear membrane is also present, but otherwise the nucleus is strikingly different in appearance to that of *Dytiscus*, for instead of being distributed in unequal masses, as in the latter insect, the chromatin occurs in the form of about twenty-five to thirty nearly equal-sized aggregations, and these generally appear to be split in one direction, giving the appearance of twin masses of chromatin.¹ The linin is inconspicuous (see figs. 11-13). When these cells have ingested foreign particles from the plasma they change their shape, gradually drawing in their polar extensions and becoming more or less round (see figs. 6-9 and 12-14). Both in the fresh and well-fixed blood of *Dytiscus* and *Hydrophilus* it can be seen that the majority of the phagocytes which contain no food-particles or recent food-vacuoles in their cytoplasm possess the polar prolongations. At all times the phagocytes may exhibit short and thin pseudopodia extruded from various parts of the cytoplasm, but the polar extensions, although of a more permanent nature, are themselves only pseudopodia, and are distinctive of that phase in the life of the cell in which no ingestion and digestion occur.

The other kind of cell found in the blood is a small cell, with large nucleus and very little cytoplasm (see figs. 10 and 18). These cells, for want of a more convenient term, we designate as small round-cells. As in the case of the phagocytes,

¹ This arrangement of the chromatin in twin groups is apparently characteristic of the somatic cells of *Hydrophilus*. It can be seen, for instance, in the Malpighian tube cells, in the cells of the glands of the mid-gut, and in the spermatogonia.

the cytoplasm of these cells is coarser in *Dytiscus* than in *Hydrophilus*. Small round-cells are present in the blood in much smaller number than are phagocytic cells, varying from one in fifty to one in thirty of the total number in *Dytiscus*, and amounting to one in fifty or less in *Hydrophilus*.

In the phagocytic cells, a series of interesting changes follow the ingestion of solid particles, which may now be described in some detail.

In *Dytiscus* the ingestive activity of these cells is very great. Thus, if a solution of Indian ink be injected into the abdominal cavity, it can be seen that after a few hours most of the phagocytes have particles of the ink in their cytoplasm, as is illustrated by fig. 4 (four and a half hours after injection). As digestion proceeds a clear area appears round each particle, becoming a well-defined vacuole later on. (see figs. 4, 6 and 8). A part of the ingested matter is not digested, being eventually ejected into the plasma. The vacuoles slowly contract until they become indistinguishable in the schaumplasma. The nucleus undergoes the following changes during the digestion of ingested matter. The chromatin becomes more plentiful (cf. figs. 6 and 7 with 1 and 4); the nuclear membrane invaginates here and there and eventually the nucleus becomes multilobulate (see fig. 8), some cells being even polynuclear (see fig. 9).

In *Hydrophilus* the changes which take place in the cytoplasm after the ingestion of solid particles are similar to those of *Dytiscus*. The nucleus undergoes only a slight change, the chromatin becoming more diffuse and the linin strands more apparent (cf. fig. 12 with figs. 13 and 14). Lobulation of the nucleus does not occur. As in *Dytiscus* some of the granules, representing probably indigestible portions of the ingesta, are extruded from the cytoplasm and discharged into the surrounding blood-plasma. The staining reaction of the solid ingesta changes, for in the cytoplasm at first these take the acid stain, later they are stained by a mixture of the acid and basic stains, and ultimately, at the

time of extrusion, the undigested residue becomes increasingly basic in its staining reaction.

On examining with dark background illumination a drop of blood immediately after removal, both forms of cells, phagocytes and small round cells, which are observed to be very granular, are seen to exhibit very numerous fine pseudopodia, about $\cdot 2 \mu$ in diameter and of length occasionally surpassing that of the cell. No amœboid movements are seen, but after a time the granules of the protoplasm exhibit Brownian movement, which ultimately ceases when death has occurred, without the cell, however, becoming vacuolated as do human leucocytes. At the time of death the pseudopodia have become somewhat indistinct, their situation being indicated by granules apparently derived from the protoplasm.

The mode of division of the phagocytes does not always appear to be the same. In *Dytiscus* only amitotic division was seen (fig. 5), and that comparatively rarely. In *Hydrophilus*, however, mitotic divisions were fairly common (see figs. 15 and 16), but no amitotic forms were met with. It is quite possible, however, that both forms of division occur in the two genera examined, but we have nevertheless been unable to observe either mitotic division in *Dytiscus*, or amitotic division in *Hydrophilus*. We have not been able to observe cell-division in the small round-cells.¹

The origin of the free cells in the blood of insects has been attributed to various sources by different writers. Cuénot (10) and Balbiani (10) derive the leucocytes from the pericardial cells. Schäffer (10) derives at least some of these cells from the fat-body cells, but Kowalewsky (10) denies any leucocyte formation to either the pericardial or fat-body cells, asserting that the blood-cells arise from special nests or islands of tissue near the heart.

¹ Hollande (loc. cit.), in dealing with other Coleoptera, *Coccinella*, *Mysia* and *Epilachnia*, describes phagocytes similar to, but considerably smaller than, those above described. These reproduce by mitosis. The other class of cells which he described in those species, viz. the "cellules à sphérules," is entirely absent in *Dytiscus* and *Hydrophilus*.

Our own observations do not permit us to make any confident statement as to the origin of the blood-cells from any of the tissues of the body. It appears to us, however, that the evidence adduced in favour of the above suggested modes of origin is altogether inadequate, for both the pericardial and fat-body cells are totally different in aspect from the blood-cells. Thus the pericardial cells of *Dytiscus* and *Hydrophilus* are considerably larger than the largest blood-cell, and their nuclei are relatively smaller, while their cytoplasm is very abundant. Again, if the blood-cells arose from any of the above sources, transitional forms would be present; these we have failed to observe.

On the other hand, whatever other mode of origin of the blood-cells may exist, it is clear, since division figures occur in *Hydrophilus* and *Dytiscus* (mitotic and amitotic), that the supply of these cells is kept up, in part at any rate, by multiplication in the blood-plasma.

COMPARISON WITH MAMMALIAN BLOOD.

In the above investigation we have applied the term "blood" to designate the circulating fluid of Coleoptera. This fluid presents, however, several important points of difference from the blood of the higher mammalia. It will, therefore, be of advantage briefly to contrast the characters of these two fluids so far as the present limitations of knowledge permit a comparison to be made.

The blood of *Dytiscus* and *Hydrophilus* resembles mammalian blood in so far as it consists of an albuminous fluid containing cells. The fluid part resembles mammalian blood-plasma in containing proteid, coagulable by heat. Whether more than one form of heat-coagulable proteid is present cannot as yet be stated, but it may be observed that a globulin precipitable by dilution with distilled water, and therefore held in solution by the saline constituents of the fluid, is also present as in mammalian blood. The cellular elements of mammalian blood are represented in the blood of *Dytiscus*

and *Hydrophilus* by phagocytes and small round-cells. In the phagocytes of *Dytiscus* fragmentation of the nucleus occurs, which is comparable to that seen in polynuclear leucocytes.

Turning now to points of difference, the low osmotic pressure of the blood of *Dytiscus* and *Hydrophilus*, represented by a $\frac{M}{23}$ to $\frac{M}{28}$ (undissociated) solution, contrasts with that of mammalian blood, represented by a $\frac{M}{7}$ (dissociated) solution. A more striking point of difference is the absence of cells containing hæmoglobin in the blood of the former, which is also free from dissolved hæmoglobin. Moreover, the white cells in *Dytiscus* and *Hydrophilus* are scanty, relatively to those of mammalian blood; no oxyphile granules can be recognised in the phagocytes when fixed and stained by Leishman's method, nor are platelets present. Mitoses are not uncommon in the phagocytic cells of *Hydrophilus*. In both *Dytiscus* and *Hydrophilus* no spontaneous coagulation of the blood occurs on standing. In both insects oxygenation appears to occur by direct transfer to the tissues of oxygen supplied by the tracheal vessels, the blood-plasma not serving as a medium of exchange.

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10. Quoted by Packard, *loc. cit.*, pp. 419–423.

EXPLANATION OF PLATE II,

Illustrating the paper by Dr. J. O. Wakelin Barratt and Mr. George Arnold entitled “A Study of the Blood of certain Coleoptera: *Dytiscus marginalis* and *Hydrophilus piceus*.”

[The drawings are made using a 2 mm. apochromatic oil-immersion objective with 8 and 18 compensating oculars, are drawn without the aid of a projection apparatus, and are represented as stained by methylene-blue and orange G. Magnification 1250 diameters.

Figs. 1–9.—*Dytiscus*. Phagocytic cells are represented in figs. 1–8; small round-cells in fig. 10.

Figs. 1–3.—Phagocytic cells in the active condition with pseudopodia. Figs. 1 and 3 viewed from above; fig. 2 seen on edge. Nucleus entire, chromatin inconspicuous. Cytoplasm contains no food-vacuoles in figs. 1 and 3.

Fig. 4.—Phagocytic cell with recently ingested particles of Indian ink in the cytoplasm. Vacuoles have not yet formed round the particles.

Fig. 5.—Phagocytic cell exhibiting amitotic division, nearly completed.

Figs. 6 and 7.—Phagocytic cells exhibiting active digestion. In fig. 6 the ingested matter is in close contact with, and depressing, the

nucleus; the remains of a food-vacuole are seen below. In fig. 7 the process is more advanced; vacuoles surround two of the ingested particles, which are being digested.

Fig. 8.—Phagocyte after digestion. Vacuoles empty. The nucleus has become lobulate.

Fig. 9.—Phagocyte in the resting condition, showing numerous small pseudopodia. Nucleus fragmented.

Fig. 10.—Small round-cells. The characteristic feature of these cells is the small amount of cytoplasm, which forms a thin layer round the nucleus.

Figs. 11-18.—*Hydrophilus*. Phagocytic cells are represented in figs. 11-17; small round-cells in fig. 18.

Fig. 11.—Phagocytic cell, with pseudopodia, seen on edge. A small particle surrounded by a vacuole lies in one pole.

Figs. 12-14.—Phagocytic cells with ingested particles. After a time these particles become enclosed in vacuoles, and, as digestion proceeds, lose their staining reaction.

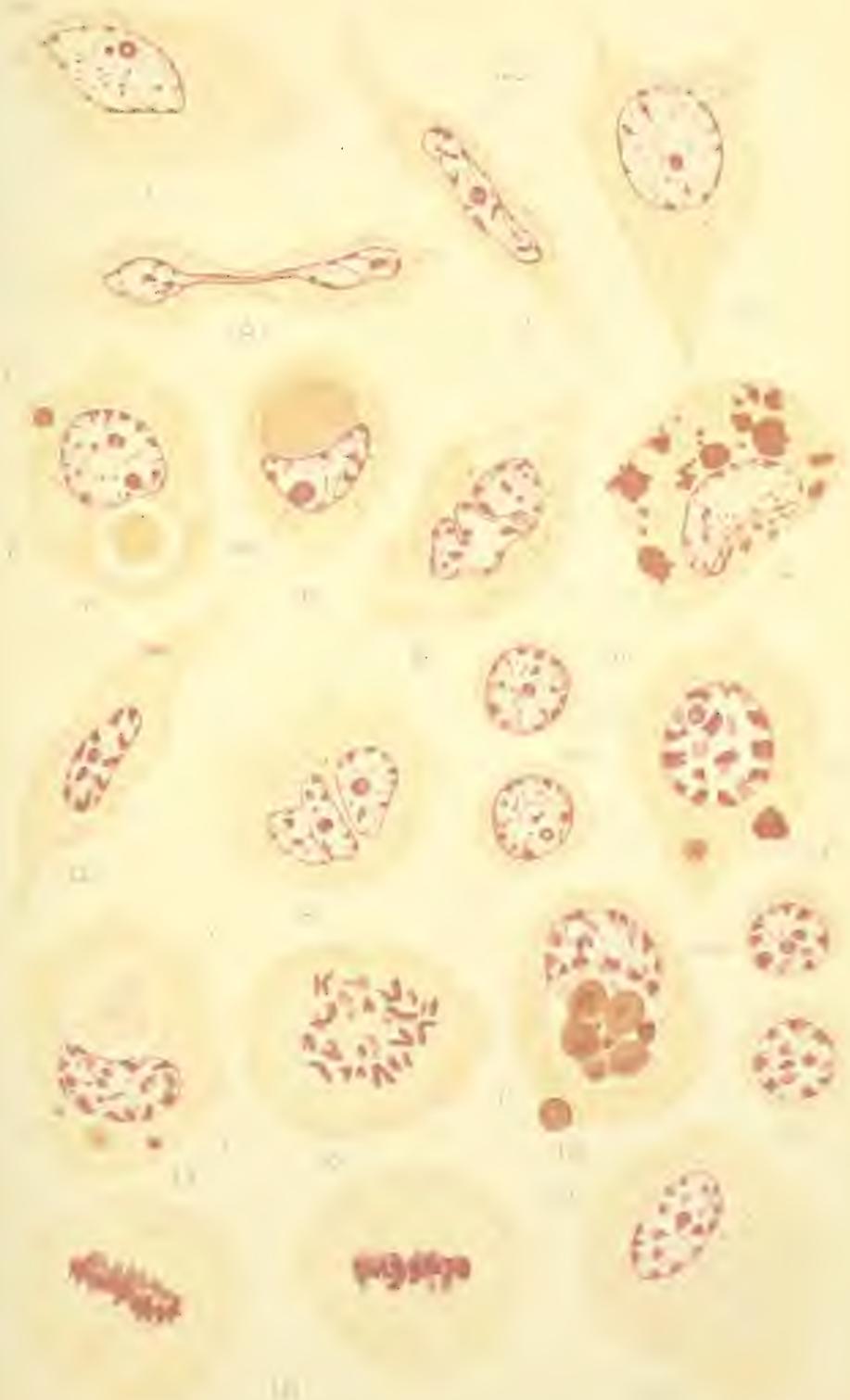
Fig. 15.—Phagocytic cell. Polar view of mitotic division-figure.

Fig. 16.—Phagocytic cell. Equatorial view of mitotic division-figures.

Fig. 17.—Large phagocyte with abundant cytoplasm containing numerous flaky granules.

Fig. 18.—Small round-cells. The characteristic feature of these cells is the scanty cytoplasm surrounding the nucleus.

nu. Nucleolus. *f.* Ingested material.



On the Morphology of the Cranial Muscles in
Some Vertebrates.

By

F. H. Edgeworth, M.D., D.Sc.,
Professor of Medicine, University of Bristol.

With 100 Text-figures.

IN the following paper I have described and compared the development of some of the cranial muscles in *Scylium canicula*, *Squalus acanthias*, *Acipenser sturio*, *Lepidosteus osseus*, *Amia calva*, *Salmo fario*, *Ceratodus Forsteri*, *Triton cristatus*, *Rana temporaria*, *Alytes obstetricans*, *Bufo lentiginosus*, *Pelobates fuscus*, *Chrysemys marginata*, *Lacerta agilis*, chick, rabbit, and pig.

The object has been to determine as far as possible the morphology of the muscles, and so to deduce a morphological classification of the motor nuclei of the cranial nerves, more especially in mammals and man.

A subsidiary object has been to ascertain what evidence is afforded by these muscles in regard to the speculations of zoologists on the phylogenetic relations of the various vertebrate groups.

The paper is a sequel to one previously published on some of the cranial muscles of Sauropsida.

The adult anatomy of the muscles has been described by Vetter, Fürbringer, Pollard, McMurrich, Allis, Jaquet, Drüner, Ecker and Gaupp, Mivart, Krause, and others referred to in the paper. In the majority of cases observation of the development of the muscles merely serves to support deductions as to their homologies already made from consideration

of their adult forms, but in some cases it suggests corrections.

In the case of *Lepidosteus* only the hypobranchial muscles have been described—by Fürbinger, and the names for other muscles have been as far as possible those used by Vetter and Allis in Ganoids and Teleostei. The cranial muscles of *Polypterus* have been described by Pollard, and the hypobranchial muscles by Fürbringer. They did not state the species examined. Those of *Polypterus senegalus* (specimens $7\frac{1}{2}$ to $9\frac{1}{2}$ cm. long) differ in a few particulars from the descriptions given by those authors. The cranial muscles of *Salmo fario* have not hitherto been described, but the researches of Vetter in other Teleostei made identification and nomenclature possible.

Van Wijhe described the early stages of the development of the cranial muscles of *Scyllium*; and Miss Platt those of *Necturus*; observations otherwise have been limited to the development of individual muscles or muscle-groups.

The nomenclature employed by previous writers has, in general, been followed. In cases where different names have been applied to homologous muscles in related animals a choice has been made, and this has necessitated some changes.

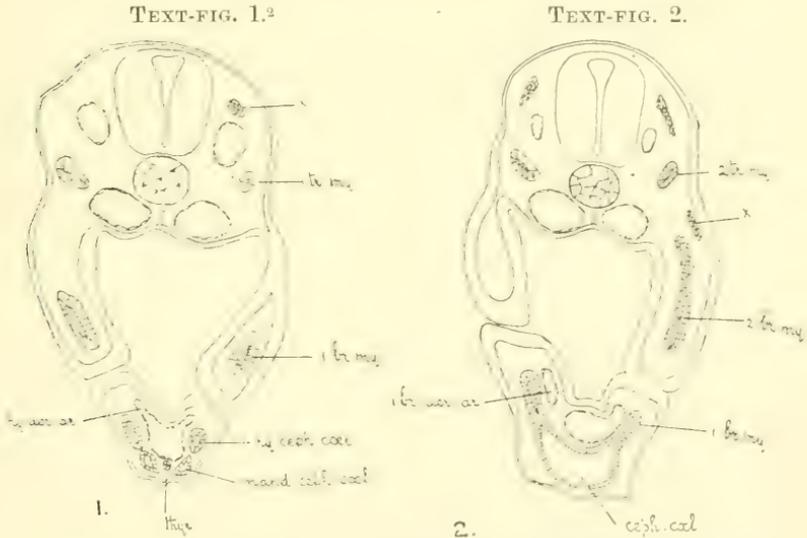
The paper is divided into the following sections: (1) The segmentation of the head; (2) mandibular muscles; (3) hyoid muscles; (4) eye muscles of the rabbit; (5) branchial muscles; (6) œsophageal, laryngeal, and pharyngeal muscles; (7) muscles derived from trunk myotomes passing to the upper ends of the branchial bars; (8) hypobranchial spinal muscles; (9) lingual muscles; (10) some phylogenetic speculations; (11) on Fürbringer's theory of the skull; (12) a suggested morphological classification of the motor centres of the mid- and hind-brain in man.

THE SEGMENTATION OF THE HEAD.

In the body region of *Scyllium* embryos it is found that there is an unsegmented part below enclosing the coelom, and

a segmented part above, the somite, which subsequently separates from the dorsal edge of the cœlom and develops into myotome and sclerotome.

In the head the conditions in the mandibular and hyoid segments are different from those in the branchial segments; in the latter the lateral plates of mesoderm are at first continuous ventrally with the wall of the pericardium or cephalic cœlom¹ (Text-fig. 2); in the mandibular and hyoid segments



Text-figs. 1 and 2.—*Scyllium*, embryo 7 mm., transverse sections.
Text-fig. 1 is the more anterior.

the lateral plates of mesoderm are continuous ventrally with the walls of the mandibular and hyoid sections of the cephalic cœlom. These differ from the branchial sections in that no communication from side to side takes place (Text-fig. 1). In the rabbit, however (Text-fig. 76), the cephalic cœlom is continuous from side to side in the mandibular and hyoid segments, just as it is in the branchial segments.

¹ The latter name is perhaps preferable, as probably the heart originally lay behind the branchial region.

² For reference letters on Text-figures see p. 314.

The lateral plate of the hyoid segment extends upwards and forwards lateral to the alimentary canal between the first and second gill-clefts; its upper end in $6\frac{1}{2}$ and 7 mm. embryos (= stages H and I of Balfour) is continuous above with the "fourth myotome" of van Wijhe.

Van Wijhe says that the "fourth myotome" in stage J is separated from the lateral plate and is very rudimentary, also that it atrophies towards the end of that period; further, that "bis in dieselbe Höhe aber mehr lateral verlangen sich in späteren Stadien die Wände der jetzigen Hyoidhöhle. Mit dieser Verlängerung darf das vierte Myotome nicht verwechselt werden." The Anlage of the rectus externus, regarded by van Wijhe as the third myotome, was stated to be continuous in stage I with the solid cell mass in the hyoid arch, and in stage J to be no longer so.

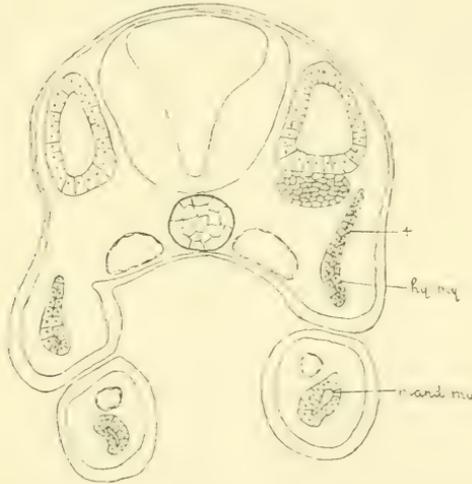
In the embryos of $6\frac{1}{2}$ and 7 mm. in length (= stages H and I) examined, it was found that the Anlage of the rectus externus was continuous behind with the upper end of the epithelium-lined cavity in the hyoid arch, i. e. with the fourth myotome of van Wijhe. In embryos of 9 mm. (Text-fig. 3) and 10 mm. it was found that the epithelial walls of the hyoid cavity had come together, so that the cavity had disappeared, that the now solid cell column had extended upwards, and that the Anlage of the rectus externus was continuous behind with this cell column some little distance from its upper end, at a site corresponding with the original upper end of the hyoid cavity. No trace of a separated "fourth myotome" was seen. It is therefore possible, on the analogy of what takes place in the trunk, to regard the whole of the "lateral plate" and "fourth myotome"—which do not become separate—as together forming the hyoid myotome.

This theory is supported by the difficulty of finding any structure in the body region which is homologous with the lateral plates of the head. Ziegler was of opinion that they were homologous with the "Urwirbelkommunikation" (of Rabl), the "Ursegmentteil" (of Felix). But it is scarcely possible that epithelial structures of the head, which develop

into muscles, can be homologous with epithelial structures in the body, which are the "Mutterboden für den verschiedenen Harnkanälchen" (Felix). Nor do the lateral plates of the head appear to be homologous with the "Seitenplatten" (of Felix) in the body, for this term denotes the epithelium lining the cœlom.

The Anlage of the rectus externus may be regarded as a

TEXT-FIG. 3.



3.

Seyllium, embryo 9 mm., transverse section. The line attached to the mark * shows the point of junction of the Anlage of the external rectus with the hyoid myotome.

very early anterior prolongation of the upper end of the hyoid myotome.

Ziegler was of opinion that the eye-muscles gave no evidence of the primary segmentation of the head, but attributed the Anlage of the rectus externus to the mandibular segment.

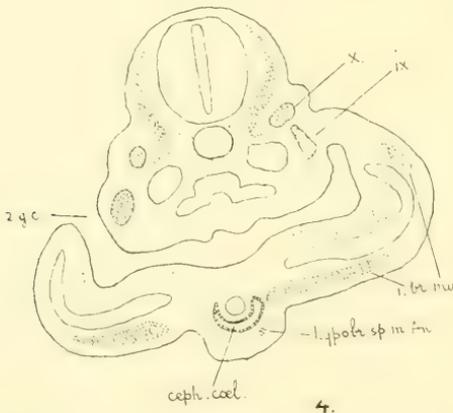
The Anlage of the obliquus superior was regarded by van Wijhe as the second myotome of the head. On the analogy of the rectus externus it may be looked upon as simply a forward projection of the upper end of the mandibular

myotome. Both these Anlagen are anterior projections from the upper ends of their respective myotomes, to add to the musculature of the eye, which is primarily formed from the pre-mandibular segment.

As above stated, the lateral plate of the mandibular segment, which is serially homologous with that in the hyoid segment, may be regarded as the myotome of that segment.

In the case of the five branchial segments, the epithelium-lined cavities—the lateral plates of van Wijhe—are continuous below with the cephalic cœlom. Above, they are

TEXT-FIG. 4.



Scyllium, embryo 14 mm., transverse section.

stated by van Wijhe to be continuous with myotomes—his sixth, seventh, eighth, and ninth. These myotomes were stated to separate from the lateral plates, and to undergo various changes, the fifth atrophying, the sixth becoming very rudimentary, and the seventh, eighth, and ninth forming “Vom Schädel zum Schultergürtel ziehende Muskeln nebst dem vordersten Theile des sterno-hyoideus.” In regard to this asserted continuity between the myotomes and lateral plates, it is noteworthy that Ziegler says it is “schwierig den unten Ursprung des Ursegments mit dem oben sichtbaren Myotom in Verbindung zu bringen, und dies ist bis jezt keinem einzigen Forscher in der richtigen Weise gelungen.”

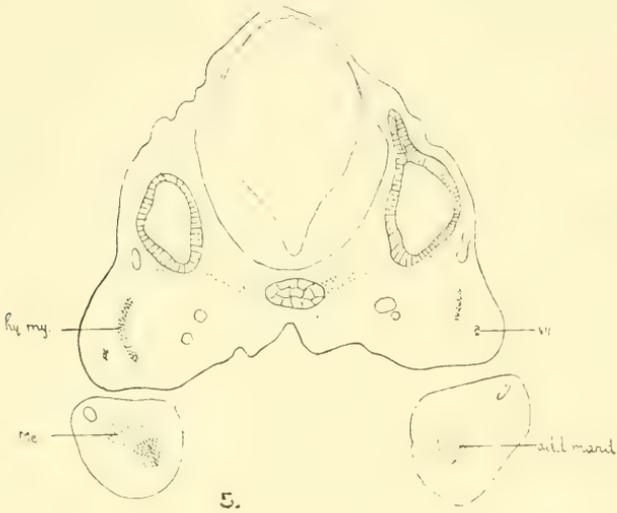
It might be expected that if the lateral plates are the splanchnic elements of myotomes above, their relation to the latter would be constant. But this is not so; for instance, in the figure given by Ziegler of a 6.5 mm. *Torpedo* embryo, two lateral plates (those of the second and third branchial arches) lie beneath three myotomes—his fifth, sixth, and seventh (= sixth, seventh, and eighth of van Wijhe), and the lateral plate of the fourth branchial arch lies below his eighth. In *Ceratodus*, according to Greil, the lateral plates of the first three branchial segments are continuous above with one (the first) myotome, and those of the fourth and fifth branchial segments are continuous above with one myotome (the second). In *Rana esculenta*, with five to six somites (Corning, '99, Taf. ix, figs. 7 and 11), the lateral plates of the first and second branchial segments lie in front of the first myotome. To this may be added that in rabbit embryos 3 mm. long (Text-fig. 78) the first branchial lateral plate lies in front of the first trunk myotome, and the second and third branchial lateral plates (as yet not separated) lie beneath the first trunk myotome.

Secondly, it might be expected that the lateral plate would in all animals be at first continuous with a myotome above, but in *Necturus* embryos (Text-figs. 51–53) and rabbit embryos (Text-fig. 78) it is not possible to see any continuity.

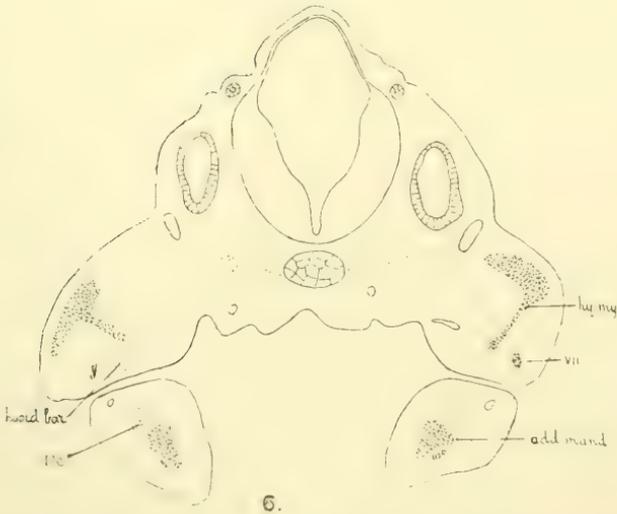
Both these points have been emphasised by Agar in a recent criticism of the theory of van Wijhe. Agar's theory is that the fourth somite of van Wijhe represents the condensed somatic portion of the hinder palingenetic head somites, and he points out that some observers have described more than one somite in this situation, e. g. Brauss, two in *Spinax*, and Miss Platt, three in *Acanthias*. The difficulty in accepting this theory is that, as above stated, this fourth somite is, in *Scyllium*, continuous with the lateral plate of the hyoid segment.

Ziegler throws doubt on the existence of van Wijhe's fifth myotome—the one which lies above the first branchial segment—on the ground that in *Torpedo* its cavity is no more

TEXT-FIG. 5.



TEXT-FIG. 6.



Text-figs. 5 and 6.—*Scyllium*, embryo 16 mm., transverse sections. Text-fig. 5 is more anterior. In the sections the right side is slightly anterior to the left.

than a cleft, and that it quickly becomes rudimentary. He concludes that it is only a small cavity in the mesenchyme and of no theoretic importance. It is difficult to share this opinion, for the corresponding structure in *Scyllium* is lined by epithelial cells and closely resembles the next following myotome (Text-figs. 1 and 2).

Ziegler counts three myotomes in *Torpedo* (his fifth, sixth, and seventh) corresponding to three lateral plates, regards the vagus as a "zusammengesetzte Nervencomplex" corresponding to three segments, and is of opinion that it is in correspondence with this that the next following myotome (his eighth = van Wijhe's ninth) is the foremost to have any nerve-roots, an anterior one only. The difficulties in accepting such a view are: first, as stated above, the want of antero-lateral correspondence between the myotomes and lateral plates; and secondly, in *Scyllium*, and probably also in *Torpedo* at a later stage, a fifth branchial lateral plate is formed, *i. e.* there would be an overlapping of the territories of the vagus and spinal roots.

Greil's views as to the nature of the mesoderm of the head of *Ceratodus* are very different from the foregoing. He holds that the musculature of the branchial region is derived from downgrowths of the first two myotomes, that in the first three branchial arches being derived from the first myotome, that in the fourth and fifth arches from the second myotome. The cells forming these downgrowths can be distinguished from the immediately subjacent lateral plates by the shape of their nuclei and by the later absorption of their yolk-granules. The downgrowths increase in vertical depth from behind forwards. The lateral plates of the branchial arches degenerate into connective tissue. The dorsal portions of the first three arches, *i. e.*, those which are formed from the first myotome, develop dorsally into the levatores arcum branchialium and ventrally into the second and third interbranchiales and the ceratohyoideus; that of the fourth arch into the fourth levator and fourth interbranchialis; that of the fifth arch anteriorly into the dorso-laryngeus and the fifth inter-

branchialis, and posteriorly into the fifth levator and levator scapulæ. The musculature of the hyoid and mandibular arches cannot be regarded as derivations either of a somite or of a lateral plate; it never takes on lateral-plate characteristics.

A difficulty in accepting this theory is that it does not seem to be of general applicability in Vertebrates. Thus, as stated above, in the rabbit the lateral plate of the first branchial arch is in front of the first trunk myotome, and those of the second and third arches lie below the first trunk myotome; and there is a gap between the dorsal edges of the lateral plates of the second and third arches and the ventral edge of the first myotome. A difference in the shape of the cell-nuclei and in the rate of absorption of yolk-granules does not appear to be of sufficient importance to justify a separation into upper and lower portions of a structure which in other animals has been called lateral plate. As far as I have been able to observe, the ventral end of the lateral plate, taken in its usual sense, does not degenerate into connective tissue, but becomes converted into muscles.

These difficulties lead to the following theory of the segmentation of the head. It is probable that it originally consisted of five segments only—the premandibular, mandibular, hyoid, first branchial, second branchial—each having a myotome, which, in the case of the four latter, contains a slit-like, epithelium-lined cavity continuous with the cephalic cœlom below. To each myotome passed a nerve—the IIIrd, Vth, VIIth, IXth, and Xth.¹ The gill-clefts are intersegmental. New segments were added, one by one, behind the second branchial, the head extended back into the body-region, and

¹ According to Neal ('98) only one encephalomere corresponds to the vagus—a fact which, if the encephalomes have segmental, or rather inter-segmental, worth, agrees with the theory that the vagus is not a "zusammengesetzte Nervencomplex," but primarily, as regards its motor branches, of one segment only—the second branchial—and that additional branches were developed as the number of gill-clefts and branchial segments was added to.

the added myotomes necessarily lie beneath the anterior body myotomes, with their upper ends at varying distances from the ventral surface of the latter, with which they may or may not agree in antero-posterior extent. In *Rana esculenta*, with five to six somites, the most anterior trunk myotome lies above the third branchial, i. e. the first added myotome; a little later there is a relative shifting forward of the trunk myotomes, so that the first trunk myotome comes to lie above the first branchial myotome (see Corning's figures, Taf. ix, figs. 7, 11, and 26). In *Scyllium* this overlapping is, secondarily, antedated in development, so that the first branchial myotome never lies in front of the first trunk myotome. In the rabbit it does so.

The backward growth of the head into the body by this process of metameric increase leads to the non-development of the cœlomic portion of the anterior trunk-somites. In *Scyllium*, for instance, the first four trunk-somites have no cœlomic portions.

In the head, as in the body, each myotome is at first an epithelium-lined cavity, which is continuous below with the cœlom. The differences between the myotomes of the body and those of the head are: (1) Whereas in the body the myotomes extend dorsally to the mid-dorsal line, and, subsequently, ventrally outside the somatopleure wall of the cœlom to the mid-ventral line, neither of these secondary phenomena takes place in the head; (2) whereas the cœlom is large in the body and contains the alimentary canal and other viscera, it is small in the head and lies entirely ventral to the alimentary canal; (3) in correlation with this the myotomes of the body lie, at first, dorso-lateral to the alimentary canal, those of the head lie dorso-lateral and lateral to it; (4) the sclerotome elements of the body-myotomes are formed by ventro-medial outgrowths, those of the head from scattered cells given off from the premandibular, mandibular, and hyoid myotomes. These differences are intimately associated with the development of gill-clefts and a cranium in the head, of viscera and a vertebral column in the body.

If, following Fürbringer, it be supposed that primitively the myotomes lay exclusively lateral to the chorda dorsalis, it would follow that they have taken different paths of development in the head and body, resulting in the conditions above stated.

According to the generally accepted theory, certain Selachii, e. g. *Heptanchus*, are the most primitive of gnathostome Vertebrates, in that they have the greatest number of branchial segments; and the lessened number of branchial segments in Teleostomi, Amphibia, and Dipnoi is due to a disappearance of the hinder ones. If, however, it be supposed that the original number of branchial segments was two, i. e. first and second, and that these were added to by a process of metameric increase, the interesting question arises as to the least number present in these Vertebrate groups, for this may be supposed to have been possessed by some primitive form. The Amphibia have four branchial segments, Dipnoi and most Teleostomi five (though *Polypterus* has only four), and most Elasmobranchs five, though they may have as many as seven. It may therefore be supposed that the original number present in Amphibia was added to in the other groups. This harmonises with the conclusion, stated later, that the condition of the muscles of the head in Amphibia is more primitive than in Dipnoi, Teleostomi, and Elasmobranchs.

The lessened number present in Sauropsida and Mammalia may be supposed to have resulted from reduction of the hinder of these four branchial segments. In the later stages of Reptiles, previously investigated, only two branchial myotomes were seen, but the early stages of *Chrysemys marginata* show four. In *Gallus* only two are present, even in the early stages. In *Lepus* only the first three are developed.

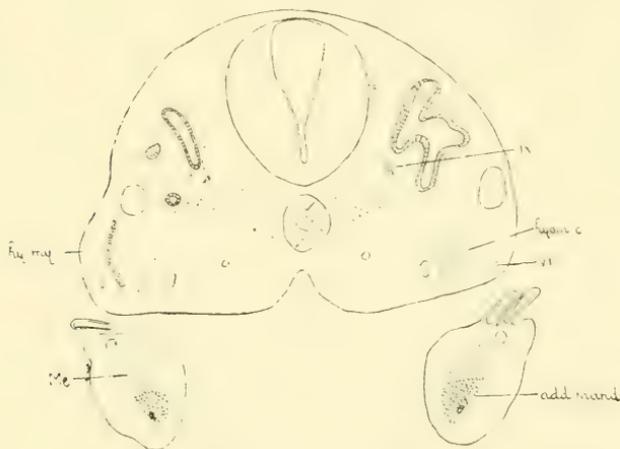
The cephalic cœlom disappears in the mandibular and hyoid segments early in development, and its walls develop into the intermandibularis and interhyoideus, which are at first continuous with the mandibular and hyoid myotomes. The lower ends of the branchial myotomes separate from the wall of the branchial portion of the cephalic cœlom, and they

develop into the branchial muscles. No muscles are directly formed from the walls of the branchial portion of the cephalic cœlom, which subsequently retreats from the head.

MANDIBULAR MUSCLES.

Scyllium.—On the formation of the palato-quadrate, in 16 mm. embryos, the mandibular myotome lies outside of and across the palatine process, and then separates into an upper

TEXT-FIG. 7.



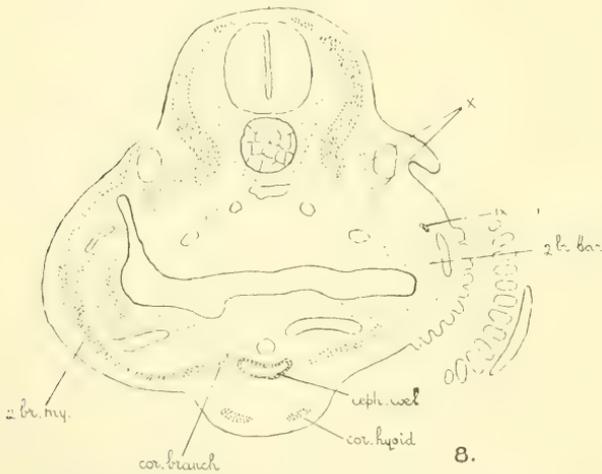
Scyllium, embryo 17 mm., transverse section. The right side of the section is slightly anterior to the left.

levator maxillæ superioris and lower adductor mandibulæ, the process beginning in 20 mm. embryos (Text-fig. 11). The separation of these two muscles by the palato-quadrate is complete, and this is also the case in *Acanthias*, where, according to Marion, the hinder portion of the levator maxillæ superioris—forming a separate first dorsal constrictor—is inserted into the lower jaw. This must consequently be the result of secondary downgrowth. The upper edge of the adductor mandibulæ gains, anteriorly, an additional origin from the suborbital cartilage in 30 mm. embryos (Text-fig. 17), and this anterior portion of the adductor separates, in 40 mm.

embryos, forming the levator labii superioris (or add. β) of Vetter. In 45 mm. embryos the add. γ of Vetter is beginning to be delaminated from the outer face of the adductor, and the hindmost fibres of the adductor have grown down into the intermandibularis, forming a band similar to that described in *Acanthias* by Vetter.

The intermandibularis (Cs_2 of Vetter, C_2mv of Ruge) is formed from the ventral part of the mandibular cavity,

TEXT-FIG. 8.



Scyllium, embryo 17 mm., transverse section.

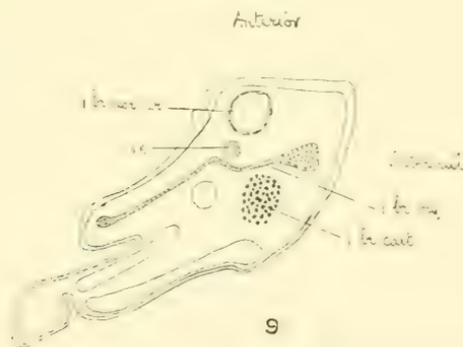
which, as mentioned above, does not meet its fellow in the mid-ventral line, but passes backwards ventro-median to the ventral end of the hyoid cavity to open into the front end of the cephalic cœlom. It results from this that there is no developmental stage in which the intermandibularis lies altogether in front of the interhyoideus. It gradually extends backwards, underlying the interhyoideus, so that in 23 mm. embryos its hinder edge lies posterior to the ventral ends of the ceratohyals (Text-fig. 12).

The nictating muscles of *Scyllium* (Ridewood) consist of a levator palpebræ nictitantis, retractor palpebræ superioris,

and constrictor spiraculi—all innervated by the Vth. Ride-wood supposed that the first-named was differentiated from the same tract as the levator maxillæ superioris and that the second belonged to the same category, whilst the constrictor spiraculi appeared to belong to a purely dermal system of muscles.

According to Harman, the musculature of the eyelids of *Mustelus* arises “from two original sources—one a superficial dermal layer, the other a portion of a deeper dermal muscle layer,” the former originating from “a mass which appears

TEXT-FIG. 9.

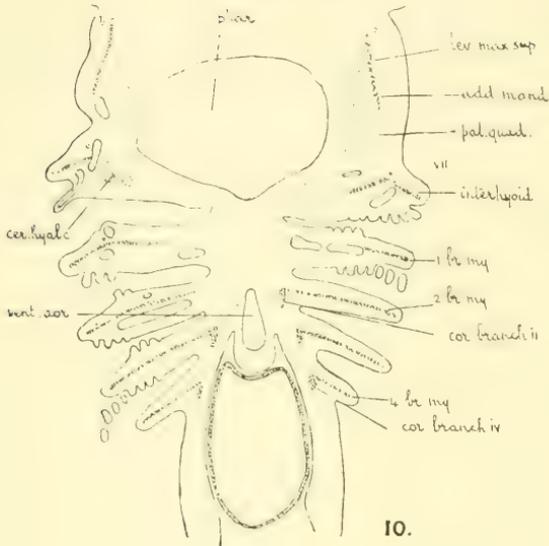


Scyllium, embryo 20 mm., longitudinal horizontal section through first branchial segment on left side.

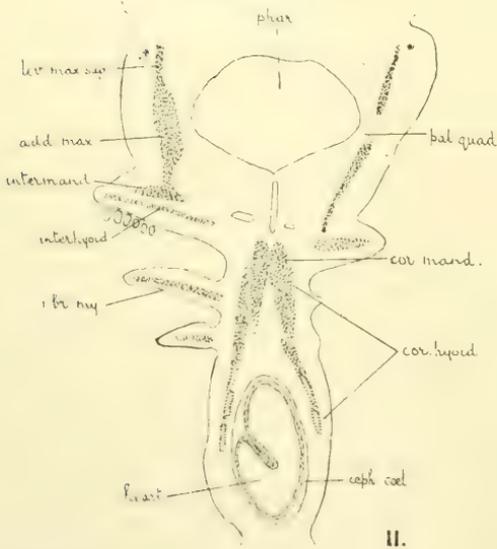
first in the region of the branchial bar which is the second after the spiracle,” the latter from a mass which separates into “a maxillary mass and a spiracular mass.” Harman also stated that “the progressive growth of the spiracle muscles in the service of the face can be traced to their full development in the facial muscles of man.”

In *Scyllium* the Anlage of the nictating muscles can be seen in 30 mm. embryos (Text-figs. 16, 17) as a mass of cells being proliferated from the outer surface of the upper end of the levator maxillæ superioris. In 40 mm. embryos the mass has become partially divided into a deeper and a more superficial layer—the former is the Anlage of the levator

TEXT-FIG. 10.



TEXT-FIG. 11.



Text-figs. 10 and 11.—Scyllium, embryo 20 mm. Text-fig. 10 is the more dorsal.

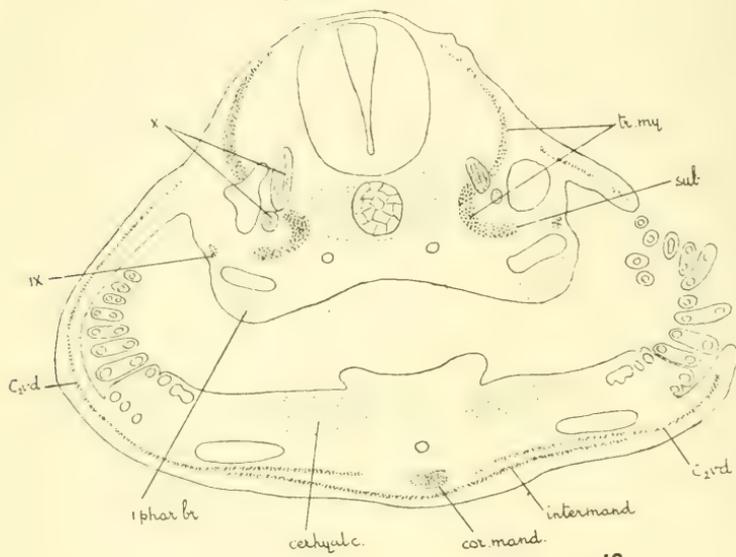
palpebræ nictitantis, the latter that of the retractor palpebræ superioris. In 45 mm. embryos, the latter sends down behind the spiracle an offshoot which develops into the constrictor spiraculi. All these muscles in *Scyllium* are thus developed from an Anlage budded off from the levator maxillæ superioris. It may be added that the facial muscles of mammals are hyoid in origin, formed from the upgrowing interhyoideus (pp. 221 and 222), and consequently are not homologous with the eyelid muscles of *Scyllium*.

The myotome of the mandibular segment in Teleostoman embryos lies at first across and outside the palatine process of the quadrate (Text-fig. 23) and then divides into parts above and below this. The division takes place in *Acipenser* in $7\frac{1}{2}$ mm., in *Lepidosteus* in $8\frac{1}{2}$ mm., in *Amia* in $6\frac{1}{2}$ mm., and in *Salmo* in 5 mm. embryos. The part above the palatine process is the levator maxillæ superioris, the part below the adductor mandibulæ. From the upper end of the levator maxillæ superioris of *Lepidosteus*, *Amia* (Text-fig. 28), and *Salmo*, is given off the dilatator operculi, which extends backwards below the first gill-cleft into the opercular fold, and the remainder forms the levator arcus palatini, which is inserted into the palato-quadrate (Text-fig. 28). In *Acipenser* the levator maxillæ superioris does not divide into levator arcus palatini and dilatator operculi; it grows backwards, without having had any temporary insertion into the palato-quadrate, and becomes inserted into the hyomandibula, forming the protractor hyomandibularis (Text-figs. 18, 19, 20).

The dilatator operculi is partially inserted into the hyomandibula in *Lepidosteus*, and wholly in *Polypterus*, ? species, described by Pollard,¹ but in *Polypterus senegalus* it passes backwards in the outer wall of the spiracle and is inserted into skin only (Text-figs. 35, 36). The adductor mandibulæ, at first passing from the palato-quadrate to Meckel's cartilage,

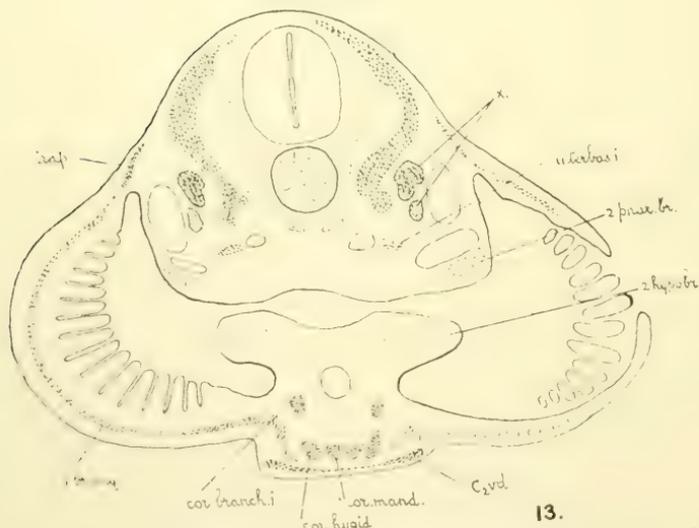
¹ Protractor hyomandibularis of Pollard, but not homologous with the protractor hyomandibularis of *Acipenser*, as he describes a levator maxillæ superioris (i. e. levator arcus palatini), and this is also present in *Polypterus senegalus*.

TEXT-FIG. 12.



12.

TEXT-FIG. 13



13.

Text-figs. 12 and 13.—Scyllium, embryo 23 mm. Text-fig. 12 is the more anterior.

undergoes various changes in the specimens examined. In *Acipenser* embryos up to the length of 11 mm. it remains undivided, but in the adult (Vetter) it has additionally spread up to the skull. In *Salmo* it additionally spreads backwards to the hyomandibula. In *Lepidosteus*, *Amia* (Text-figs. 28, 34), and probably (vide infra) *Polypterus*, the adductor mandibulæ divides into internal and external portions. In *Lepidosteus* (Text-figs. 26, 27) the internal portion extends upwards outside the palato-quadrate to gain an additional origin from the cranial wall; whilst the external portion, keeping its original origin, arises from the external surface and upper end of the quadrate outside the insertion of the levator arcus palatini. In *Amia*¹ the internal portion (in 11 mm. embryos) sends forwards a projection from its upper end which forms the muscle connected with the olfactory chamber (LAP₅ of McMurrich, LMS₄ of Allis); in 14 mm. embryos the remainder of the internal portion extends upwards above the level of the palato-quadrate and divides into three parts (LAP_{2, 3, 4} of McMurrich, LMS_{2, 1, 3} of Allis). The external portion of the adductor additionally extends backwards to the hyomandibula in 9 mm. embryos, and divides into the parts described, as parts of the adductor, by Allis.

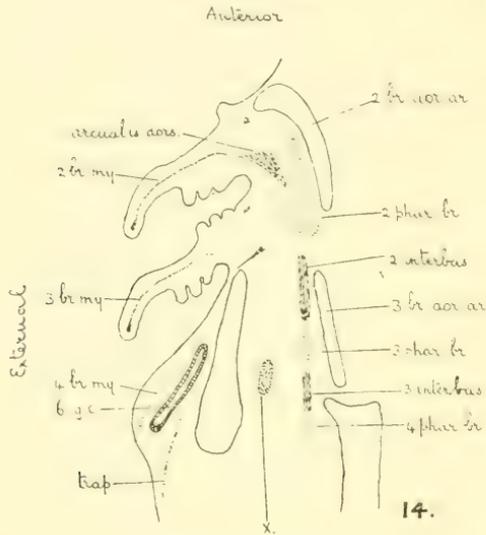
In *Polypterus*, Pollard described the adductor as consisting of three parts, the pterygoid, temporal, and masseter²; of

¹The adult condition of the mandibular muscles of *Amia* has been described by McMurrich and by Allis. McMurrich stated that they consist of a levator arcus palatini and an adductor mandibulæ, and that the former is divided into five parts—from behind forwards LAP_{1, 2, 3, 4, 5}; of these LAP₁ is inserted into the metapterygoid with some of its hindmost fibres into the operculum, LAP_{2, 3, 4} join the adductor, and LAP₅ is in connection with the olfactory chamber. Allis separated LAP₁ into a dilatator operculi and a levator arcus palatini, whilst LAP_{2, 3, 4, 5} he called the second, first, third and fourth divisions of the levator maxillæ superioris. He suggested that LAP₄ and ₅ (LMS₃ and ₄) are derived from add. β of Selachians, and that (his) levator arcus palatini and dilatator operculi are the homologue of add. γ of Selachians. From the embryological findings mentioned above it would appear that a new nomenclature for LAP_{2, 3, 4, 5} (= LMS_{2, 1, 3, 4}) is needed—in terms of an internal adductor.

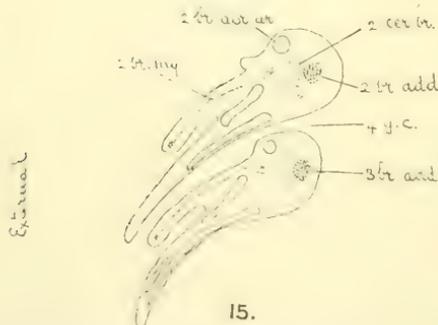
² Pollard thought that the pterygoid and temporal were the homologue

these the pterygoid and temporal are together the homologue of the internal adductor, and the masseter the homologue of the external adductor, of *Lepidosteus* and *Amia*.

TEXT-FIG. 14.



TEXT-FIG. 15.



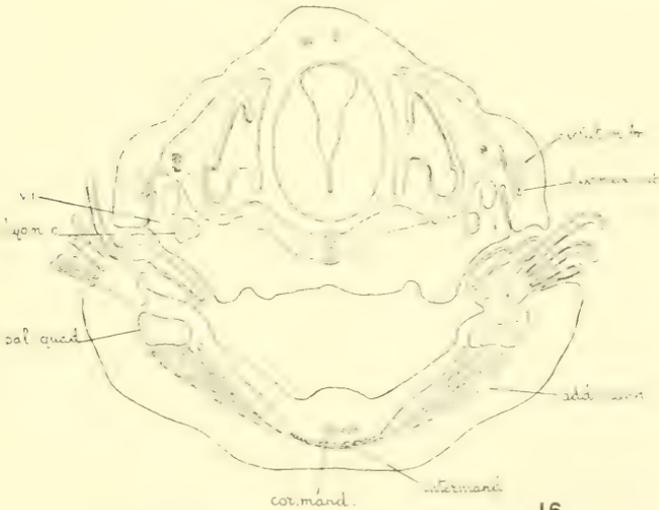
Text-figs. 14 and 15.—*Scyllium*, embryo 28 mm., longitudinal horizontal sections. Text-fig. 14 is the more dorsal, through second, third, and fourth branchial segments on left side.

of Add. β of Selachians, but this is not possible. In Text-figs. 35 and 36 I have denoted his "masseter" by the term "external adductor," and his pterygoid and temporal might be called parts of the internal adductor.

The intermandibularis of Teleostoman embryos forms, at first, with its fellow, a transverse muscle attached laterally to Meckel's cartilage (Text-figs. 23, 24, 28).

In *Salmo* it does not extend backwards, but does so in *Acipenser*, *Lepidosteus*, *Amia* and *Polypterus*, and partially underlies the fore part of the interhyoideus. In *Salmo*, *Polypterus*,¹ and *Lepidosteus*, it remains single, in *Acipenser* and *Amia* it divides into anterior and posterior parts. In

TEXT-FIG. 16.



16.

Seyllium, embryo 30 mm., transverse section.

Acipenser the intermandibularis anterior (Cs_6 of Vetter) is attached laterally to Meckel's cartilage, and the intermandibularis posterior (Cs_1 and Cs_2 of Vetter) spreads upwards, laterally, towards the skull.² In *Amia* both intermandibularis anterior³ and posterior⁴ are attached laterally to

¹ Intermaxillaris anterior of Pollard.

² All these parts are innervated by the Vth (Allis).

³ Intermandibularis of Allis.

⁴ Superficial or inferior portion of geniohyoid of Allis; the muscle has, however, no genetic relation to the superior portion of the geniohyoid (called in this paper "hyomaxillaris") which is developed in the hyoid segment.

Meckel's cartilage (the process of separation into anterior and posterior portion beginning in $9\frac{1}{2}$ mm. embryos and being completed in 14 mm. embryos).

In *Ceratodus* the myotome of the mandibular segment spreads upwards lateral to the Gasserian ganglion (Text-fig. 39), and separates from the lateral half of the intermandibularis between stages 40 and 42 (of Semon). It divides into outer and inner portions—pterygoid¹ and temporal²—the former of which, in stage 48 (Text-fig. 46), arises from the trabecular wall, and the latter from the anterior and outer surface of the quadrate. The intermandibularis³ joins its fellow in a median raphé and becomes attached laterally to Meckel's cartilage; its posterior edge extending backwards underlies the fore part of the interhyoideus (Text-figs. 41, 45).

In *Necturus* (Miss Platt) the mesothelium of the mandibular arch (here interpreted as "myotome") divides into an internal part, the temporal (here called, following Drüner, the "pterygoid"), and an external part, the masseter. In *Triton* the myotome of the mandibular segment also divides into an internal and an external part; the upper end of the internal, pterygoid, part extends up to the side of the skull; the external part, at first arising from the suspensorium only, divides into an outer portion, the masseter, which keeps this origin, and an inner portion, the temporal, which extends up to the auditory capsule.

The intermandibularis of *Necturus*⁴ remains single, its posterior edge underlies the anterior interhyoideus (Text-fig. 55); in *Triton* the intermandibularis (in larvæ between the lengths of $8\frac{1}{2}$ and 10 mm.) divides into anterior and posterior parts,⁵ the latter of which partially underlies the interhyoideus.

¹ Pterygoid of Jaquet.

² Adductor mandibulæ seu digastricus of Jaquet.

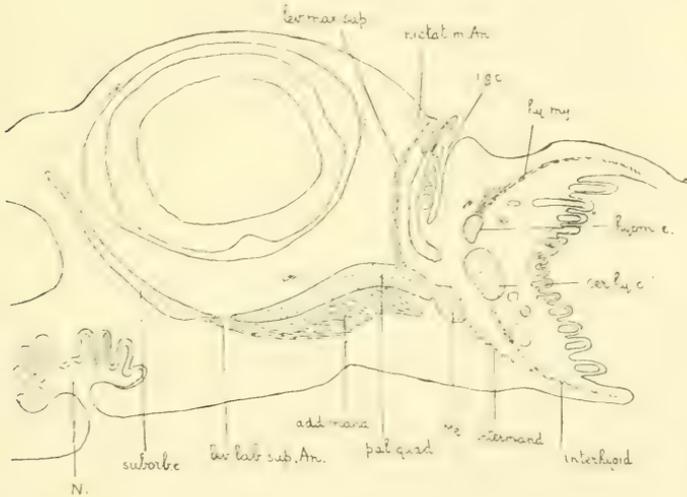
³ C₂mv of Ruge; mylohyoideus pars anterior of Jaquet.

⁴ Mylohyoideus anterior of Mivart and Miss Platt.

⁵ Intermaxillaris anterior and posterior of Wiedersheim; intermandibularis anterior and posterior of Drüner.

The myotome of the mandibular segment of *Rana* separates from the lateral half of the intermandibularis in 5 mm. embryos; it extends backwards in 7 mm. embryos, dividing into internal and external portions (Text-fig. 58). The myotome thus comes to lie in a nearly horizontal position internal to the muscles developed in the hyoid segment. The internal portion develops into the pterygoid muscle, the external into the temporal, sub-temporal, extra-temporal, and masseter

TEXT-FIG. 17.

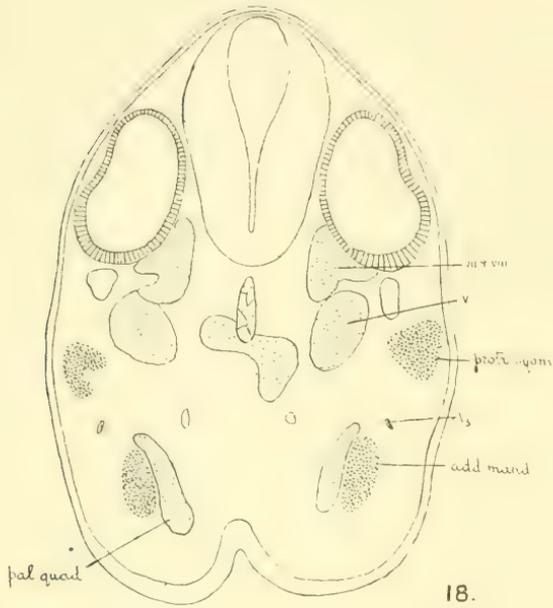


17.

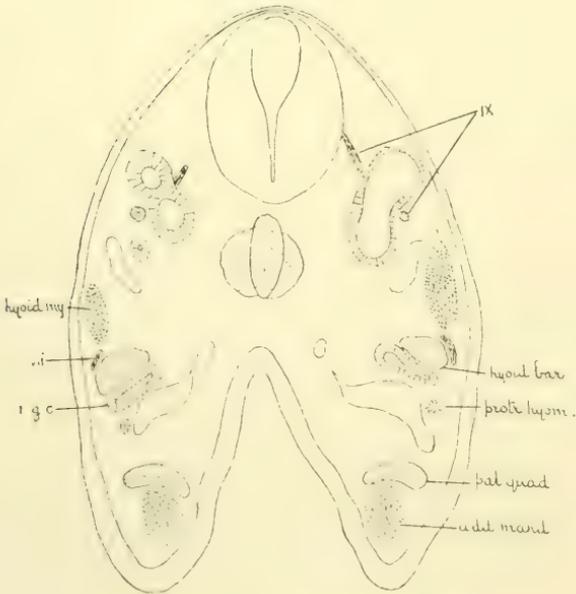
Scyllium, embryo 30 mm., longitudinal vertical section.

(Text-figs. 59, 60). The masseter and extra-temporal arise from the internal surface of the processus muscularis of the palatoquadratus bar. The anterior end of the pterygoid shifts outwards beneath the anterior ends of the other muscles and is inserted into the outer end of Meckel's cartilage. The temporal is inserted into the inner end of Meckel's cartilage; the masseter is inserted into Meckel's cartilage a little distance from its outer end; the subtemporal is inserted, by two tendons, into Meckel's cartilage and the superior labial cartilage; the extra-temporal divides into two portions, one of

TEXT-FIG. 18.



TEXT-FIG. 19.



Text-figs. 18 and 19.—*Acipenser*, embryo 8 mm. Text-fig. 18 is the more anterior.

which joins the temporal (Text-fig. 60) and the other the sub-temporal.

The muscles of *Alytes*, *Bufo lentiginosus*, and *Pelobates*¹ are similar to those of *Rana* (Text-fig. 63), except that the extra-temporal is inserted only into the superior labial cartilage.

The Anlage of the levator bulbi is given off from the upper surface of the hinder part of the temporal in 9 mm. larvæ; its outer end becomes inserted into the skin and upper edge of the palato-quadrate bar; it remains relatively undeveloped until late in metamorphosis. On the development of the lower eyelid a slip is separated from the levator bulbi, forming the depressor palpebræ inferioris.

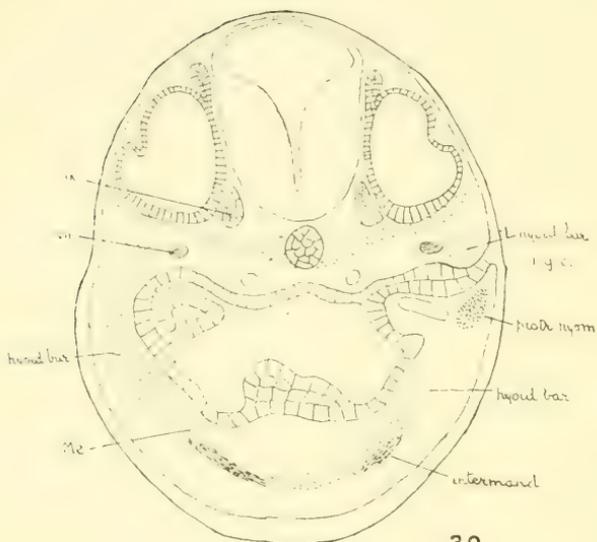
At metamorphosis, on the atrophy of the superior labial cartilage the sub-temporal and extra-temporal fuse with the temporal, and the muscles become more vertical in position on the rotation of the palato-quadrate bar.

The Anlage of the intermandibularis of *Rana* divides in 7 mm. embryos into three parts—the submentalis, the mandibulo-labialis, and the submaxillaris. The submentalis develops later than the other two muscles; in 12 mm. embryos it forms a mass of small round cells lying beneath and extending backwards from the inferior labial cartilages, and at the beginning of metamorphosis forms a layer of transversely directed muscle-fibres connecting together the inferior surfaces of the inferior labial cartilages (Text-fig. 60). The mandibulo-labialis, arising from the inner aspect of the transversely directed Meckel's cartilage, passes down external to the genio-hyoid and is partially inserted into skin, partially interlaces with the muscle of the opposite side (Text-fig. 60).

The submaxillaris arises from the under surface of Meckel's cartilage. The conditions in larvæ of *Bufo lentiginosus*

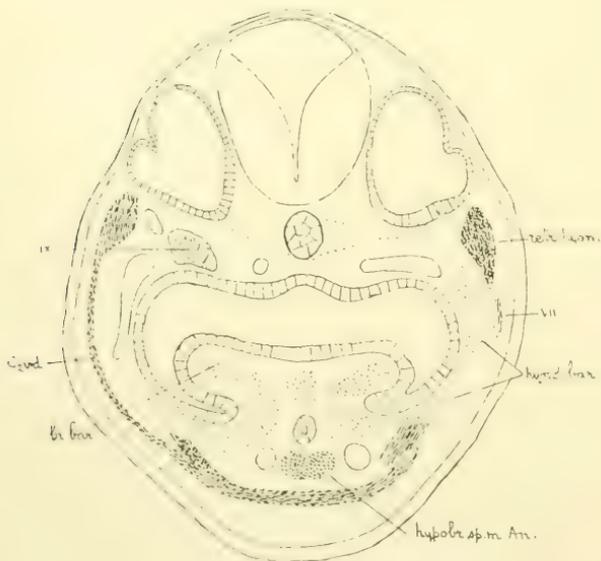
¹ This account differs from that of Schultze, in that the subtemporal is stated to be inserted into Meckel's cartilage as well as into the superior labial cartilage, and in the description of an extra-temporal. The results were obtained from serial sections of larvæ, 10, 13, 22, and 30 mm. long.

TEXT-FIG. 20.



20.

TEXT-FIG. 21.



21.

Text-figs. 20 and 21.—*Acipenser*, embryo $8\frac{1}{2}$ mm. Text-fig. 20 is the more anterior. The right side of the sections is slightly anterior to the left.

are similar to those of *Rana*; in *Alytes* the submaxillaris arises, like the mandibulo-labialis, from the inner aspect of Meckel's cartilage, so that the two muscles are much more continuous than is the case in *Rana*, *Bufo*, and *Pelobates*. The condition in 10 mm. larvæ of *Pelobates* is similar to that of 12 mm. larvæ of *Rana*; in 13 mm. larvæ the mandibulo-labialis has spread additionally into the upper lip, the condition described by Schultze. He states that the submentalis is attached to the inner aspect of Meckel's cartilage, but up to the stage of 30 mm. it is attached, as in *Rana*, *Bufo*, and *Alytes*, to the inferior labial cartilages, as a very minute transverse muscle.

At metamorphosis in *Rana*, the attachment to the skin of the mandibulo-labialis is lost, and the muscle forms one sheet with the submaxillaris.¹

Observations on the development of the mandibular muscles have been made by Reuter in pig-embryos, and in regard to the tensor tympani by Futamura in human embryos. Reuter stated that the mandibular muscles are first visible in pig embryos measuring 16 mm. in "Nacken-Steisslänge"² in the form of an inverted Y, the two limbs of which lie on either side of the lower jaw. The temporal develops from the upper limb, the masseter from the lower external limb, and the two pterygoids from the lower internal limb. No mention is made of the tensor tympani or the palatine muscles. According to Futamura the tensor tympani and tensor veli palatini form a "ganz einheitlichen Muskel" in human embryos of seven weeks. This Anlage and the levator veli palatini are developed about the branches of the palatine nerves from a "Muskelblastengewebe" which "deutlichen Zusammenhang mit dem tiefen Teil der Platysmaanlage erkennen lässt." "Die Nervenäste für diese

¹ Submaxillaris of Ecker and Gaup.

² This stage is an advanced one, as the figures show that the ossification of the lower jaw has begun. The Anlage of the mandibular muscles was quite evident in a pig embryo of 8 mm. crown-rump measurement, from which Text-fig. 98 was taken.

Muskeln lassen sich leicht vom Facialis hervorfolgen."¹ He also states that in pig embryos the levator veli palatini and *M. uvulæ* develop as in man from "Gewebe des *Platysma colli* das von der vorderen Seite des Oberkieferfortsatzes nach seiner medialen Seite zieht."

In 2 mm. embryos of the rabbit the cells which will form the myotome of the mandibular segment cannot be differentiated from the other cells occupying the segment. In 3 mm. embryos (Text-fig. 76) the myotome is visible, and the walls of the mandibular section of the cephalic cœlom are beginning to come together, forming the intermandibularis. The myotome separates from the lateral edge of the intermandibularis in 7 mm. embryos. In 13 mm. embryos it has partially separated into external and internal portions, which form the two limbs of a Λ -shaped mass, the apex of which lies just below the Gasserian ganglion (Text-figs. 94, 95); the external portion is the Anlage of the temporal masseter and external pterygoid muscles; it extends up to the skull in 16 mm. embryos, the external pterygoid is cut off from the internal surface of the lower end of the temporal. The internal portion separates into internal pterygoid and tensor tympani. The intermandibularis forms the mylohyoid of the adult; it is covered over, in 10 mm. embryos, by the forward growing interhyoideus.

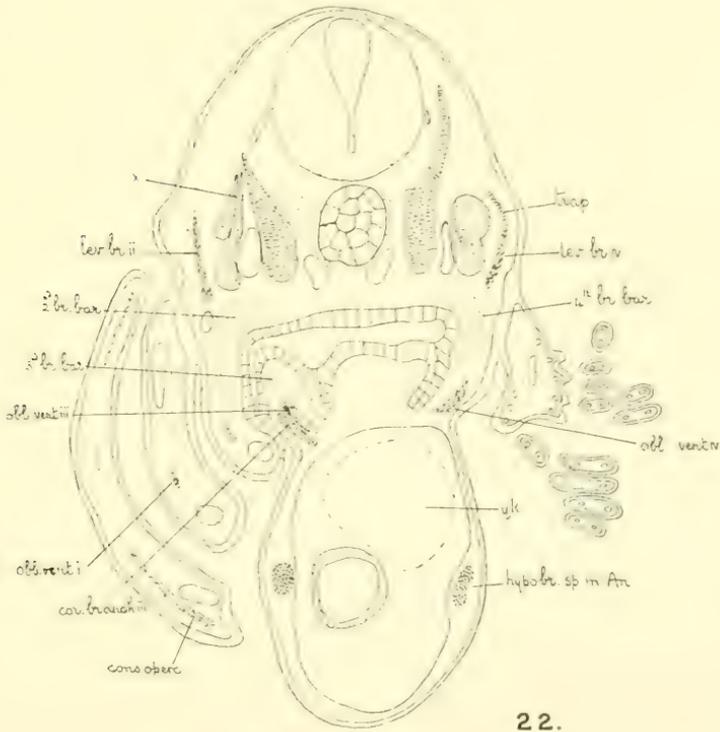
The Homologies of the Mandibular Muscles.—Comparison of the various ways in which the myotome of the mandibular segment develops shows that they may be reduced to two types: (1) That in which the myotome does not divide into upper and lower portions—*Ceratodus*, *Necturus*, *Triton*, *Rana*, *Alytes*, *Bufo lentiginosus*, *Pelobates*, *Lepus*. (2) That in which the myotome divides into portions above and below the palato-quadrate, into levator maxillæ superioris and adductor mandibulæ—*Scyllium*, *Acipenser*, *Lepidosteus*, *Amia*, *Salmo*, *Sauropsida*.

Drüner supposed that a portion homologous with the

¹ Beever and Horsley showed, however, that no movement of the palate is produced in the monkey on intra-cranial stimulation of the VIIth.

levator maxillæ superioris of Selachians disappears in Amphibia.¹ There is, however, no trace of this in the ontogeny of Amphibia. According to Gaupp the pterygoid process of Amphibia presents features which lead to the

TEXT-FIG. 22.



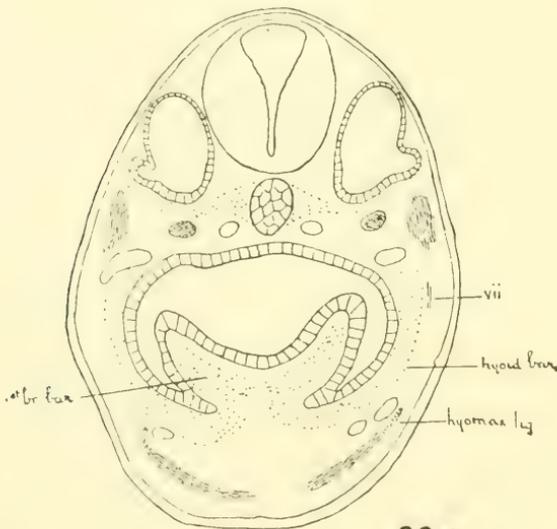
Acipenser, embryo $8\frac{1}{2}$ mm. The left side of the section is slightly anterior to the right.

suggestion that it is in process of "Rückbildung." If this be so, and if the pterygoid process of Amphibia be homologous with that of Selachians—a matter which Gaupp says

¹ The levator maxillæ superioris "ist wohl mit der Verwandlung der Streptostylie in die Monimostylie der Urodela verloren gegangen."

is not certain—it might be supposed that a muscle strip which formerly divided into upper and lower portions now by some atavistic process no longer does so. On the other hand, the fact that, in all the animals of the second class, the myotome, undivided, lies at first across and unattached to the palato-quadrate, i. e. shows a condition which is the permanent one in Amphibia and *Ceratodus*, suggests that the con-

TEXT-FIG. 22A.



Acipenser, embryo 9 mm.

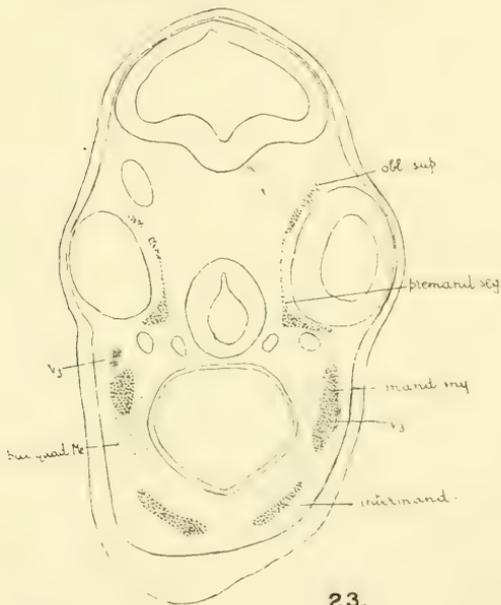
dition in Amphibia, *Ceratodus*, and Mammalia is the primary one, and that the one present in Selachii, Teleostomi, and Sauropsida is a secondary one. It would follow that the palatine or pterygoid process of the quadrate was not primarily a process for attachment of muscles nor an upper jaw.

Fürbringer divided Vertebrates into two classes with regard to the connection of the quadrate with the skull—those with movable quadrates (streptostylic), and those with immovable quadrates (monimostylic). The latter condition,

he thought, was secondary to the first. "Die Monimostylie allgemein von der Streptostylie ableitet."

The development of the mandibular muscles in the Sauropsida suggests that in them there are two streptostylic conditions—a primary streptostylic pterygo-quadrates in birds, and a secondary streptostylic quadrates in *Lacerta vera*,

TEXT-FIG. 23.



23.

Lepidosteus, embryo 8 mm., transverse section.

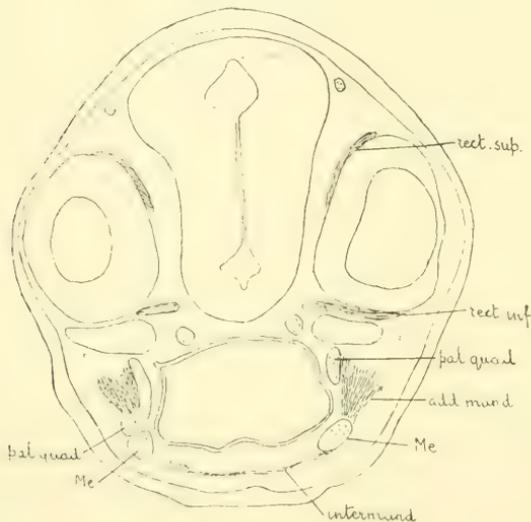
Rhoptoglossa, and Ophidia, and that the monimostylic condition of Chelonia, Crocodilia, and Rhynchocephalia was developed—and probably independently—from a primitive streptostylic pterygo-quadrates which has been preserved in Birds (*loc. cit.*).

The development of the mandibular muscles in Amphibia and *Ceratodus* affords no evidence that the monimostylic condition there present has been derived from a streptostylic one, and a fixed quadrates would appear to be a necessary

correlative of an undivided mandibular myotome, to form a point d'appui for the lower jaw.

It would follow that the streptostylic condition present in Selachians, Teleostomi, and Sauropsidan embryos is one which developed in correlation with a division of the myotome into upper and lower parts, inserted into and arising from the palatine process of the quadrate.

TEXT-FIG. 24.



24.

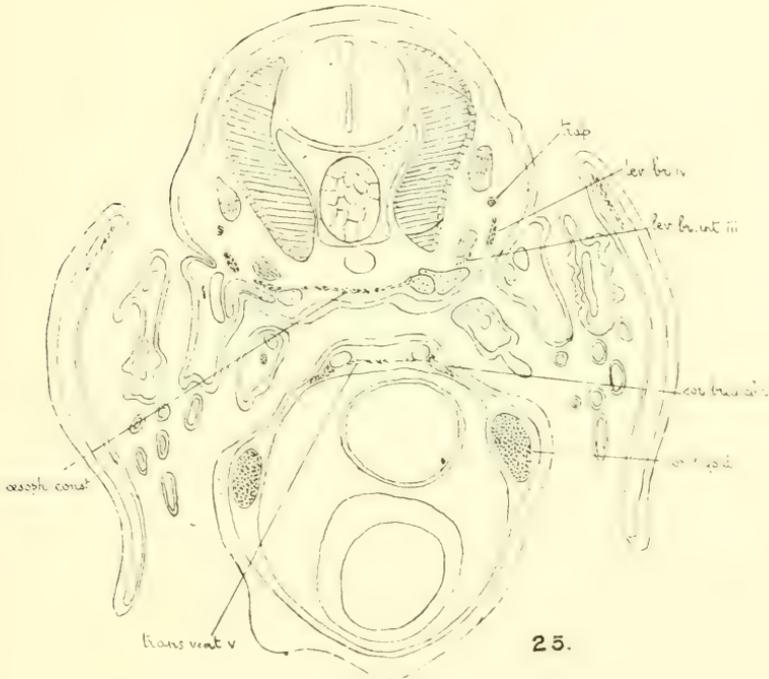
Lepidosteus, embryo 12 mm., transverse section.

In *Ceratodus*, *Amphibia*, and *Lepus*, where the mandibular myotome does not become divided into upper and lower parts, it separates into internal and external portions. In the Anuran larvæ the outer division divides into parts, some of which have a temporary insertion into the superior labial cartilage, and the whole myotome assumes a nearly horizontal position in correlation with that of the palato-quadrate bar; at metamorphosis both bar and muscles rotate into a more vertical position. In the rabbit the inner division separates into the internal pterygoid and the tensor tympani

muscles, the outer division into the temporal, masseter, and external pterygoid.

Secondary changes take place in the levator maxillæ superioris and adductor mandibulæ in all the animals investigated; no one preserves them as such. In *Scyllium* the

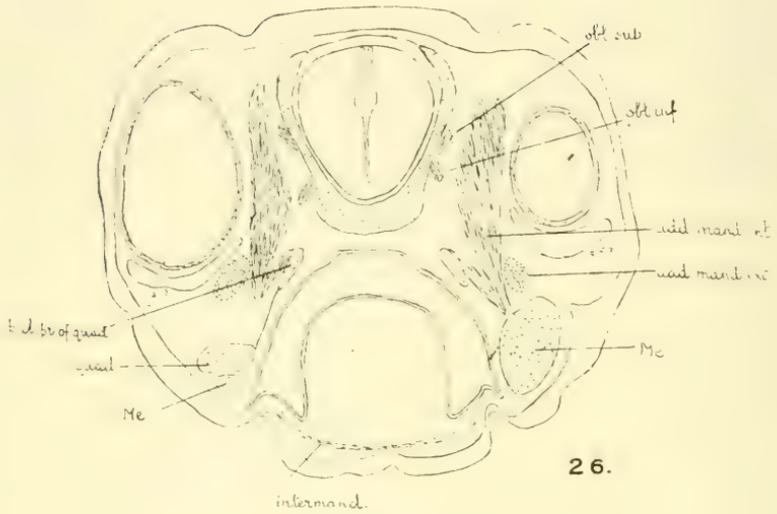
TEXT-FIG. 25.



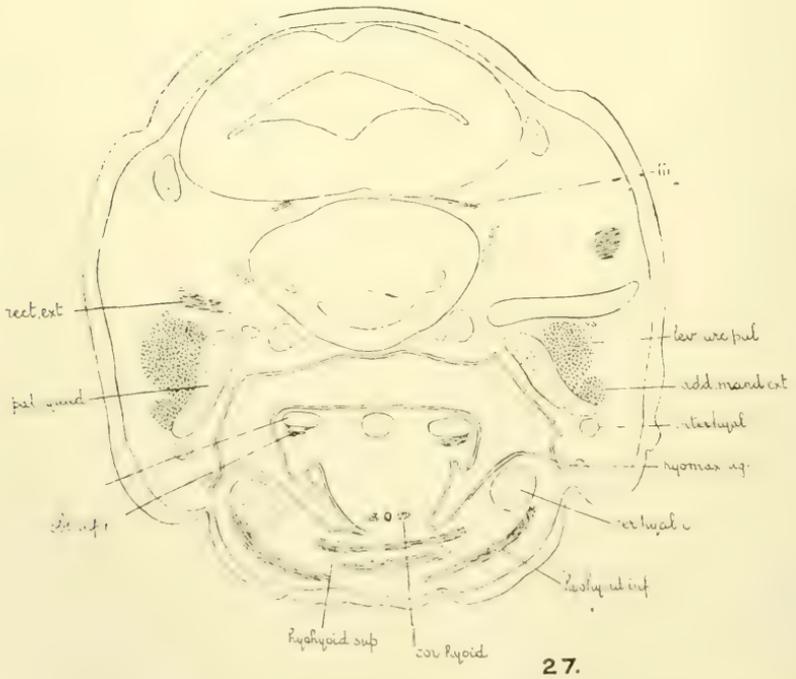
Lepidosteus, embryo 14 mm., transverse section.

Anlage of the nictating muscles is proliferated from the levator maxillæ superioris, and add. β and add. γ are separated from the adductor. In Teleostomi the levator maxillæ superioris either forms a protractor hyomandibularis or divides into a dilatator operaculi and levator arcus palatini; and the adductor may either remain single as in *Salmo*, or divide into external and internal portions, of which either the internal (*Lepidosteus*), or both (*Amia*, *Polypterus*), or ? the

TEXT-FIG. 26.



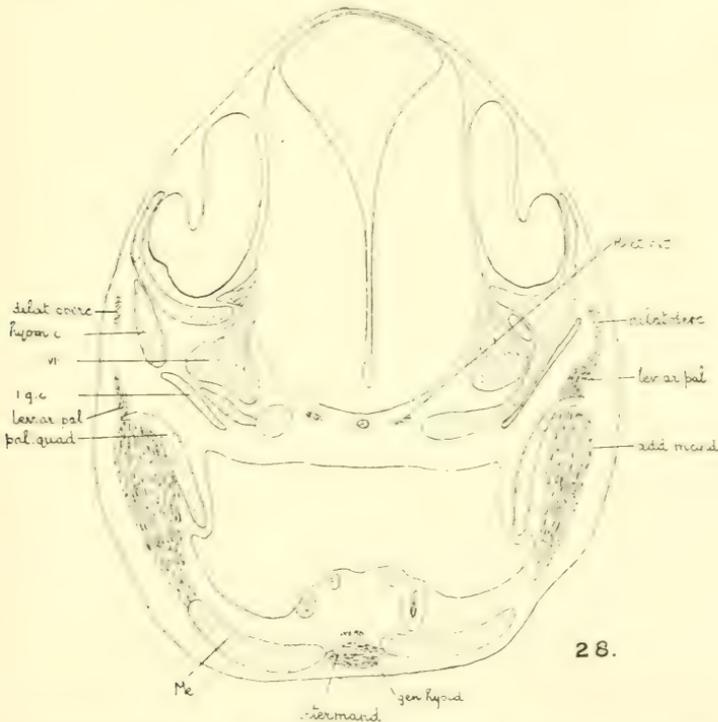
TEXT-FIG. 27.



Text-figs. 26 and 27.—*Lepidosteus*, embryo 19 mm. Text-fig. 26 is the more anterior.

external (*Acipenser*), grows up to the skull. In Sauropsidan embryos the depressor palpebræ inferioris is given off from the anterior margin of the levator maxillæ superioris, which becomes inserted into the palato-quadrata—this is preserved in birds, whereas in reptiles various changes, modifications

TEXT-FIG. 28.



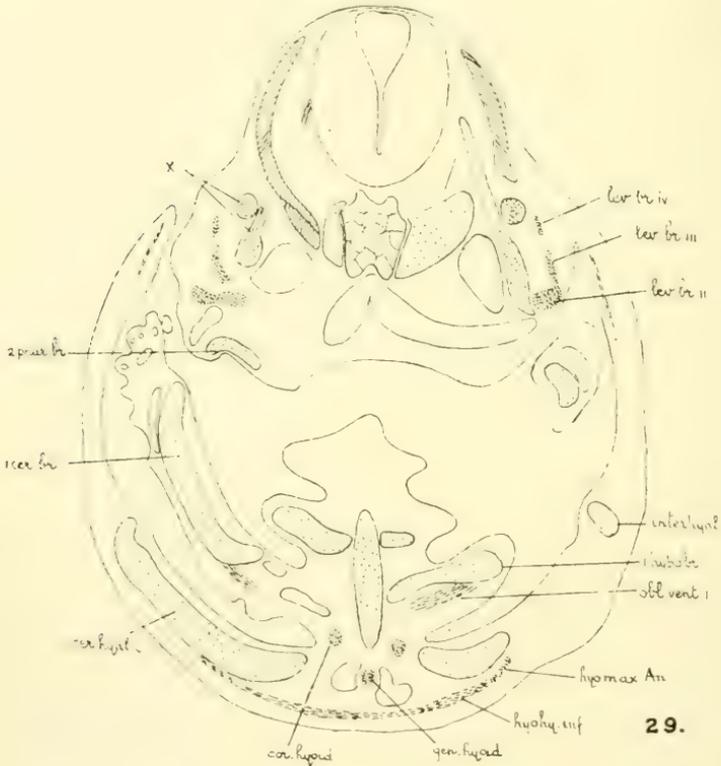
Text-figs. 28-33.—*Amia*, embryo 8½ mm. Text-fig. 28 is the most anterior.

or atrophy, occur; and the adductor mandibulæ divides into external and internal portions, of which the former grows up to the skull, whilst the primitive origin of the latter was probably to the palato-quadrata and the hind end of the palato-ptyergoid bar—this is preserved in *Chelonia*, but is variously modified in other groups (*loc. cit.*).

A comparison of the various forms of the intermandibularis

shows that its primitive condition is that of a transverse sheet passing from one ramus of the lower jaw to the other. This exists only in *Salmo*. In *Necterus*, *Triton*, *Ceratodus*, *Scyllium*, *Acanthias*, *Polypterus*, *Lepidosteus*, and *Amia* it extends backwards, underlying the fore part of the inter-

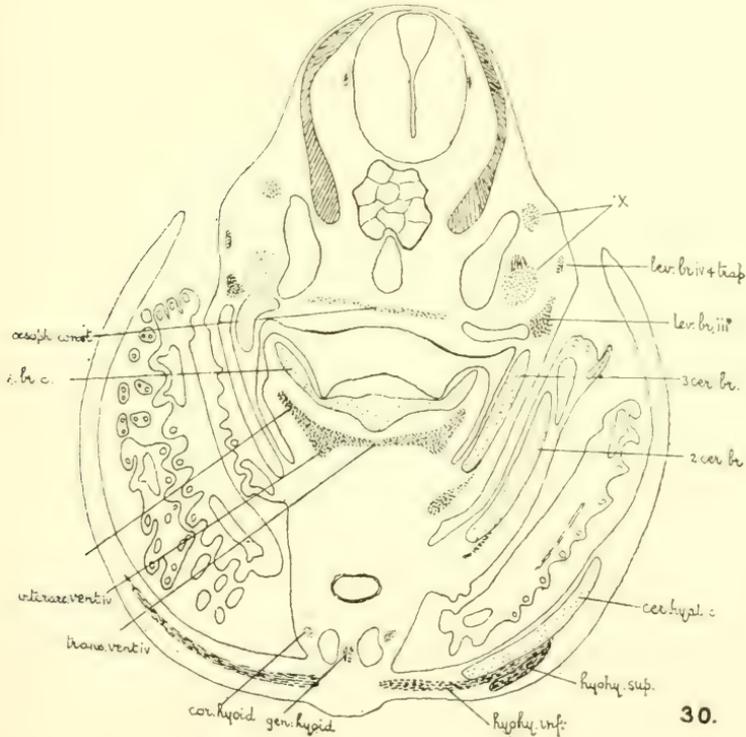
TEXT-FIG. 29.



hyoideus, and in *Amia* and *Triton* it divides into anterior and posterior portions. In Anuran larvæ it divides into submentalis, mandibulo-labialis and submaxillaris, of which the first has a special relationship to the inferior labial cartilages. In Sauropsida it forms a continuous sheet with the interhyoideus and C_2 vd. In *Lepus* it is overlapped by the forward-growing interhyoideus.

The intermandibularis, in correlation with its development in the mandibular segment, is usually innervated by the Vth cranial nerve. Vetter, however, found that in *Scyllium* and *Prionodon* the portion immediately behind the symphysis of the jaws was innervated by the Vth, and the greater portion

TEXT-FIG. 30.

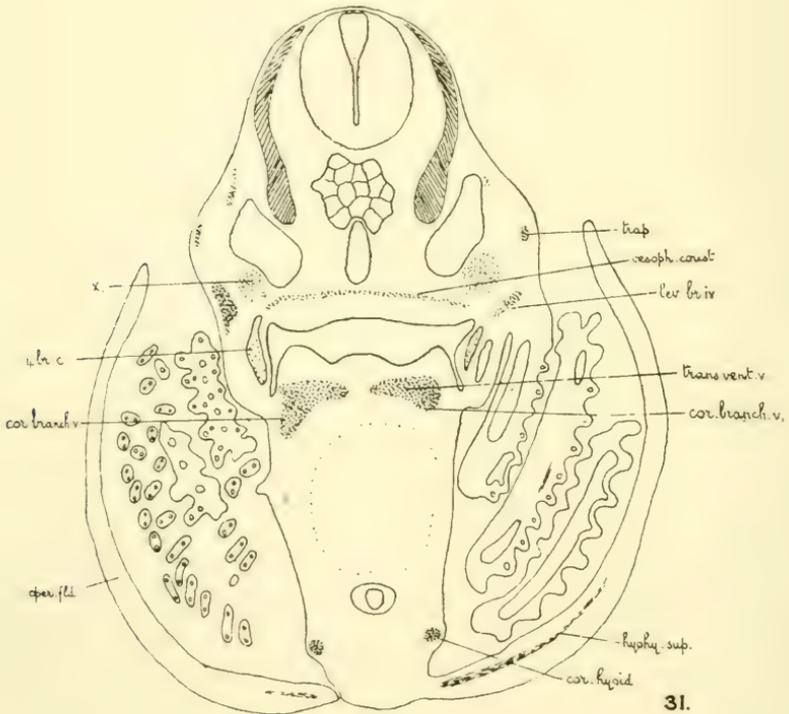


30.

of the muscle by the VIIth, and that in *Acanthias*, *Heptanchus*, and *Scymnus* the whole of the muscle was innervated by the VIIth. He concluded that in the former the greater part, and in the latter the whole, of the intermandibularis (Csv_1) had disappeared, and had been replaced by the interhyoideus (Csv_2), which had gained a secondary insertion into the lower jaw. But this opinion, which was founded on adult

anatomy only, is at variance with the phenomena of development; both in *Scyllium* and *Acanthias* a well-marked intermandibularis is formed in the mandibular segment, and spreads back below the interhyoideus and fusing with it behind the hyoid bar. Its partial or total innervation by the VIIIth must consequently be a secondary phenomenon.

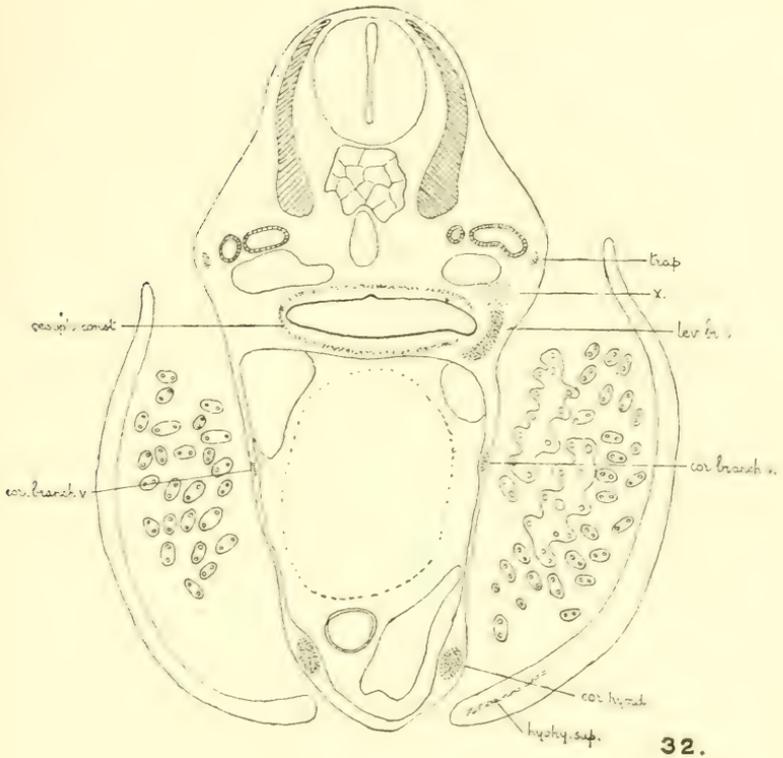
TEXT-FIG. 31.



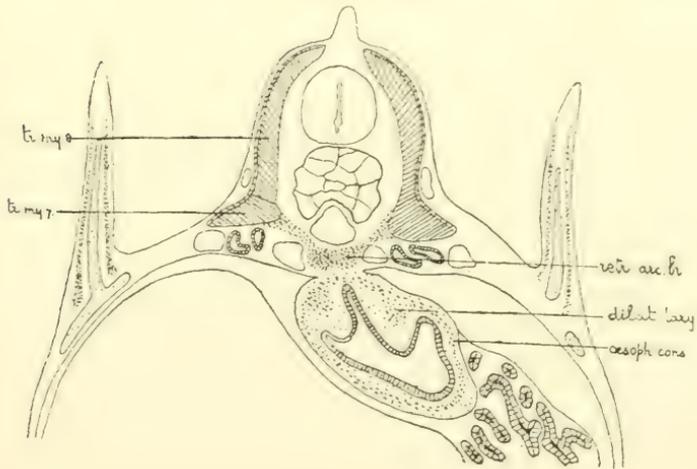
The intermandibularis of *Ceratodus* is also innervated by the VIIth (Ruge), and its hinder part in *Triton* (Drüner).

Ruge held that what is here called the intermandibularis is a facial muscle, and that its innervation from the Vth is secondary, but in *Ceratodus*, as in all the vertebrates examined, it is developed in the mandibular segment. Ruge's theory was based on the idea that "Es liegt auch nicht der geringste

TEXT-FIG. 32.



TEXT-FIG. 33.



33.

Grund vor um an der Ursprunglichkeit der Einrichtungen bei den Notidaniden zu zweifeln." Study of the comparative embryology of the cranial muscles, however, leads to considerable doubt on this matter.

HYOID MUSCLES.

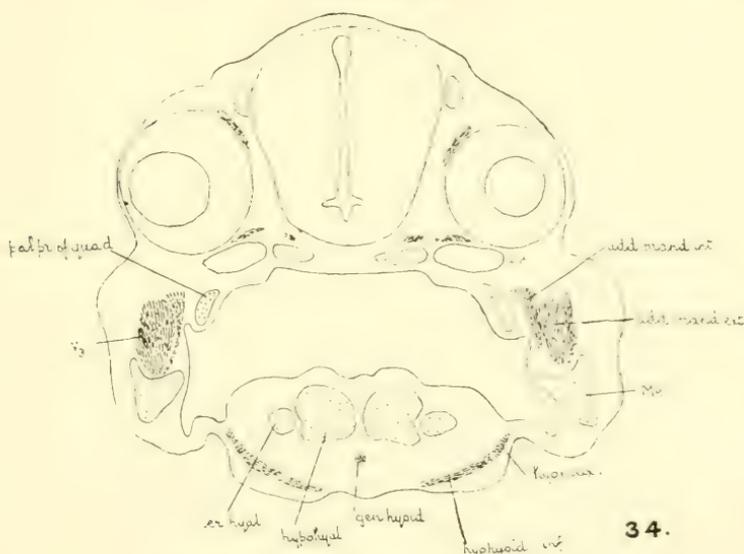
In *Scyllium* the ventral end of the hyoid myotome becomes continuous with the lateral edge of the future interhyoideus in 14 mm. embryos. In 16 mm. embryos the formation of the hyoid bar begins by aggregation of the mesoblast cells, forming a pro-cartilaginous tract lateral to the alimentary canal, and the myotome is at first partly continuous with the interhyoideus, partly inserted into the upper end of the bar (Text-figs. 5 and 6), forming a levator hyoidei. In 17 mm. embryos the hyoid bar extends upwards towards the auditory capsule (Text-fig. 7), partly covered by the myotome, which is inserted into its lateral surface (C_2hd of Ruge). It is only later, in embryos between the lengths of 23 and 30 mm., that the hyoid bar separates into ceratohyal and hyomandibula, as in *Acanthias* (Gaupp). The continuity of the myotome and the interhyoideus becomes lost, and the lateral edge of the latter is inserted into the ceratohyal. In 23 mm. embryos (Text-figs. 12, 13, cf. Text-figs. 10 and 11) backward extension of the myotome and interhyoideus takes place, so that a continuous dorso-ventral sheet (C_2vd of Ruge) is formed behind the hyoid bar. Later on, in 40 mm. embryos, the myotome extends forwards, completely covering the hyomandibular cartilage, and its anterior edge is inserted into the quadrate.

In the Teleostomi the relations of the fore part of the hyoid myotome (retractor or adductor mandibulæ) to the hyomandibular cartilage are different from those existing in Selachii. The retractor of *Acipenser* is inserted into its hinder edge, and of *Polypterus* into its inner surface, and the adductor of *Lepidosteus*, *Amia*, and *Salmo* is inserted into its inner surface. Further, the VIIth nerve (hyoid branch of VIIth in *Polypterus*) winds round the cartilage in

Acipenser and *Polypterus*, pierces it in *Lepidosteus*, *Amia*, and *Salmo*.

The development is not yet known in *Polypterus*. In the first stages, hitherto described, of *Acipenser ruthenus* (Parker), *Lepidosteus* (Parker), and *Salmo trutta* (Stöhr), the hyomandibula is stated to abut against the auditory capsule. Rutherford¹ states that in the brown trout a down-growth of no great size, from the periotic capsule at the edge

TEXT-FIG. 34.



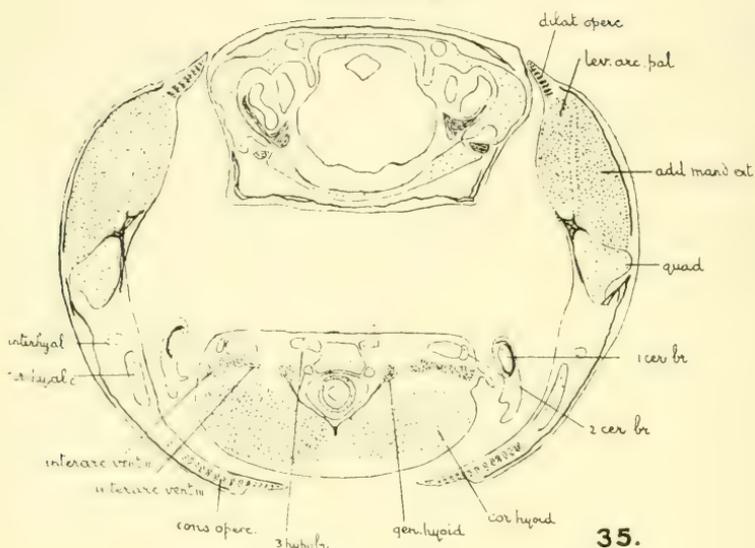
Amia, embryo 10 mm., transverse section.

of the fenestra ovalis, joins with the symplecticum in front of the VIIth nerve, and finally unites with the primitive hyomandibula.

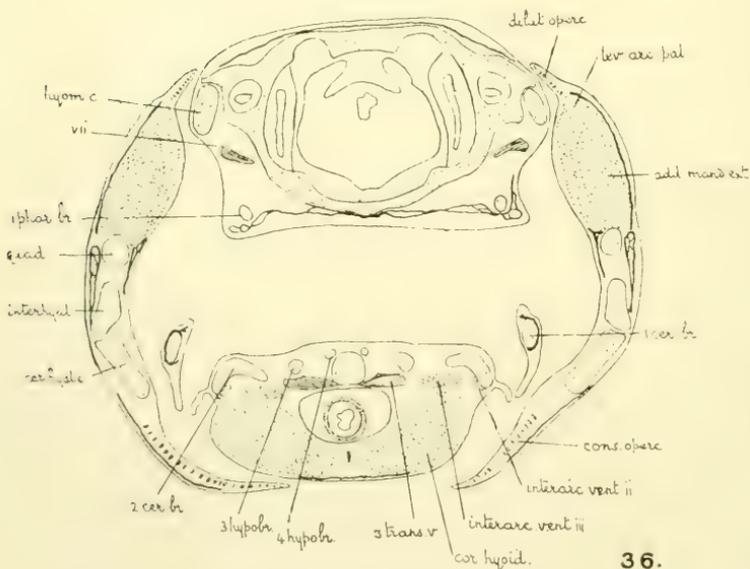
In 8 mm. embryos of *Acipenser* the hyoid bar, in a pro-cartilaginous condition and unsegmented, does not extend up to the auditory capsule. The VIIth nerve passes over the upper end of the bar, and then downwards outside it (Text-fig. 19). In 8½ mm. embryos the hyoid bar extends up towards

¹ The paper is as yet only published in abstract.

TEXT-FIG. 35.



TEXT-FIG. 36.



Text-figs. 35-37.—*Polypterus*, larva 7½ cm. Text-fig. 35 is the most anterior.

the auditory capsule and in front of, and outside, the VIIIth nerve, which now winds round it (Text-figs. 20 and 21). The hyo-mandibular cartilage is formed in part from the upper portion of the bar present in 8 mm. embryos, and in part from the upward extension. The hyoid muscles in 8 mm. embryos consist of a hyoid myotome, the anterior part of which is inserted into the upper end of the hyoid bar (Text-

TEXT-FIG. 37.

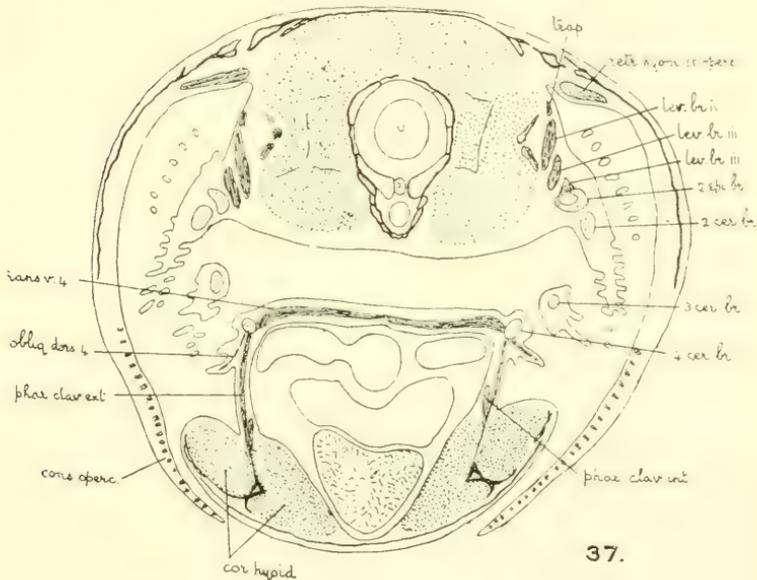


fig. 19), forming a levator hyoidei, and the posterior part of which forms a dorso-ventral sheet—homologous with C_{2vd} of Selachians—continuous with the posterior part of the inter-hyoideus (Text-fig. 21), whilst the anterior part of the inter-hyoideus is inserted laterally into the hyoid bar.

The sequence of events in the other Teleostomi examined is similar to that occurring in *Acipenser*, the upgrowth of the hyoid bar to the auditory capsule taking place in 8 mm. embryos of *Lepidosteus*, $6\frac{1}{2}$ mm. embryos of *Amia*, and $5\frac{1}{2}$ mm. embryos of *Salmo fario*. In no case was any downgrowth

from the periotic capsule found. In *Lepidosteus*, *Amia*, and *Salmo*, the VIIIth nerve, at first winding round the hyoid bar, subsequently pierces the hyomandibula owing to chondrification spreading round it; the more primitive condition is preserved in *Acipenser* and *Polypterus*.

The adult condition of the hyoid muscles in these Teleostomi is not quite uniform. In all the dorso-ventral sheet C_{2vd} divides into dorsal and ventral portions. In *Polypterus* the anterior and posterior portions of the myotome do not separate from each other, but form one muscle, the retractor hyomandibularis et opercularis. In the others separation takes place; the anterior part, i. e. the original levator hyoidei, forms a retractor hyomandibularis in *Acipenser*, and an adductor hyomandibularis in *Lepidosteus*, *Amia*, and *Salmo*. The posterior part, i. e. the upper part of C_{2vd} , forms a *M. opercularis* in *Acipenser* and *Lepidosteus*, an adductor and levator operculi in *Amia* and *Salmo*. In $9\frac{1}{2}$ mm. embryos of *Salmo* the adductor mandibularis additionally spreads forwards, forming the adductor arcus palatini.

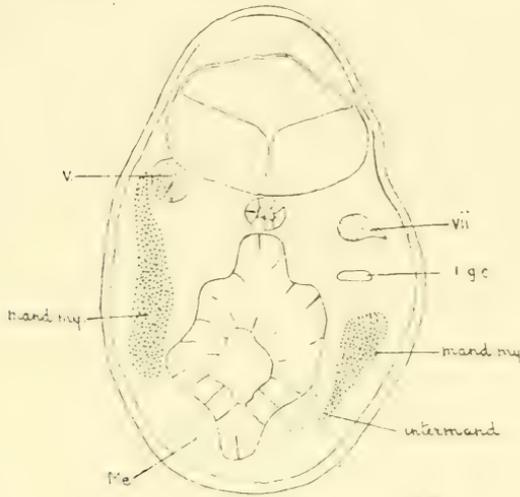
The fore part of the interhyoideus of *Acipenser* forms the hyoideus inferior (Cs_5 of Vetter), the hinder part, i. e. the lower part of C_{2vd} , forms a constrictor operculi (Cs_3 and Cs_4 of Vetter). In *Polypterus* the condition is similar.¹ In *Lepidosteus*, *Amia*, and *Salmo*, the fore part forms the hyoideus inferior; the hinder part becomes attached laterally to the hyoid bar (only partially so in *Lepidosteus*), and forms the hyoideus superior. The median raphé of these muscles is preserved in *Acipenser*, *Lepidosteus*, and *Polypterus*; in *Salmo* and *Amia* it is lost, and the hyoideus inferior becomes attached to the hypohyals of the same and opposite side.

In $8\frac{1}{2}$ mm. embryos of *Amia* the Anlage of the hyomaxillaris² muscle becomes separated from the upper edge of the hyo-hyoideus inferior (Text-fig. 29); it grows forward to Meckel's

¹ Intermaxillaris posterior and mantle muscle of Pollard.

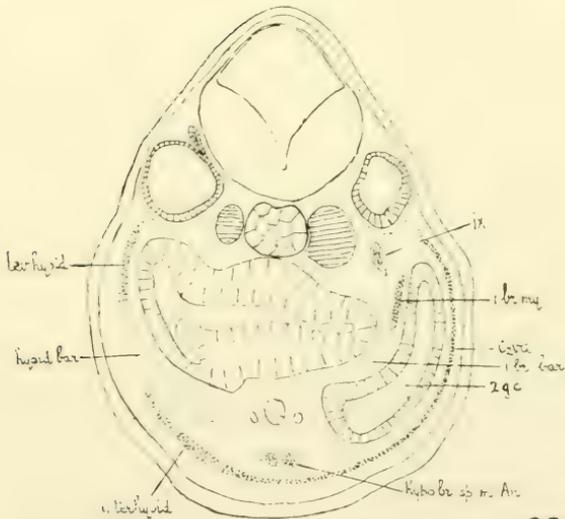
² Superior deeper portion of the genio-hyoid of Allis. A different terminology is used in this paper, as the word "genio-hyoid" is generally used to denote the anterior element of the hypobranchial spinal muscles.

TEXT-FIG. 38.



38.

TEXT-FIG. 39.

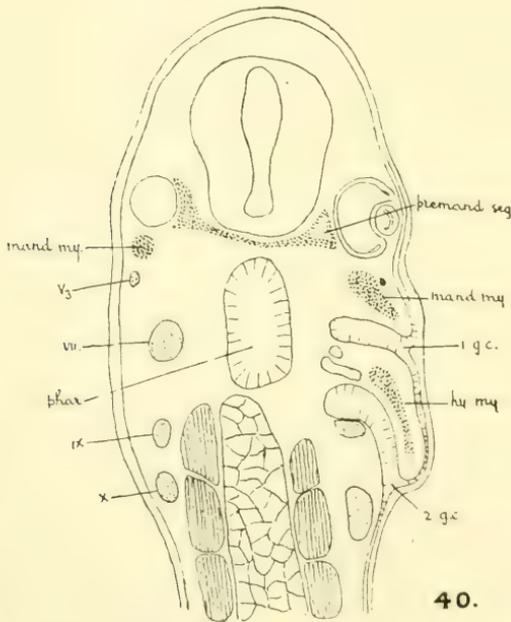


39.

Text-figs. 38 and 39.—*Ceratodus*, embryo stage 40; transverse sections. Text-fig. 38 is the more anterior; the left side of the sections is slightly anterior to the right.

cartilage dorsal to the intermandibularis posterior. A similar muscle is formed in *Salmo*; it grows forward ventral to the inter-mandibularis. In $9\frac{1}{2}$ mm. embryos of *Lepidosteus* and 9 mm. embryos of *Acipenser* (Text-fig. 22 A) a similar Anlage is formed from the dorsal edge of the hyoideus inferior or Cs_5 and develops into the hyomaxillaris ligament.¹ In *Polypterus*, ? species, Pollard described, but did not name, a small muscle

TEXT-FIG. 40.

Text-fig. 40.—*Ceratodus*, stage 40, longitudinal horizontal section.

“at the angle of the jaw in the substance of the ligament which binds the hyomandibula quadrata and stylohyal,” innervated by the opercular branch of the hyoid branch of the VIIth. In *Polypterus senegalus* only a ligament is present.

In *Ceratodus*, between the stages of 38 and 40, the hyoid myotome and interhyoideus spread backward in the opercular

¹ Mandibulo-hyoid ligament of van Wijhe.

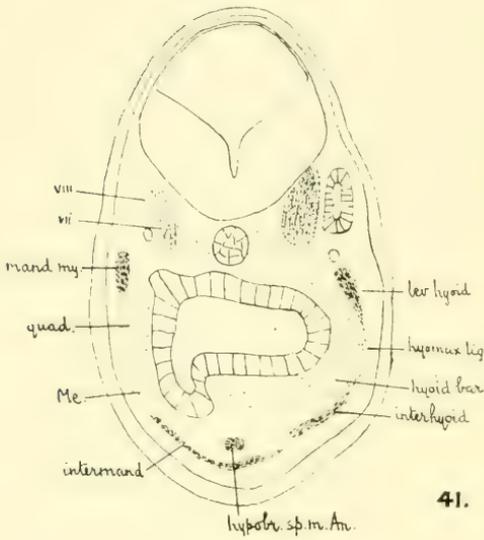
fold (Text-fig. 40), and form in the latter stage (Text-fig. 38) anteriorly a levator hyoidei inserted into the upper end of the hyoid bar, and an interhyoideus, and posteriorly a continuous ventro-dorsal sheet C_2vd in the operculum. In stage 42 the Anlage of the hyomaxillaris ligament is cut off from the upper edge of the interhyoideus and spreads forwards to the hind end of Meckel's cartilage (Text-fig. 41). In stage 42 (Text-fig. 41) the hyoid bar extends upwards and inwards towards the under surface of the pro-cartilaginous tract connecting the parachordal plate with the auditory capsule, forming the hyomandibula, the original hyoid bar forming the ceratohyal and hypohyal.

The upper part of the originally pro-cartilaginous hyomandibula chondrifies; the lower forms a fibrous tract connecting its outer end with the upper end of the ceratohyal (Text-fig. 50). In stage 48 a downgrowth occurs from the outer edge of the auditory capsule, external to the hyomandibular branch of the VIIth (Text-fig. 47), and becomes separated, forming a cartilage abutting against the outer end of the hyomandibula (Text-fig. 50), and a second more dorsally situated piece is subsequently cut off from the auditory capsule (Text-fig. 50).¹ The insertion of the levator hyoidei into the upper end of the ceratohyal is preserved in the oldest embryo examined, but is not present in the adult (Ruge); it is, however, retained in *Protopterus* (vide description by Ruge).

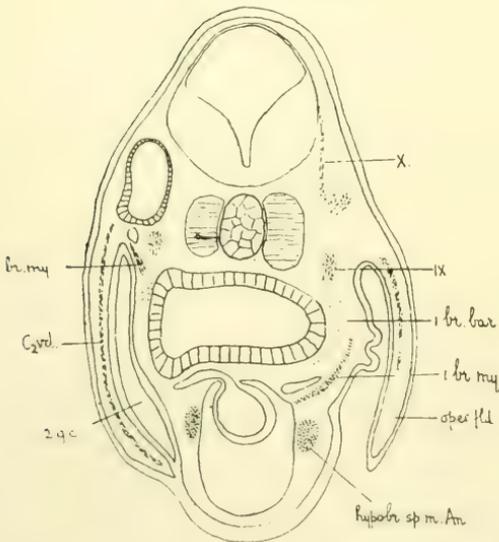
In *Necturus* Miss Platt stated that the "hyoid mesothelial tissue" (here interpreted as "myotome") divided into a dorsal digastricus, which "is ultimately connected with the posterior extremity of the mandibular bar by a long tendon"

¹ This description of the hyomandibula coincides with and amplifies that of K. Fürbringer. The cartilage (or cartilages) cut off from the auditory capsule is probably, from its relation to the hyomandibular branch of the VIIth, that described by Huxley, Ridewood, Ruge, and Sewertzoff as the "hyomandibula." From the descriptions given it would appear probable that no part of a true hyomandibula is preserved in the adult, and that (*vide* Ridewood) the cartilages cut off from the auditory capsule are variable.

TEXT-FIG. 41.



TEXT-FIG. 42.

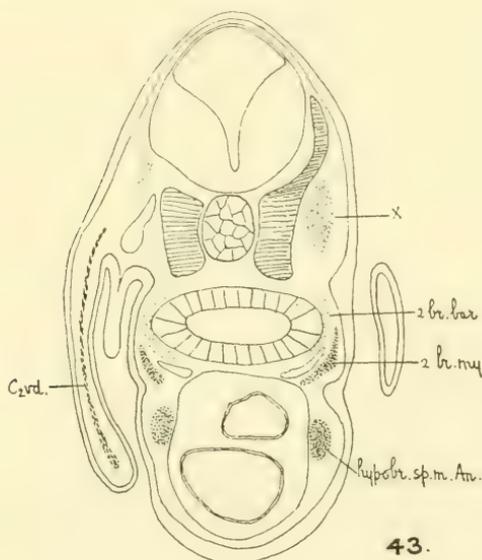


42.

Text-figs. 41-43.—*Ceratodus*, embryo stage 42; transverse sections. Text-fig. 41 is the most anterior; the left side of the sections is slightly anterior to the right side.

and a ventral ceratohyoideus externus. Examination of sections of $14\frac{1}{2}$ mm. embryos shows that there is a stage in which the future digastricus (the "cephalo-dorso-mandibularis" of Drüner) forms a levator hyoidei, inserted into the upper end of the hyoid bar, whilst below the cerato-hyoideus externus the Anlage of the hyomaxillaris ligament is formed (Text-fig. 55).

TEXT-FIG. 43.



It is of interest to note that in *Siren* (Drüner) there is a levator hyoidei, a slip of the cephalo-dorso-mandibularis, i. e. in that animal the primary insertion of the muscle is not wholly lost.

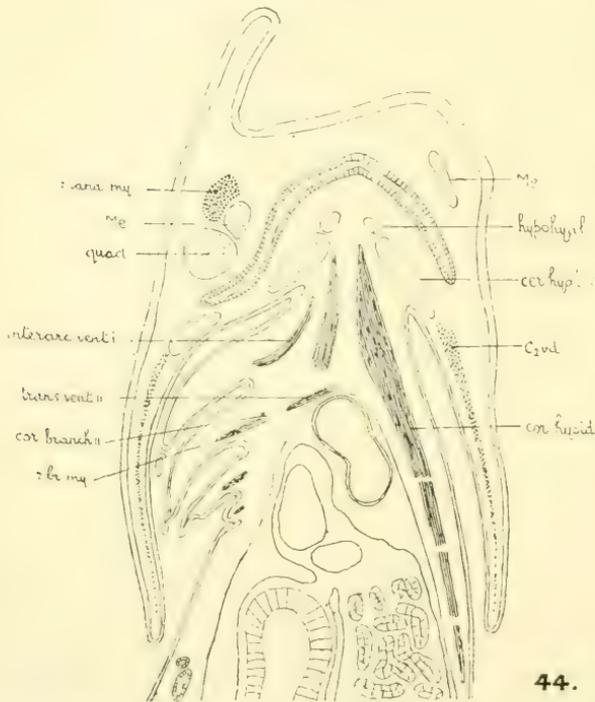
The interhyoideus¹ of *Necturus* spreads backward behind the hyoid bar in the opercular fold, and this posterior part, at first entirely ventral, spreads upwards laterally external to the cerato-hyoideus externus and depressor mandibulæ. There is a similar development of the interhyoideus in *Triton*, and

¹ Posterior mylohyoid of Mivart and Miss Platt.

the hinder part gains an attachment to the first branchial bar.¹

Schultze described the hyoid muscles of the larvæ of *Pelobates fuscus* as consisting of an orbito-hyoideus, suspensorio-hyoideus, cerato-hyo-angularis, quadrato-angularis,

TEXT-FIG. 44.



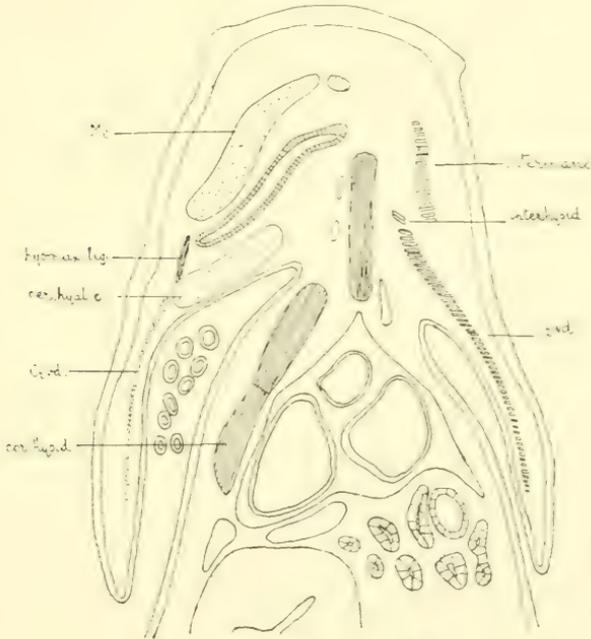
Text-figs. 44 and 45.—*Ceratodus*, embryo stage 46; longitudinal horizontal sections. Text-fig. 44 is the more dorsal; the left side of the sections is slightly dorsal to the right side.

and suspensorio-angularis. In *Rana* the myotome of the hyoid segment separates from the interhyoideus in 6 mm. embryos. It divides in 7 mm. embryos into an upper and lower portion (Text-fig. 58); the former develops into the orbito-hyoideus, which passes from the processus muscularis of the

¹ First interbranchial of Drüner.

palato-quadrate to the ceratohyal; the latter, which is the hyomaxillaris, grows forwards to the outer end of Meckel's cartilage and divides into the cerato-hyo-angularis, suspensorio-angularis, and the quadrato-angularis (Text-fig. 59). In *Bufo lentiginosus* the upper portion of the myotome forms only an orbito-hyoideus, as in *Rana*; in *Pelobates* and *Alytes* it forms an orbito-hyoideus and suspensorio-hyoideus. In *Pelo-*

TEXT-FIG. 45.



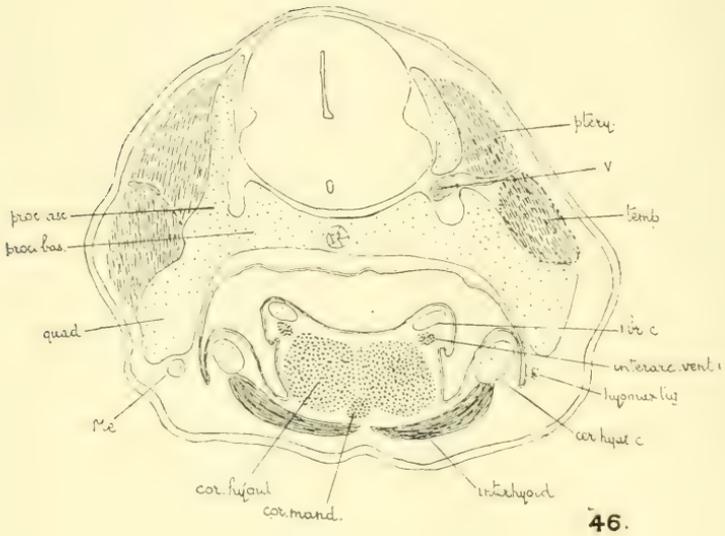
45.

bates and *Bufo lentiginosus* the hyo-maxillaris divides into three muscles as in *Rana*, in *Alytes* into two only, the cerato-hyo-angularis and quadrato-angularis (Text-fig. 63).

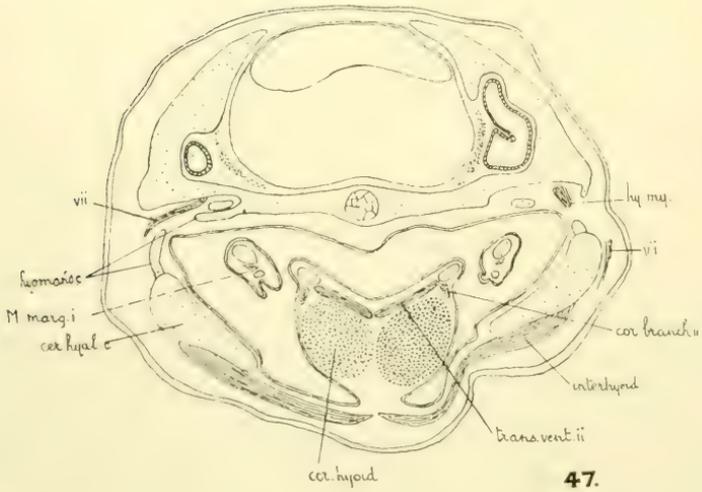
In *Rana*, at metamorphosis, the upper end of the orbito-hyoideus extends upwards on the atrophy of the processus muscularis of the palato-quadrate; subsequently, on rotation backward of the palato-quadrate, the lower end of the orbito-

¹ The coraco-mandibularis in this drawing is unfortunately not named.

TEXT-FIG. 46.



TEXT-FIG. 47.

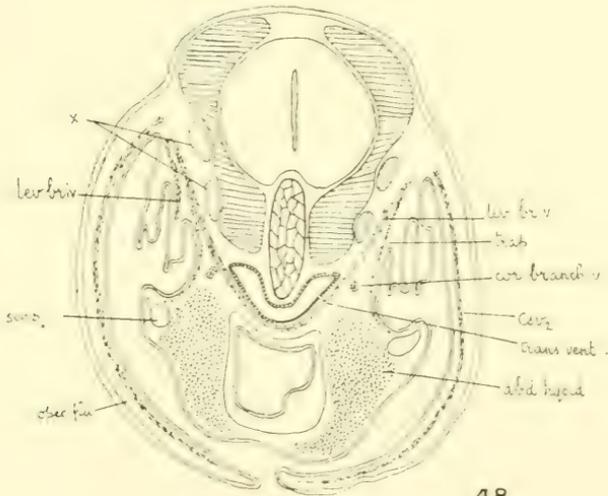


Text-figs. 46-48.—*Ceratodus*, embryo stage 48. Text-fig. 46 is the most anterior.

hyoideus gains a new insertion to the hind end of the lower jaw. The cerato-hyo-angularis, suspensorio-angularis, and quadrato-angularis assume a more vertical position on the rotation of the palato-quadrato, and form the inner portion, whilst the original orbito-hyoideus forms the outer portion, of the depressor mandibulæ.

The interhyoideus of the larvæ of *Rana*¹ forms a transverse band connecting together the two ceratohyals (Text-fig. 57), and this is also the case in *Alytes*. In *Pelobates* and *Bufo*

TEXT-FIG. 48.



48.

lentiginosus it extends backwards below the branchial cavity, and this hinder part forms the sub-branchialis (of Schultze), and the diaphragmato-branchialis (of Schultze) is separated from the median edge of this muscle.

The digastric muscle² of Mammals is either monogastric (*digastricus spurius*), inserted into the jaw, or digastric (*digastricus verus*), with a tendon between the two bellies which may or may not be connected with the hyoid bone.

¹ Subhyoideus of Ecker and Gaup, and of Schultze.

² This bare outline only is given, as the matter has been so thoroughly discussed by Bijvoet.

The anterior belly of a digastricus verus is innervated by the Vth, the posterior belly by the VIIIth. A digastricus spurius has a deeper or superficial tendinous inscription, and is also innervated by both Vth and VIIIth.

Anatomists who have investigated the adult conditions of the muscle have expressed various views as to its origin; some, e. g. His and Chaine, thought that the muscle was developed from one mass; others, e. g. Humphrey, Gegenbauer, Rüge, Fürbringer, Bijvoet, have held that it was developed from two masses.

Bijvoet, the latest investigator of the subject, was of opinion that the condition present in Monotremes—a *M. depressor mandibulæ anterior*, and a *M. stylohyoideus*—is the "Ausgangsform," that from this was developed a digastricus verus, and that a digastricus spurius was a secondary condition. He was also of opinion that a *M. intermandibularis* separated into a *M. mylohyoideus* and a *M. depressor mandibulæ anterior*. The only descriptions of the development of the digastric muscle which have hitherto been given are those by Futamura of human and pig embryos. He says that the common Anlage of the digastricus, stylohyoideus, and stapedius is visible in twenty-seven to thirty day human embryos as a "medialwärts sich anhäufende dicht gedrängte Muskelblastengewebe," which is continuous laterally with the platysma Anlage, the whole forming a single mass. In embryos a little older the platysma and digastric Anlagen separate. In six weeks' embryos the digastric Anlage passes forward to the anterior border of the lower jaw. The mylohyoid nerve at this date passes to the mylohyoid muscle only; it does not innervate the anterior belly of the digastric until the age of seven weeks. The stylohyoid and stapedius begin to separate from the posterior belly of the digastric at the age of six weeks. The digastric is thus solely a facial muscle, the anterior part of which receives a secondary innervation from the trigeminal nerve.

Bijvoet doubted this account given by Futamura, as it appeared to him to disagree with the results of comparative anatomy.

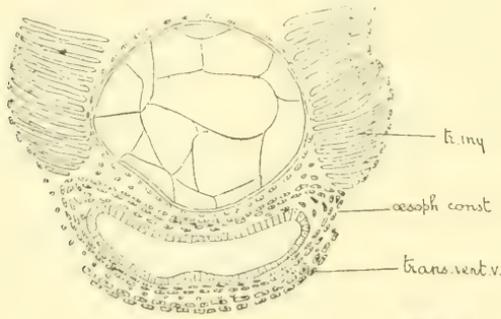
Futamura's account of the development of the facial and platysma muscles is that their Anlage separates, as stated above, from the outer aspect of the common facial Anlage; in thirty-one to thirty-four day embryos it spreads aborally almost to the shoulder region, forming the platysma colli, and in six-week embryos also forwards, in two directions separated by the outer ear, forming a platysma occipitalis and a facial portion. The latter passes forwards over the edge of the lower jaw to the forehead, eyelid, upper lip, and temporal region, separating into a deep and a superficial layer.

In 3 mm. embryos of the rabbit the hyoid myotome is continuous below with the epithelium lining the hyoid section of the cephalic cœlom (Text-fig. 77). In 4 mm. embryos the walls of the cephalic cœlom have come together, and the Anlage of the interhyoideus is formed from its epithelial wall; each half is continuous laterally with the lower end of the myotome (Text-fig. 81). In $7\frac{1}{2}$ mm. embryos the myotome is partially separated into an upper and a lower part; the former is the Anlage of the posterior digastric, stylohyoid, and stapedius, the latter that of the anterior digastric (Text-fig. 89). In embryos between 8 and 9 mm. the hyoid bar is formed,¹ and in its whole extent simultaneously, so that there is no stage in which the Anlage of the posterior digastric, stylohyoid, and stapedius is inserted into the upper end of a hyoid bar which has not yet extended up to the auditory capsule. In 9 mm. embryos (Text-figs. 92 and 93) the lateral edge of the inter-

¹ The "body of the hyoid" in the rabbit is formed from the ventral ends of the hyoid and first branchial bars. No basihyal nor basi-branchial is developed. In 9 and 13 mm. embryos a column of cells—the remains of the glosso-thyroid duct—extends downwards from the foramen cœcum in the middle line between the hyoid and first branchial bars. Antero-posterior fusion of the ventral ends of these bars to form the body of the hyoid, and the commencement of formation of a joint between the body and the first branchial cornua, take place in embryos between the stages of 13 and 17 mm. Chondrification takes place later in the "body" than in the hyoid and first branchial cornua. These findings confirm the suggestions of Parsons; in the rabbit, however, there is no basihyal.

hyoideus has separated from and extended upwards outside the anterior digastric, which has grown a little forwards. In 10 mm. embryos the anterior end of the anterior digastric has reached Meckel's cartilage, the interhyoideus has extended forwards, and also backwards in the neck forming the platysma colli, and the stapedius has separated from the Anlage of the posterior digastric and stylohyoid. In 13 mm. embryos the Anlage of the posterior digastric and stylohyoid has separated into those muscles, and the lateral edge of the fore part of the interhyoideus (Text-fig. 96) has extended upwards and for-

TEXT-FIG. 49.



49.

Text-fig. 49.—*Ceratodus*, embryo 15 mm.; portion of transverse section taken between the fifth branchial segment and the lung.

wards forming the platysma occipitalis and platysma faciei. The posterior digastric forms a tendon (vide Krause).

There is thus in the rabbit a stage of development prior to the first described in man by Futamura, in which the Anlage of the platysma faciei, platysma occipitalis, and platysma colli is a structure homologous with the interhyoideus of lower forms. The anterior digastric is homologous with the hyo-maxillaris of some Amphibia and Teleostomi.

The Homologies of the Hyoid Muscles.—In *Alytes*, *Bufo*, *Rana*, *Pelobates*, and *Lepus*, the lower end of the hyoid myotome becomes separated from the part above and forms a longitudinal muscle—the hyo-maxillaris—connecting the hyoid bar with the lower jaw. An homologous muscle is

formed in *Amia* and *Salmo*, but differs in that it is separated from the upper end of the hyoideus inferior. The first mentioned method of formation is probably the primitive one, for serially homologous muscles, interarcuales ventrales, are formed from the lower ends of branchial myotomes where there are no muscles homologous with the interhyoideus; the method of formation in these Teleostomi is probably related to the secondary position of the adductor hyomandibularis internal to the upper part of the hyoid bar. In Urodela (*Necturus*, *Triton*), *Ceratodus*, *Lepidosteus*, and

TEXT-FIG. 50.



50.

Text-fig. 50.—*Ceratodus*, advanced embryo, length ?; portion of transverse section, to show relations of the hyomandibular cartilage.

Acipenser a corresponding Anlage develops into a hyo-maxillaris ligament. In *Selachii* (*Scyllium*, *Acanthias*) and in *Sauropsida* no hyo-maxillaris Anlage is formed.

The developmental history of the hyo-maxillaris suggests that its primitive condition was that of a longitudinal muscle passing from the hyoid bar to the hind end of Meckel's cartilage, but this is not preserved in any of the animals investigated. In *Teleostomi* (*Amia* and *Salmo*) the front end of the muscle extends forwards along Meckel's cartilage.

In *Chimæra* there is a similar muscle (hyoideus inferior of Vetter) extending from the hyoid bar along the lower jaw.

In *Rana*, at metamorphosis, the muscle becomes more vertical in position, and forms, with the orbito-hyoideus, the depressor mandibulæ; this stage is preceded by a larval one, in which the muscle, having a longitudinal direction, is in part attached to the palato-quadrate bar. In Mammals the hyo-maxillaris (anterior digastric) may lose its attachment to the hyoid bar and form part of a digastricus verus with a tendon not attached to the hyoid bar, or part of a digastricus spurius.

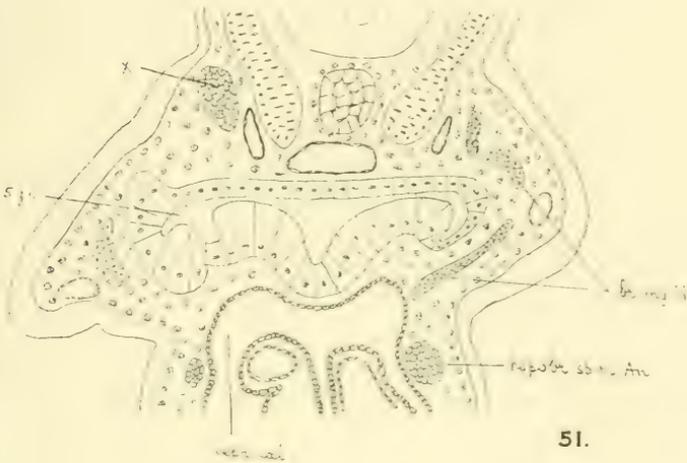
The part of the hyoid myotome above the hyo-maxillaris in cases where this is formed, or above this and the cerato-hyoideus externus (in the Urodela), or the whole myotome, where either or both of these muscles is not formed, is at first wholly or partially (*Scyllium*) inserted into the upper end of a hyoid bar, which has, or has not yet, extended up to the auditory capsule, forming a levator hyoidei. This holds for *Scyllium*, *Acipenser*, *Lepidosteus*, *Amia*, *Salmo*, *Ceratodus*, *Necturus*, *Triton*, *Rana*, and other Anuran larvæ, *Chrysemys*, and *Gallus*. In the Amphibia the hyoid bar remains in this primitive condition, in the other groups it extends upwards to the auditory capsule.

According to Kingsbury and Reed, the columella auris of Urodela is the homologue of the hyomandibula of other forms. If this be so, its dorsal relation to the seventh nerve would suggest that it is homologous with the hyomandibula of Teleostei, where a similar relation holds, rather than with that of Selachii, *Ceratodus*, and Sauropsida, which lies ventral to the VIIIth.

Against such homology, however, it might be urged that in *Scyllium*, *Acipenser*, *Lepidosteus*, *Amia*, *Salmo*, *Ceratodus*, *Chrysemys*, and *Gallus* (Geoffry Smith), the hyomandibula is formed by a secondary segmentation occurring in a hyoid bar, which extends continuously up to the auditory capsule, whereas this is not stated by Kingsbury and Reed to occur in Urodela.

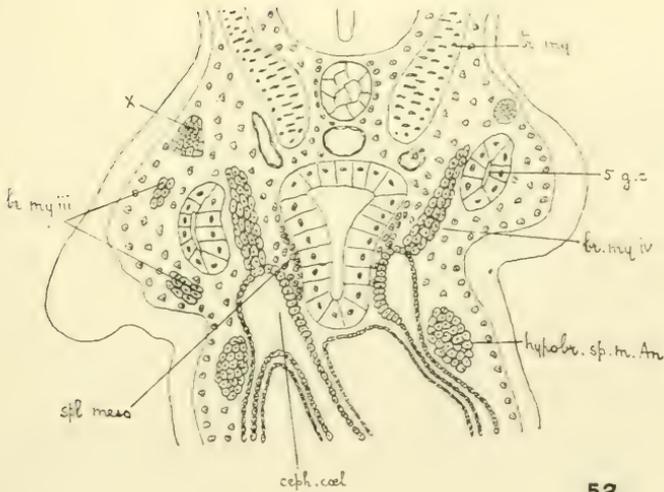
A levator hyoidei—attached to the upper end of a hyoid bar—is not preserved to adult life in any of the animals examined. In Urodela and Sauropsida it extends downwards

TEXT-FIG. 51.



51.

TEXT-FIG. 52.



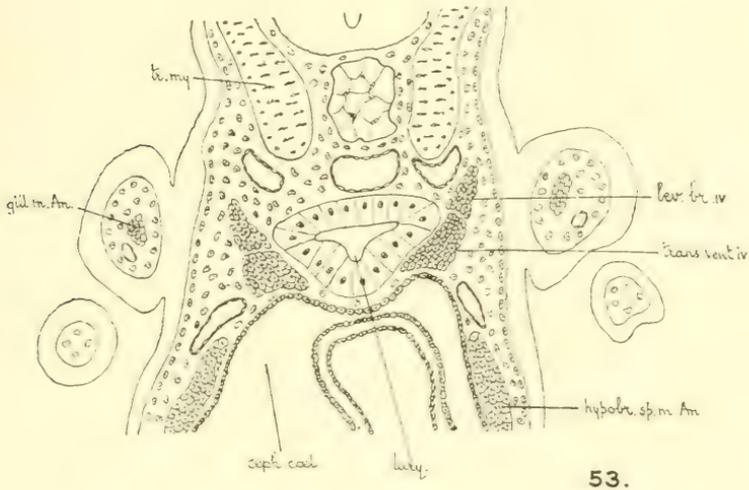
52.

Text-figs. 51 and 52.—*Necturus*, embryo 12 mm. Text-fig. 51 is the more anterior.

to Meckel's cartilage, forming the depressor mandibulæ. In *Rana* this also occurs at metamorphosis, the muscle forming the outer part of the depressor mandibulæ. In *Scyllium* the levator hyoidei, on the upgrowth of the hyoid bar, is inserted into its outer surface, and in very late stages of development extends forwards to the quadrate. In Teleostomi, on the upgrowth of the hyoid bar, the levator hyoidei becomes inserted into its posterior edge or inner surface, and in *Salmo* additionally spreads forward to the palato-quadrate. In *Ceratodus* the insertion of the levator hyoidei to the upper end of the ceratohyal is lost, but it is preserved in *Protopterus* (vide description by Ruge). In *Lepus* there is no stage in development in which there is a levator hyoidei inserted into the upper end of a hyoid bar, for this is formed simultaneously in its whole length, but the portion of the myotome above the Anlage of the hyo-maxillaris (anterior digastric) is a mass, which is homologous with the levator hyoidei of other forms, and lies outside the hyoid bar; it subsequently divides into stapedius, stylohyoideus and digastricus posterior. It is homologous with the *M. styloideus* of *Monotremes*. The upper end of the levator hyoidei is attached to the auditory capsule; this attachment, in *Anura*, is preceded by a larval stage, in which the upper end of the muscle takes origin from the processus muscularis of the palato-quadrate bar.

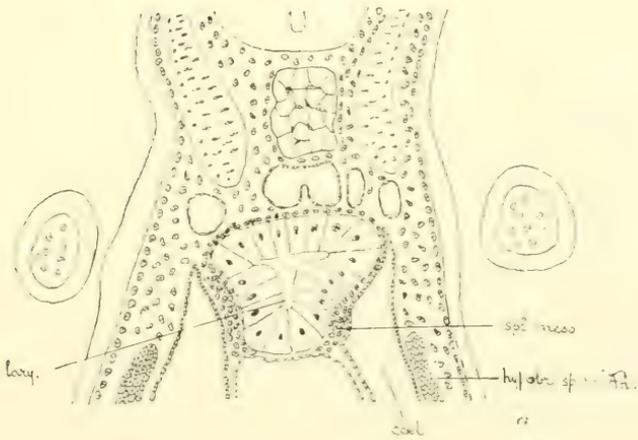
The primary form of the interhyoideus—formed from the walls of the obliterated hyoid section of the cephalic cœlum, and serially homologous with the intermandibularis—would appear to have been a transverse band connecting the two hyoid bars, but in all forms examined, other than *Rana* and *Alytes*, it extends backwards. In *Urodela*, *Anura*, and rabbit there is a backward extension of the interhyoideus only, but in *Selachii*, *Teleostomi*, *Ceratodus*, and *Sauropsida*, of both myotome and interhyoideus, forming a continuous ventro-dorsal sheet (C_{2vd} of Ruge) behind the hyoid bar. The backward extension of the interhyoideus, or of this and of the myotome, takes place in the opercular fold in *Urodela*, *Anura*, *Ceratodus*, and *Teleostomi*; in part in the opercular

TEXT-FIG. 53.



53.

TEXT-FIG. 54.



54.

Text-figs. 53 and 54.—*Necturus*, embryo 13 mm., transverse section. Text-fig. 53 through the fourth branchial segment, Text-fig. 54 a little more posterior.

fold (only existing ventrally) in Sauropsida and rabbit; and in Scyllium entirely in the wall of the head, no opercular fold, even as an atrophying Anlage, being developed.

The backward extension of the interhyoideus forms the hinder part of the interhyoideus of Urodela, and the sub-branchialis of Anura. In the rabbit the attachment of the interhyoideus to the hyoid bar is lost, and it spreads down the neck, forming the platysma, and also upwards and forwards, forming the superficial occipital and facial muscles.

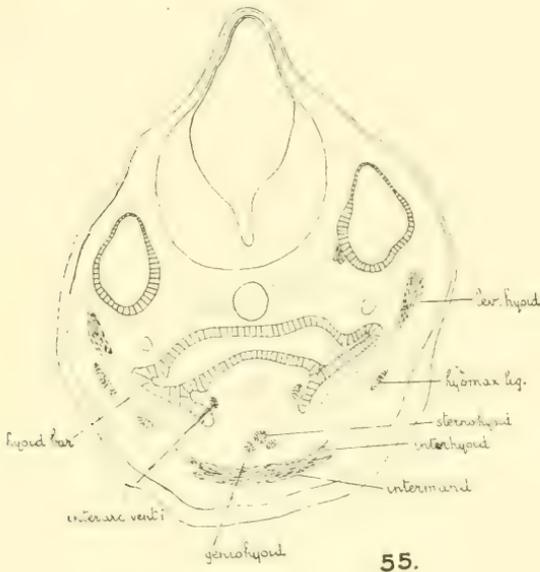
C₃vd persists as a continuous ventro-dorsal sheet in Scyllium, Ceratodus, and Sauropsida; in the latter group, in correlation with the atrophy of the gill-clefts, it spreads backwards in the neck, forming the constrictor colli; in Teleostomi it separates into dorsal and ventral portions, the former developing into the opercular muscle or muscles, the latter into a constrictor operculi (Acipenser and Polypterus), or hyohyoideus superior (Lepidosteus, Amia, Salmo).

The hinder part of the interhyoideus of Urodela and the platysma of the rabbit imitate, to some extent, the constrictor colli of Sauropsida, but this is due to a dorsal extension of their lateral edges over the hyoid myotome after their formation from the interhyoideus only.

It will be shown, later, that the probable primitive condition of the muscles of each branchial arch was, a *M. levator*, a *M. marginalis*, a *M. interarcualis*, and a *M. transversus ventralis*. The levator hyoidei is serially homologous with the first, and the hyo-maxillaris with the third of these. The cerato-hyoideus externus is serially homologous with the *Mm. marginales* and their homologues the Anlagen of the gill-muscles, and may possibly be derived from a hyoidean *M. marginalis*, but there is no trace of such a muscle in other groups. There are no homologues of the *transversi ventrales* in the mandibular and hyoid segments.

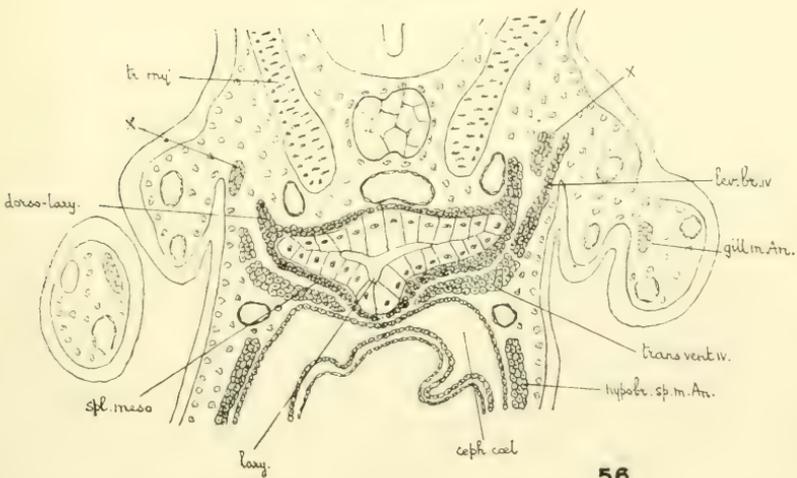
The primitive condition of the mandibular and hyoid muscles was probably one in which the former lay wholly in front of the latter. This condition exists in the embryo but does not persist; during development, in correlation with the

TEXT-FIG. 55.



55.

TEXT-FIG. 56.



56.

Text-figs. 55 and 56.—*Necturus*, embryo 14½ mm., transverse sections. Text-fig. 55 through the hyoid segment, Text-fig. 56 through the fourth branchial segment.

increase in size of the jaws, there is a gradual overlapping of the hyoid bar by the mandibular skeletal structures, with associated secondary changes in the muscles. Thus, the levator hyoides shifts its insertion from the hyoid bar to the hind end of Meckel's cartilage in Amphibia and Sauropsida, and the intermandibularis partially underlies the interhyoideus in Selachii, Teleostomi, Ceratodus, and Urodela. It is probably in connection with this overlapping that the hyomaxillaris Anlage becomes a ligament in Urodela, Ceratodus, and some Teleostomi, and is not developed in Selachii and Sauropsida.

EYE MUSCLES OF THE RABBIT.

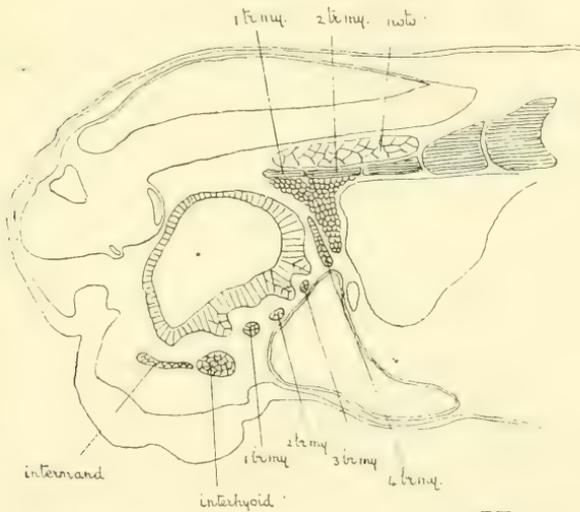
The development of the external ocular muscles in Teleostoman and Amphibian embryos was not followed.

In pig embryos of 10 mm. maximum and 6 mm. Nackenlänge, Reuter found the Anlage of the eye muscles as an "untrennbarer Zellcomplex," which, he held, "als Anhäufung von morphologisch ursprünglich Keimzellen aus dem Mesenchym hervorgeht."

In 3 mm. embryos of the rabbit the premandibular Anlage is developed, on each side, as a hollow outgrowth of the fore end of the alimentary canal (Text-fig. 75). This outgrowth becomes solid by proliferation of its walls as it is constricted off, and in $3\frac{1}{4}$ mm. embryos (Text-fig. 79) it forms a solid mass connected with its fellow by a solid string of cells lying across the fore end of the alimentary canal just below the anterior down-turned end of the notochord. The connecting string of cells has disappeared in $4\frac{1}{4}$ mm. embryos. The premandibular Anlage forms a crescent-shaped mass in 4 mm. embryos (Text-fig. 84); and from this the recti superior, inferior, internus, and obliquus inferior are formed (Text-figs. 96, 97). The obliquus superior is formed from the anterior extremity of the upper end of the mandibular myotome in $3\frac{1}{4}$ mm. embryos (Text-fig. 78). The development of the abducens Anlage is doubtful; in $3\frac{1}{4}$ mm. embryos it is visible lying

behind the premandibular Anlage and internal to the mandibular myotome (Text-fig. 79). In $2\frac{1}{2}$ mm. embryos it apparently is represented by a group of cells lying above the first gill-cleft and continuous behind with cells lying in the hyoid segment—cells which in 3 mm. embryos have differentiated into myotome and surrounding mesoblast. It is probable, therefore, that the abducens Anlage is formed from the mesoblast of the hyoid segment before the myotome is

TEXT-FIG. 57.



57.

Rana, embryo 6 mm., longitudinal vertical section.

formed. The abducens Anlage begins to separate into rectus externus and retractor oculi in 13 mm. embryos (Text-fig. 96).

These observations suggest that perhaps in pig embryos of a younger age than those investigated by Reuter a similar series of events may occur. In the later stages of development there is also a difference between the pig and the rabbit; in the former, according to Reuter, the retractor oculi is formed from all four recti, in the latter it is formed from the abducens Anlage only, as is usual in Vertebrates.

BRANCHIAL MUSCLES.

The homologies between the branchial muscles of various vertebrates are obscured by the absence of uniformity in nomenclature. The word "interbranchial" has been applied to many quite different muscles. It was used by Vetter to denote the layer of muscle-fibres which lies external to the branchial bars and internal to the superficial constrictor in Selachii. It was subsequently employed by Bronn and by Schultze to denote longitudinal muscles between the ventral ends of branchial bars in Amphibia; by Drüner, to denote transverse muscles in Amphibia; by K. Fürbringer and by Greil to denote slender vertical muscles in *Ceratodus*.

In this paper the word "interbranchial" is used exclusively in the Selachii in the sense of Vetter; longitudinal muscles between the ventral ends of branchial bars are called "interarcuales ventrales" (vide infra); transverse muscles are called "transversi ventrales," and the vertical muscles of *Ceratodus* are called *Mm. marginales*, as they are exactly homologous with the *Mm. marginales* of Anuran larvæ described by Schultze.

Fürbringer included two sets of muscles under the term "hypobranchial spinal musculature"¹—(1) Those which are sometimes called ventral longitudinal muscles, e. g. *coraco-mandibularis*, *coraco-hyoideus*, *genio-hyoideus*, *sterno-hyoideus*; (2) the *coraco-branchiales* of Elasmobranchs, *Teleostomi*, and *Dipnoi*.

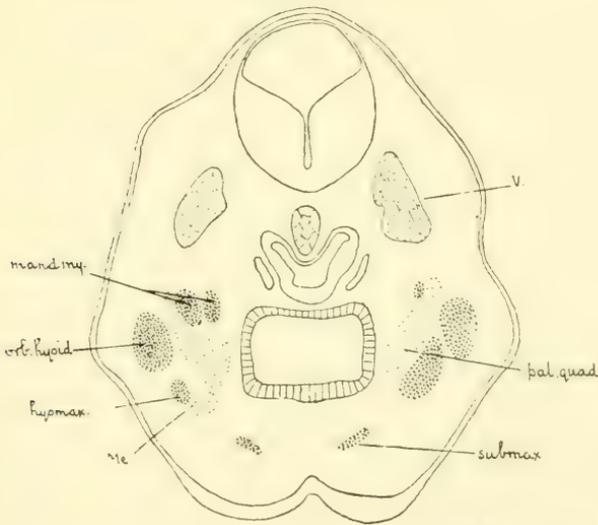
Investigation of the development of these muscles, however, shows that whereas the ventral longitudinal muscles are derived from trunk-myotomes, the *coraco-branchiales* are derived from the ventral end of one or more branchial myotomes, i. e. are of cranial origin. The above-mentioned terminology would therefore appear to be in need of revision,

¹ The name "hypoglossal musculature" employed by Neal and by Corning has nearly, but not quite, the same meaning as Fürbringer's "hypobranchial spinal musculature," e. g., it would include the *ceratohyoideus* (*interarcualis ventralis I*) of *Sphenodon*.

and in this paper longitudinal muscles derived from trunk-myotomes are called "hypobranchial spinal muscles," and longitudinal muscles derived from branchial myotomes are called "hypobranchial cranial muscles."

The Anlagen, which in some Vertebrates grow backwards and form coraco-branchiales, are homologous with Anlagen in other Vertebrates, which form muscles passing between the ventral ends of the branchial bars. Many different

TEXT-FIG. 58.



58.

Rana, embryo 7 mm., transverse section.

names have been given to the latter muscles, e.g. interbranchiales s. constrictores arcuum branchialium (Bronn), constrictores arcuum (Mivart), interbranchiales (Schultze), subarcuales (Drüner).

Vetter called certain muscles in the branchial arches of *Acipenser* "interarcuales ventrales," and gave the name of "obliqui ventrales" to exactly homologous muscles in Teleostei. One or other term is superfluous, and following Allis, that of "obliqui ventrales" is used in this paper for the Teleostomi generally. The name "interarcuales ventrales"

is used for the longitudinal muscles passing between the ventral ends of the branchial bars.

The foremost "interarcualis ventralis" passing from the first branchial to the hyoid bar has received many names, e. g. ceratoidien latéral on petit ceratoidien (Cuvier), intercornalis (Owen), cerato-hyoideus internus (Fürbringer, Miss Platt, Drüner), cerato-hyoideus (Osawa), hyoideus lateralis (Bendz), kerato-hyoideus (Ellenberger and Baum), inter-hyoideus (Dubois), kerato-thyro-hyoideus (Jaquet). The name "branchio-hyoideus" was applied to this muscle in *Sphenodon* (loc. cit.), and is employed in the case of other animals, when it occurs, in this paper.

In *Scyllium*, van Wijhe described the coraco-branchialis and coraco-mandibularis as developing from the walls of the obliterated anterior prolongation of the pericardium, and from "Nebenzweige," given off from the "Unterenden der Wände der Visceralbogenhöhlen."

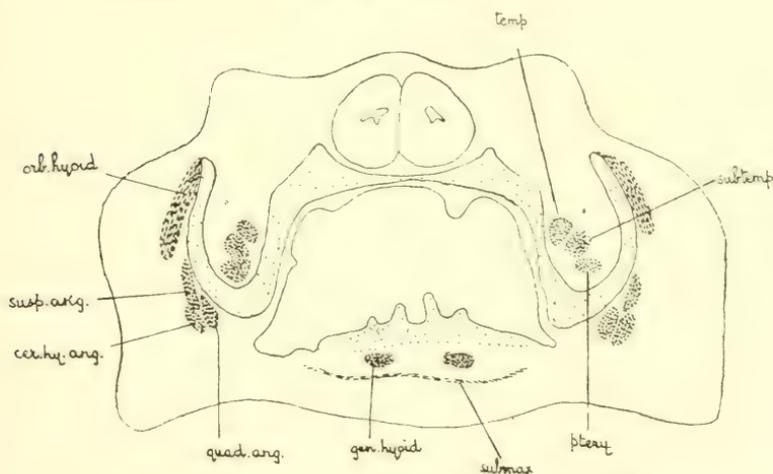
It will be shown later (p. 268) that the coraco-mandibularis and coraco-hyoideus are developed from the fourth to eighth trunk-miotomes. The five branchial miotomes form at first epithelium-lined cavities continuous below with the cephalic cœlom (Text-fig. 2). They separate from the cephalic cœlom in 14 mm. embryos, and their cavities become obliterated (Text-fig. 4). In 17 mm. embryos the lower ends of the branchial miotomes grow backwards, and become cut off from the remainder of the miotomes, forming coraco-branchiales (Text-figs. 8, 10). The (now) ventral ends of the miotomes grow downwards outside the Anlagen of the coraco-branchiales forming the ventral ends of the constrictor (Text-fig. 13). The upper ends of the miotomes, in embryos between the lengths of 17 and 20 mm., increase in antero-posterior extent (Text-fig. 14), and, fusing together, extend backward as the trapezius¹ to the shoulder-girdle. Below

¹ It is stated by van Wijhe that the seventh, eighth and ninth miotomes give rise to "Vom Schädel zum Schultergürtel ziehende Muskeln nebst dem vordersten Theile des sterno-hyoideus," but the trapezius is not specifically mentioned by him.

the Anlagen of the trapezius each branchial myotome forms a transversely broad plate in the branchial septum (Text-fig. 9). The part internal to the branchial bar forms the adductor (Text-fig. 15); the part external to the bar forms—next the bar, dorsally the arcualis dorsalis¹ (Text-fig. 14), and below that the interbranchial, whilst the external edge forms the constrictor superficialis.

In *Acipenser* the lower ends of the branchial myotomes

TEXT-FIG. 59.



59.

Rana. larva 12 mm., transverse section.

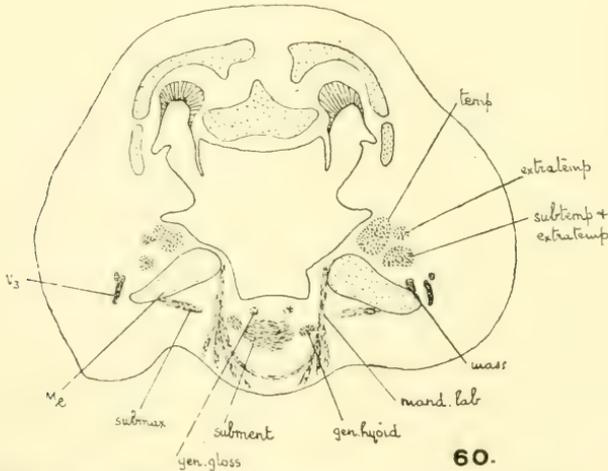
separate from the cephalic cœlom, and in $8\frac{1}{2}$ mm. embryos (Text-fig. 22) grow downwards, except in the fourth branchial segment,² forming the Anlagen of the coraco-branchiales, and also downwards and inwards dorsal to the cephalic cœlom, forming the lower parts of the obliqui ventrales. The ventral end of the lower part of obliquus ventralis I becomes attached

¹ Employing Fürbringer's terminology (vide p. 266).

² In the adult forms examined by Vetter the coraco-branchialis IV was absent, and it was not developed in the embryos examined. According to Fürbringer it is present.

to the ceratohyal, and those of the obliqui ventrales II and III to the corresponding hypobranchials, whilst the IVth and Vth meet their fellows in the median line, in the case of the IVth also becoming attached to the basibranchial. The parts of the first three branchial myotomes above the Anlagen of the coraco-branchiales form adductors internal to the branchial bars, and the upper portions of the obliqui ventrales external to the branchial bars; in the case of the fourth myotome only the upper portion of obliquus ventralis

TEXT-FIG. 60.



Rana, larva with hind legs moderately developed, transverse section.

IV. The uppermost portions of the first four branchial myotomes form levatores arcuum branchialium; in $8\frac{1}{2}$ mm. embryos the first is attached to the auditory capsule, the second, third, and fourth lie outside the trunk myotomes (Text-fig. 22); in 11 mm. embryos the upper ends of the third and fourth have also shifted to the auditory capsule with additional attachments to the second pharyngo-branchial, and the upper end of the second has become attached to the second pharyngo-branchial; all four are inserted to the corresponding epibranchials. The trapezius is given off from the fourth

levator in $8\frac{1}{2}$ mm. embryos (Text-fig. 22), and grows back to the shoulder-girdle; in 11 mm. embryos its fore part has disappeared, and the anterior end of the part remaining is attached to the skin; in the adult it is absent (Vetter). The portion of the fifth branchial myotome above the coraco-branchialis V forms a muscle attached above the fourth epibranchial and below to the fifth cerato-branchial—the fifth levator of Vetter.

In *Amia* the lower end of the fifth branchial myotome forms in $8\frac{1}{2}$ mm. embryos (Text-figs. 31, 32) the lateral half of a transversus ventralis V and a coraco-branchialis V, as in *Acipenser*; the lower end of the fourth branchial myotome forms (Text-fig. 30) the lateral half of a transversus ventralis IV and the Anlage of the interarcualis ventralis IV, which grows forward to the third branchial bar. Neither coraco-branchiales¹ nor interarcuales ventrales are formed in the first three myotomes; the lower ends of the myotomes grow downwards and inwards, forming the ventral portions of the obliqui ventrales I, II, and III. The portion of the fourth myotome, next above the Anlagen of the interarcualis ventralis IV and transversus ventralis IV, forms the obliquus ventralis of that arch, which is serially homologous with the dorsal portions of the obliqui ventrales of the first three arches. In 15 mm. embryos the hind end of the interarcualis ventralis IV grows backward to the fifth bar, and in 19 mm. embryos its front portion divides longitudinally into two (Allis), so that there are formed two longitudinal muscles extending from the third bar to the fourth and fifth respectively; both are innervated by the nerve to the fourth arch (Allis).

Allis homologised these longitudinal muscles with the lower portions of the obliqui ventrales of the first three arches, but their development shows that the latter are homo-

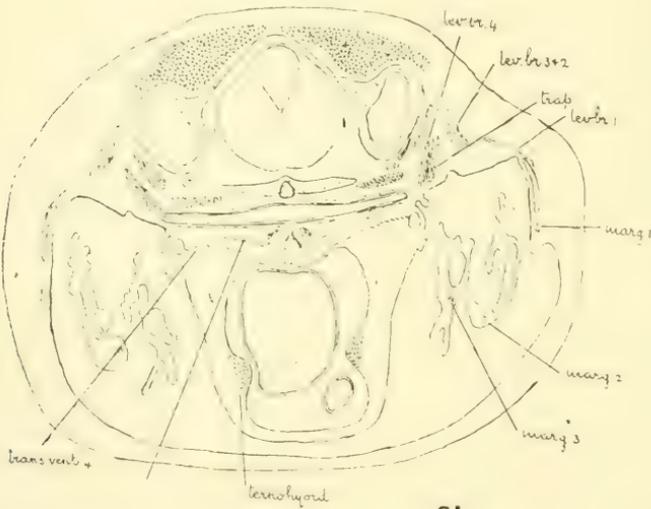
¹ Fürbringer described a coraco-branchialis II, but it is not described by Allis or McMurrich, and was not present in the embryos examined.

logous with the transversi ventrales of the fourth and fifth arches.

The coraco-branchialis V divides, in 14 mm. embryos, into pharyngo-clavicularis internus and externus.

The upper ends of the first branchial myotomes form levatores arcuum branchialium; the first two broaden transversely (Text-fig. 29) and divide into external and internal portions. The first and second externi and third and fourth levatores

TEXT-FIG. 61.



61.

Rana, larva with large hind legs, transverse section.

become attached to the first, second, third, and fourth epibranchials, the first internus to the second pharyngo-branchial, and second internus¹ to the third pharyngo-branchial constituent of the superior pharyngeal bone² of Allis (os pharyngeum superior of v. Wijhe, Pharyngealplatte of Wiedersheim). All take their origin from the auditory capsule. The

¹ Protractor laryngis of Wiedersheim.

² The os pharyngeum superior of *Amia* and *Lepidosteus* (Text-fig. 25) is formed by the union of the pharyngo-branchials of the third and fourth arches—bearing out the theory of v. Wijhe.

trapezius is formed from the fourth levator, and persists; it is the muscle described by Allis as the "fifth externus" levator, "found in 40 mm. fishes as a part of the fourth levator." The portion of the fifth branchial myotome above the coraco-branchialis V does not divide into levator and (dorsal portion of) obliquus ventralis; it forms two muscles—the second obliquus dorsalis and second adductor of Allis, passing from the fourth epi- and cerato-branchial to the fifth cerato-branchial.

The development of the branchial muscles of *Lepidosteus* is similar to that of *Amia*, the only exceptions being (1) the coraco-branchialis V does not divide into pharyngo-clavicularis externus and internus (Text-fig. 25)¹; (2) the interarcualis ventralis IV is not developed.

The differences between the branchial muscles of *Salmo* and *Amia* are that in the former (1) the interarcualis ventralis IV retains the primitive condition of a longitudinal muscle between the fourth and third bars, and does not, as in *Amia*, secondarily extend back to the fifth bar. (2) The third levator arcuum branchialium, as well as the first two, divides into external and internal portions, of which the externus is inserted into the third epibranchial, and the internus into the fourth pharyngo-branchial. (3) The portion of the fifth myotome above the coraco-branchialis V forms one muscle only (obliquus dorsalis of Vetter), passing from the fourth epibranchial to the fifth cerato-branchial. (4) No adductor is formed in the fourth arch.

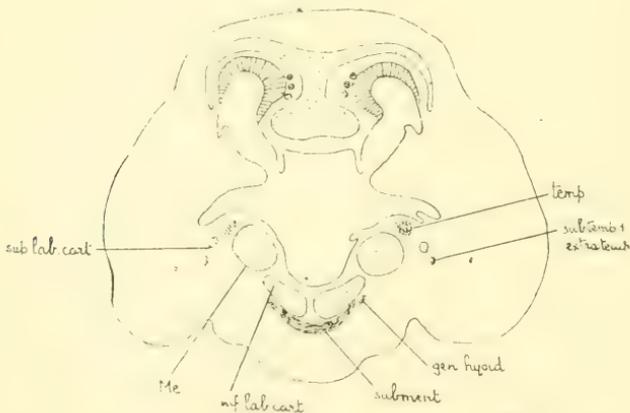
It is noteworthy that the trapezius persists in *Salmo*, as in *Menidia* (Herrick); in *Esox*, *Cyprinus*, and *Perca* it is absent in the adult (Vetter). In some Teleostei there are a greater number of interarcuales ventrales present than in *Salmo*, e. g. in *Cyprinus* an interarcualis ventralis I, and in *Esox* an interarcualis ventralis III are additionally present (Vetter).

The muscles attached to the ventral ends of the branchial bars of *Polypterus*, apparently, are very different in various

¹ According to Fürbringer, "*Lepidosteus* hat kein coraco-branchialis mehr."

species. Fürbringer stated that in *Polypterus*, ? species, there are four coraco-branchiales attached to the four branchial bars. Pollard did not describe these; he stated that in *Polypterus*, ? species, the coraco-hyoideus sends additionally a long tendon to the lower end of the first cerato-branchial, and also that there is a muscle belonging to the system of the coraco-arcuales, which, arising from the fourth, i. e. last cerato-branchial, passes horizontally forwards and affixes itself to the lower ends of the second and first cerato-branchiales. It is apparently supplied by the united first and second spinal

TEXT-FIG. 62.



62.

Rana, larva with fully formed hind legs, transverse section.

nerves. There is also "a flat muscle of small size, which takes its origin from the last cerato-branchial. It loses itself in the skin near the anterior edge of the dermal clavicle. Its innervation was not traced."

In *Polypterus senegalus* (larvæ $7\frac{1}{2}$ to $9\frac{1}{2}$ cm. long) there is a pharyngo-clavicularis externus and internus (= coraco-branchialis IV) attached anteriorly to the fourth cerato-branchial, and passing downwards through the coraco-hyoideus to the shoulder-girdle (Text-fig. 37). In front of this is a longitudinal muscle passing from the fourth to the second cerato-branchial, and innervated by the nerve to the

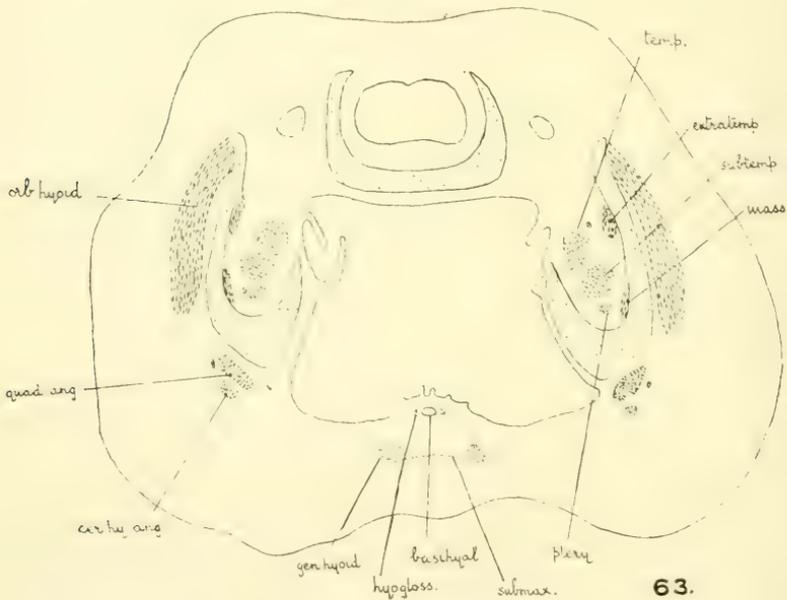
third arch; this, on comparison with the *interarcualis ventralis* IV of *Amia*, is probably an *interarcualis ventralis* III, which has additionally extended back to the fourth bar. In front of this are the *interarcuales ventrales* II and I, the former passing from the second to the first cerato-branchial, and the latter from the cerato-branchial to the ceratohyal (Text-figs. 35 and 36).

In *Polypterus senegalus* there are *transversi ventrales* III and IV (Text-figs. 36, 37); the median edges of the former are attached to the basibranchial; the latter in its anterior part forms a transverse muscle, and in its posterior part enters into relation with the *rima glottidis*, forming the dilatator of Wiedersheim. He called the fore part of the muscle *M. adductor arc. branch.*, but adductors, in the sense of Vetter, are not present in *Polypterus senegalus*, and the whole muscle is a *transversus ventralis* of the fourth arch.

In *Polypterus*, ? species, Pollard described four "*interarcuales ventrales*" (i. e. in the terminology of this paper, "*obliqui ventrales*"), one to each branchial bar. In *Polypterus senegalus* these muscles are not present in the first and second branchial segments; in the third and fourth segments their dorsal portions are present in the form of very minute muscles, the lower ends of which are attached to the *cerato-branchiales* (Text-fig. 37). Pollard described four *levatores arcuum branchialium* inserted into the upper ends of the *cerato-branchials*. In *Polypterus senegalus* the first is inserted into the first pharyngo- and epi-branchial, the second and third into the respective pharyngo-branchials, and the fourth, which has an additional head from the third pharyngo-branchial, into the fourth *cerato-branchial*. According to Pollard, there is no *trapezius* corresponding to that of *Selachians*, but he mentions that a muscular slip—presumably of the fourth levator—continues on beyond the last (fourth) *cerato-branchial*, and is inserted into the skin-ligaments in front of the shoulder-girdle. In *Polypterus senegalus* there is a *trapezius* arising in common with the fourth levator and passing back to the shoulder-girdle (Text-fig. 37).

The development of the branchial muscles of *Ceratodus*, as given by Greil, is summarised above (pp. 175 and 176). In the specimens examined the lower ends of the branchial myotomes separate from the lateral wall of the cephalic cœlom in stage 42 (Text-figs. 42, 43). In stage 46 the lower end of the first branchial myotome grows forward to the hypohyal (Text-fig. 44), forming the interarcualis ventralis I¹ s. branchio-hyoideus;

TEXT-FIG. 63.



Alytes, larva 12 mm., transverse section.

in the second, third, and fifth branchial segments the lower ends of the myotomes grow downwards, forming coraco-branchiales II (Text-fig. 47), III and V, and also downwards and inwards, forming the (lateral halves of the) transversi ventrales

¹ Cerato-hyoideus internus of Fürbringer; cerato-hyoideus of Greil; M. grand abducteur du premier arc branchial of Jaquet, who states that the hind end of the muscle is attached to the first and second branchial bars.

II,¹ III,¹ and V²; in the fourth branchial segment only a coraco-branchialis IV is formed. This condition—of an interarcualis ventralis I and coraco-branchiales II, III, IV, and V—persists till stage 63; at a later stage the hinder end of the interarcualis ventralis I grows backwards, forming, in the specimen examined, a longitudinal muscle, which is attached posteriorly to the fifth bar, and also a coraco-branchialis I. From this and the descriptions given of the adult by M. Fürbringer and by Jaquet, it may be inferred that the hind end of the interarcualis ventralis I always grows back, forming a coraco-branchialis I, and may or may not also grow back to a more posterior branchial bar.

The portions of the branchial myotomes immediately above the interarcualis ventralis I and coraco-branchiales II, III, and IV form Mm. marginales.³ No adductors are developed. The upper ends of the first four branchial myotomes and the whole of the fifth branchial myotome above the coraco-branchialis V (no fifth M. marginalis being developed) form levatores arcuum branchialium⁴ (Text-fig. 48).

The trapezius⁵ is proliferated from the outer side of the fifth levator in stage 48 (Text-fig. 48).

In *Necturus* (Miss Platt), in embryos of $12\frac{1}{2}$ mm. there is an outgrowth from the ventral part of the glosso-pharyngeal muscle—the beginning of the cerato-hyoideus internus; and there are three constrictors arcuum, the first growing forwards from the mesothelium of the first vagus arch where this joins

¹ M. chiasmique of Jaquet; second and third Mm. interbranchiales of Greil.

² The posterior margin of the transversus ventralis V in stage 63 underlies the lung.

³ M. branchialis of Jaquet; first, second, third, and fourth Mm. interbranchiales of K. Fürbringer; fourth and fifth Mm. interbranchiales of Greil. In the adult, according to Jaquet, these muscles are attached dorsally to the upper ends of the branchial bars, according to K. Fürbringer to the skull.

⁴ Cranio-branchiales of Jaquet; levatores arcuum branchialium of Greil.

⁵ M. scapulo-branchialis of Jaquet; levator scapulæ of Greil.

the wall of the pericardium, the second and third arising as a single muscle from the wall of the pericardium in the region where the mesothelium of the second vagus arch unites with the pericardial wall. Above these muscles are found the gill-muscles, and dorsally the three levatores arcuum.

This would mean, according to the theory which was suggested above, that the interarcuales ventrales I, II, and III are formed from the ventral ends of the first, second, and third branchial myotomes, the Anlagen of the gill-muscles above these, and the three levatores from the uppermost portions.

There are three other branchial muscles in *Necturus* which were not mentioned by Miss Platt—the transversus ventralis IV,¹ fourth² levator arcuum, and the trapezius.² In 12 mm. embryos there is present a fourth branchial myotome serially homologous with the first, second, and third (Text-figs. 51, 52). In 13 mm. embryos this has separated from the cephalic cœlom and divided into a fourth levator and lateral half of a transversus ventralis IV (Text-fig. 53); in 14½ mm. embryos the lower half of the transversus ventralis IV has spread inwards dorsal to the cephalic cœlom and below the developing larynx to meet its fellow in the middle line (Text-fig. 56).³ No interarcualis ventralis IV is developed. Transversi ventrales are not developed in the first three arches.³ The trapezius is proliferated from the outer surface of the fourth levator in 16 mm. embryos.

In *Triton cristatus* the events are similar; an interarcualis ventralis IV is developed, in correlation with the formation of the fourth branchial bar. The interarcuales ventrales II, III, and IV become divided into the muscles called sub-

¹ The fourth pharyngo-branchialis of Wilder; the hyo-pharyngeus of Göppert.

² The fourth levator and trapezius were described by Mivart; the latter, in the terminology of Fürbringer, is a dorso-scapularis.

³ This confirms the opinion of Göppert that his hyopharyngeus is not formed by fusion of transversi ventrales III and IV, but is only a transversus ventralis IV.

arcuales recti and obliqui by Drüner. The trapezius is a *capiti-dorso-scapularis*; it is formed by proliferation from the outer surface of the fourth levator in $8\frac{1}{2}$ mm. (just hatched) larvæ.

Drüner described in Urodela a first, third, fourth, and fifth *transversus ventralis* (called by him "interbranchial") in the territory of the first, third, fourth, and (an atrophied) fifth branchial arches. He also stated that the first is formed by a secondary attachment of the ventral facial muscles to the first branchial arch—this, which is not a true *transversus*, is described above (p. 215). In *Necturus* and *Triton cristatus* a *transversus ventralis* III is not formed; there is only a IVth. The question whether the laryngei represent a Vth is discussed below. The *transversi ventrales* were included by Drüner in the ventral head muscles, but they are not serially homologous with the *intermandibularis* and *interhyoideus*, which are developed from the walls of the cephalic cœlom in the mandibular and hyoid segments, whereas the *transversi ventrales* are formed by downgrowths of the branchial myotomes dorsal to the cephalic cœlom.

In *Rana temporaria* the ventral ends of the four branchial myotomes separate from the parts above in $6\frac{1}{2}$ mm. embryos, and form the Anlagen of the four *interarcuales ventrales*, and in the second, third, and fourth segments the Anlagen of the *transversi ventrales*. In the first three segments the middle portions of the myotomes form the *Mm. marginales* and the upper parts the levators. The portion of the fourth myotome above the *interarcualis ventralis* forms the fourth levator, no *M. marginalis* being developed.

The median ends of *transversi ventrales* II and III become attached to the posterior surface of a ventral projection of the first basibranchial (second copula), and their lateral edges to the *processus branchialis*. The lateral edges of *transversus ventralis* IV become attached to the fourth ceratobranchial (Text-fig. 61), and their median edges meet in a central raphé, which underlies the fore part of the larynx. There are similar muscles in larvæ of *Alytes*, *Bufo lenti-*

ginosus, and Pelobates.¹ In *Rana*, at the end of the metamorphosis, the *transversi ventrales* II and III disappear, whilst *transversus ventralis* IV persists.

Wilder was of opinion that *transversus ventralis* IV (*constrictor laryngis*, *hyopharyngeus* of Göppert, *Verengerer des Aditus laryngis* of Henle) was a derivative of the intrinsic ring, i. e. of the *sphincter laryngis*. Göppert, on the other hand, thought that it was homologous with the *hyopharyngeus* of *Urodela*, only differing in that it fails in the larva to be attached to the fourth bar. This homology of Göppert is confirmed by the development of the muscles. In the Anuran larvæ examined the muscle was attached to the fourth bar.

The *Anlagen* of the *interarcuales ventrales* develop into longitudinal muscles, each extending from the bar of its segment of origin to the next anterior one. In 9 mm. larvæ the *interarcualis ventralis* I s. *branchio-hyoideus* divides longitudinally into two parts, one of which connects the first branchial bar to the ceratohyal, the other forms with the *interarcualis ventralis* II a muscle extending from the second branchial bar to the ceratohyal. A similar development of the *interarcualis ventralis* I takes place in *Bufo lentiginosus*, *Alytes*, and *Pelobates*.²

The *Mm. marginales* of *Alytes*, *Bufo*, *Rana*, and *Pelobates* (vide Schultze), run along the external edges of the corresponding branchial bars; their dorsal ends are attached to the external surfaces of the upper ends (below the insertions of the levators) of their respective bars. The ventral end of the first is attached to the second bar—to the *processus branchialis* of Schultze, which is formed from the second bar, the ventral

¹ The *transversi ventrales* II and III are collectively termed "*basihyobrachialis*" by Schultze, in *Pelobates*.

² Schultze did not describe the muscle passing from the first branchial bar to the ceratohyal in older larvæ of *Pelobates*, but it persists up to the stage of 30 mm. The *interarcuales* II and III he collectively terms the "*interbranchial*"; and the muscle passing from the second bar to the ceratohyal the "*cerato-hyo-brachialis*."

ends of the second and third to the third bar, just behind its junction with the processus branchialis. In *Rana*, at the end of metamorphosis, the cerato-branchial portions of the branchial bars disappear and the *Mm. marginales* also. The upper end of the first levator becomes attached in part to the palato-pterygoid bar and in part to the periotic capsule, the second, third, and fourth to the periotic capsule only (Text-fig. 61). This is also the case in *Alytes*, *Bufo lentiginosus*, and *Pelobates*.¹

According to Wilder, in *Rana clamitans* the fourth levator is formed during metamorphosis by division of the dorso-laryngis into the fourth levator, and dilatator laryngis; and this is stated to hold generally in *Anura*. In *Rana temporaria*, however, the fourth levator is formed in 7 mm. embryos, and the dorso-laryngeus not until $7\frac{1}{2}$ mm., and the two muscles have no genetic connection; and both muscles are present in larvæ of *Pelobates*, *Alytes*, and *Bufo lentiginosus*, of 10, $12\frac{1}{2}$, and 10 mm. respectively, i. e. long before metamorphosis.

In *Rana*, late in metamorphosis, the partial origin of the first levator from the palato-pterygoid bar is given up, and, on the atrophy of the cerato-branchials and *Mm. marginales*, all four levators extend downwards, and their lower ends become attached to the body and processus posterior medius of the hyoid bar.

In 12 mm. larvæ of *Rana* a downgrowth of the lower end of the fourth levator takes place, forming the diaphragmato-branchialis lateralis² (of Schultze). Its upper end becomes attached to the fourth bar, its lower end to the diaphragm. It is innervated by the Xth. It is also formed in *Alytes*,

¹ Schultze, in older larvæ of *Pelobates*, described all four levatores as arising from the palato-quadrate bar.

² Schultze gave the name "diaphragmato-branchialis medialis" to the muscle called "sterno-hyoid" in this paper. He did not describe the innervation or development of the larval muscles of *Pelobates*; his names are purely descriptive, and do not imply that he thought that the two muscles "diaphragmato-branchialis" and "medialis" have any genetic connection.

Bufo lentiginosus, and *Pelobates*, in larvæ of about the same length. In *Rana* it disappears late in metamorphosis. The trapezius (*capiti-scapularis*, of Fürbringer; *cucullaris*, of Ecker and Gaupp) is formed early in metamorphosis from cells proliferated from the outer surface of the fourth levator (Text-fig. 61).

In 6 mm. embryos of *Chrysemys marginata* there are four branchial myotomes; in 8 mm. embryos (Text-figs. 66-69) the middle portion of the first is very slender and that of the second has disappeared, and the middle and lower portions of the third and fourth have disappeared; the upper end of the fourth has extended back a little in the neck, the upper end of the third has extended back to that of the fourth, the upper end of the second is a separate structure, and the upper end of the first is still connected with the rest of the myotome. In 12 mm. embryos the dorsal ends of the first and second have each grown backwards into the next segment, and there is thus formed a long column of cells which has grown still further backwards into the neck, forming a trapezius—the *capiti-plastralis* of Fürbringer; the middle portion of the first and the lower end of the second myotomes¹ have disappeared, whilst the lower end of the first forms the *interarcualis ventralis I*, which, extending from the first branchial bar to Meckel's cartilage, is the *branchio-mandibularis*.

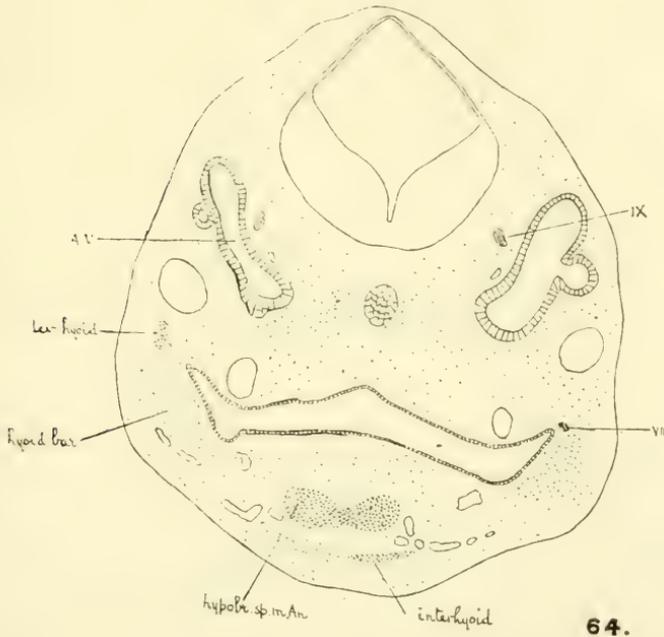
In *Lacerta agilis* the dorsal edge of the primitive trapezius extends upwards outside the trunk myotomes of the neck (Text-figs. 70, 71), and in 20 mm. embryos it has divided into dorsal and ventral portions, the *capiti-dorso-clavicularis* and *capiti-cleido-episternalis* of Fürbringer. The former is innervated solely by spinal nerves, the latter by the *accessorius vagi*. Fürbringer concluded from this innervation that the *capiti-dorso-clavicularis* is a new formation, and that

¹ The curious persistence for a time of the lower end of the second branchial myotome, after disappearance of the middle portion of the myotome, is in favour of the idea (*loc. cit.*) that ancestors of the Sauropsida may have possessed an *interarcualis ventralis II*, passing from the second to the first branchial bar.

the whole muscle is a complex of muscle-metameres,¹ but this inference is not borne out by study of its development.

In *Gallus* the upper ends of the first and second branchial myotomes separate from the parts beneath on the fourth day (Text-figs. 72, 73). They fuse together and extend backwards in the neck (Text-fig. 74) forming the trapezius

TEXT-FIG. 64.



Text-figs. 64-69.—*Chrysemys*, embryo 8 mm. Text-fig. 64 is the most anterior; Text-figs. 64 and 65 are through the hyoid segment, Text-fig. 66 through the first branchial, Text-fig. 67 through the second branchial, Text-fig. 68 through the third branchial, and Text-fig. 69 through the fourth branchial segment.

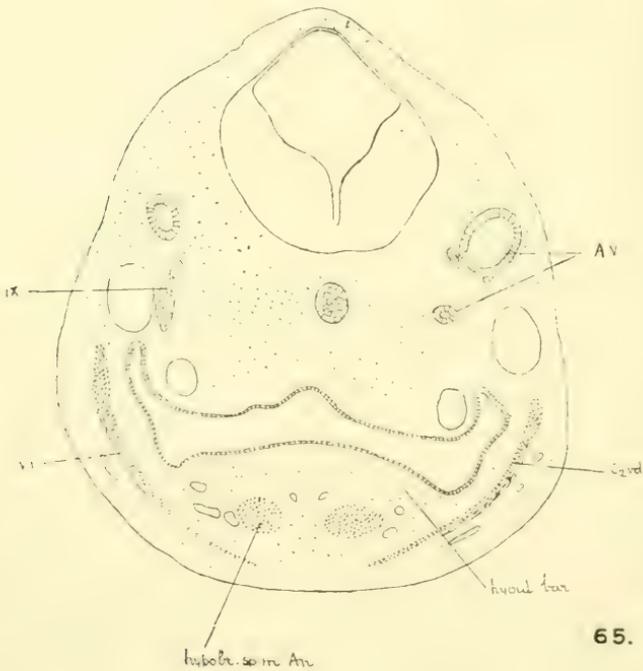
(*Cucullaris* of Fürbringer). The lower end of the first branchial myotome forms the *interarcualis ventralis I s.*

¹ "So entstand ein neugebildeter, dem ursprünglichen *M. cucullaris* nur in seinen vordersten Theile homologer, in seinen Hauptmasse aber bloß initiatorisch-homodynamer oder parhomologer Muskel."

branchio-mandibularis. The middle portion of the first and the whole of the second (below the Aulage of the trapezius) branchial myotomes disappear.

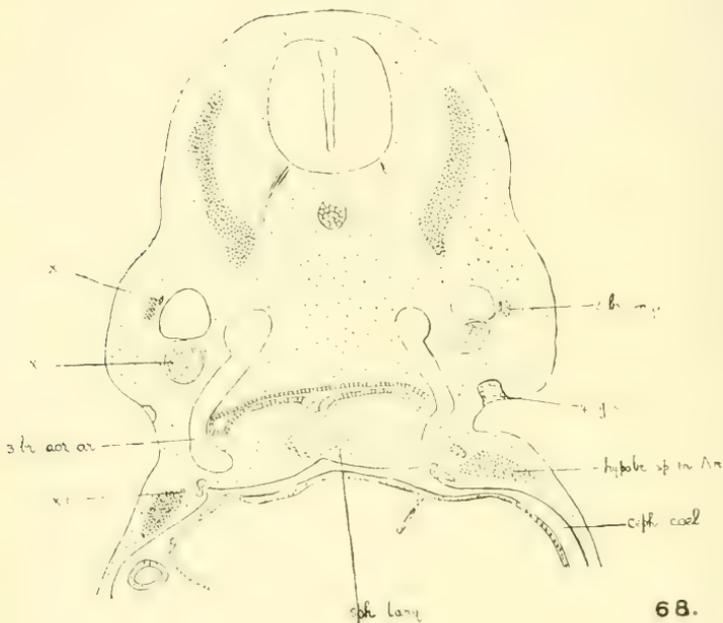
In 5 mm. embryos of the rabbit the upper ends of the first, second, and third branchial myotomes separate from the parts below, the upper end of the third grows backward in the neck, and the upper end of the second backward to join that

TEXT-FIG. 65.



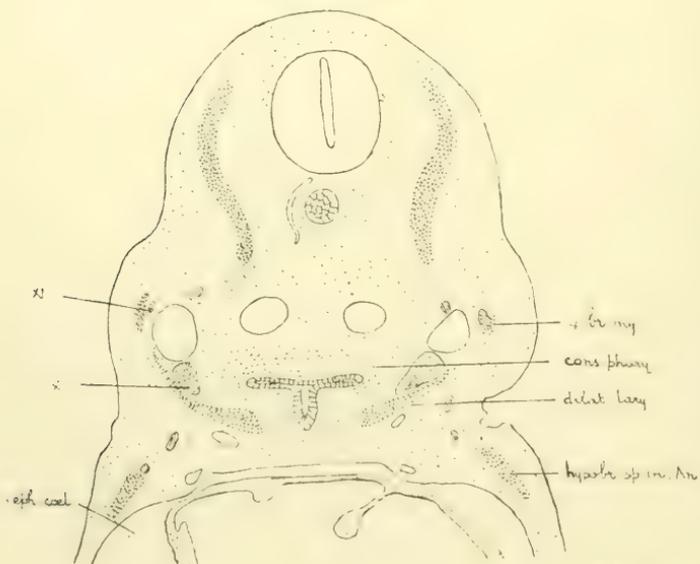
of the third (Text-figs. 85, 86, 87); in 6 mm. embryos the upper end of the first has grown back to that of the second. The hind end of the primitive trapezius, thus formed from the upper ends of all three branchial myotomes, reaches the anterior limb area in 7 mm. embryos (Text-fig. 88); its dorsal edge extends upwards in $7\frac{1}{2}$ mm. embryos (Text-fig. 90), and in 9 mm. embryos it has divided into the trapezius and

TEXT-FIG. 68.



68.

TEXT-FIG. 69.



69.

sterno-mastoid of the adult. The portions of the branchial myotomes below the Anlagen of the trapezius have disappeared in 7 mm. embryos.

In the pig the lower end of the first branchial myotome persists (Text-fig. 98), and forms the interarcualis ventralis I s. branchio-hyoideus (Text-fig. 99). This muscle is also constantly present in the dog,¹ and in Monotremes,² and is occasionally present in man.³ In Monotremes there is also an interarcualis ventralis III passing from the third to the second branchial arch.⁴

ON THE HOMOLOGIES BETWEEN THE BRANCHIAL MUSCLES OF VARIOUS VERTEBRATES.

(I) The Hypobranchial Cranial Muscles.—The lower ends of the branchial myotomes develop into longitudinal muscles—interarcuales ventrales, and coraco branchiales—in Selachii, Teleostomi, Dipnoi, Amphibia, and Mammalia. Their innervation varies. Those which remain in their segment of origin, or nearly so, extending forward to the ventral end of the next anterior branchial bar or hyoid bar (or additionally and subsequently to the next posterior bar, in *Amia*, and probably *Polypterus senegalus*) are innervated by the corresponding branchial nerve, IXth or branch of Xth, or by this and the next anterior branch. This holds for the interarcuales ventrales of *Amia*, *Polypterus senegalus*, *Salmo*, *Ceratodus*, Amphibia, and Mammalia. The interarcualis ventralis I of Sauropsida (branchio-hyoideus or branchio-mandibularis) is an exception to the rule; it is innervated by the XIIth.

A coraco-branchialis, or pharyngo-clavicularis externus and internus, developed by backward growth from the last branchial myotome, i. e. fourth in *Polypterus senegalus*, fifth

¹ Kerato-hyoideus of Ellenberger and Baum.

² Interhyoideus of Dubois.

³ Kerato-thyro-hyoideus of Shattuck.

⁴ Interthyroideus of Dubois.

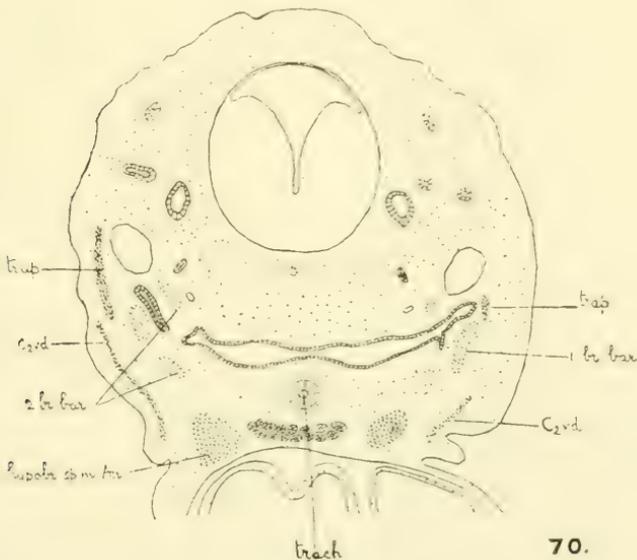
in *Amia*, *Salmo*, *Esox*, *Menidia*, may either retain its original branchial innervation from the Xth, e. g. *Amia* (Allis), *Esox* (Vetter), *Menidia* (Herrick), *Lepidosteus*, *Polypterus senegalus*, or be innervated by spino-occipital nerves, e. g. *Amieurus* (Wright), *Salmo* (Harrison). When coraco-branchiales are developed from all the branchial myotomes, they are innervated by the spino-occipital nerves, e. g. *Selachii* (Vetter, Fürbringer), *Acipenser* (Vetter), *Polypterus* ? species (Fürbringer), *Ceratodus* (Fürbringer).

The coraco-branchiales muscles have been generally classed with the hypobranchial spinal muscles, but investigation of developmental stages shows that the ventral ends of branchial myotomes may form longitudinal muscles, which either grow forwards, forming interarcuales ventrales, or backwards, forming coraco-branchiales, but not in both directions. (There are two, probably three, exceptions to the above rule; in *Amia*, at a late stage of development, the hind end of the interarcualis ventralis IV grows backward to the fifth bar; the innervation in *Polypterus senegalus* suggests that the hind end of the interarcualis ventralis III similarly grows back to the fourth bar; and in *Ceratodus* the hind end of the interarcualis ventralis I, at a late stage of development, grows back, forming the coraco-branchialis I, and also, at least in some cases, to a more posterior bar.) The first condition, that of interarcuales ventrales, is the primary one, as shown by the correspondence of cranial nerve innervation, with segment of origin. The second condition, that of coraco-branchiales, is a secondary one, in which a change of function to one very similar to that of the coraco-hyoideus is correlated, though in varying degree, with a change of innervation to one by the spino-occipital nerves.

An approximation to what was, probably, the primitive condition, is seen in Amphibia. This was a series of interarcuales ventrales, each extending from the bar of its segment of origin to the next anterior one. The hyo-maxillaris, in the hyoid segment, is serially homologous with

the branchial interarcuales ventrales. There is no homologue in the mandibular segment. These longitudinal muscles possibly date from a period where there were no median cartilages connecting the ventral ends of the hyoid and branchial bars, and formed a rectus system of the head serially homologous with that of the body, though now covered over by the latter, owing to its extension forwards into the head.

TEXT-FIG. 70.



Lacerta, embryo 8 mm., transverse section. The right side of the section is a little anterior to the left.

(1) *Transversi Ventrales*, *Mm. Marginales*, and *Obliqui Ventrales*.—In *Scyllium*, *Acanthias*, *Sauropsida*, rabbit, and pig, the hypobranchial cranial muscles are the most ventral ones formed from the branchial myotomes; no *transversi ventrales* are formed. This is also the case in the first branchial segment of *Anuran* tadpoles, the first, second, and third of the *Necturus* and *Triton*, the first and fourth of *Ceratodus*, the first and second of *Polypterus senegalus*.

But in the second, third, and fourth branchial segments of Anuran tadpoles, the fourth of *Necturus* and *Triton*, the second, third, and fifth of *Ceratodus*, and in those segments of Teleostomi in which hypobranchial cranial muscles are formed, the lower ends of the branchial myotomes also grow downwards and inwards above the cephalic cœlom, towards, or to the middle line forming the (lateral halves of the) *transversi ventrales*, or their homologues, the lower portions of the *obliqui ventrales*. In branchial segments of Teleostomi, where hypobranchial cranial muscles are not formed, i. e. first four of *Lepidosteus*, first three of *Amia* and *Salmo*, fourth of *Acipenser*, there is a similar downward and inward growth of the ventral ends of the branchial myotomes, to form the lower portions of the *obliqui ventrales*.

The hinder part of the *transversus ventralis* IV of *Polypterus* and *Amphibia*, and of the *transversus ventralis* V of *Ceratodus*, comes into intimate relations with the ventral larynx, though in varying ways, underlying it in *Amphibia* and *Ceratodus*, forming a dilatator in *Polypterus*.

The portions of the branchial myotomes next above the Anlagen of the hypobranchial cranial muscles form the Anlagen of the muscles of the external gills in the first three segments of *Necturus* and *Triton*, and the *Mm. marginales* in the first three segments of Anuran larvæ and the first four segments of *Ceratodus*. Homologous Anlagen form the upper portions of the *obliqui ventrales* in Teleostoman embryos—of the first four segments of *Acipenser*, *Lepidosteus*, *Amia*, *Salmo*, *Polypterus* (Pollard), and of the third and fourth segments of *Polypterus senegalus*; these may or may not unite with the lower portions. In some segments of Teleostomi, i. e. first three of *Acipenser*, fourth of *Lepidosteus*, *Amia*, and *Salmo*, adductors are formed from portions of the myotomes lying internal to the branchial bars; they are not developed in *Polypterus*.

In *Scyllium* the portions of the branchial myotomes next above the Anlagen of the coraco-branchiales form adductors internal to the branchial bars, and the superficial con-

strictors, interbranchials, and arcuales dorsales external to them. The lower ends of the superficial constrictors extend downwards external to the coraco-branchiales, but such downgrowths do not appear to be homologous with the transversi ventrales or inferior portions of the obliqui ventrales of Teleostomi, Ceratodus, and Amphibia.

In Sauropsida, rabbit, and pig embryos the portions of the branchial myotomes next above the Anlagen of the hypo-branchial cranial muscles, and also the lower ends where these Anlagen are not formed, atrophy.

Levatores arcuum branchialium are developed from the upper ends of the branchial myotomes in Teleostomi, Ceratodus, and Amphibia, but are not developed in Scyllium, Sauropsida, rabbit, and pig. The method of development of the trapezius—apparently a homologous muscle throughout these vertebrate groups—is intimately related to these differences. It is developed in Teleostomi and Amphibia from the fourth, in Ceratodus from the fifth, levator, i. e. from the penultimate or ultimate levator¹; whereas in Scyllium, Chrysemys, Gallus, and rabbit, it is formed from the upper ends of the branchial myotomes—five in Scyllium, four in Chrysemys, two in Gallus, and three in the rabbit.

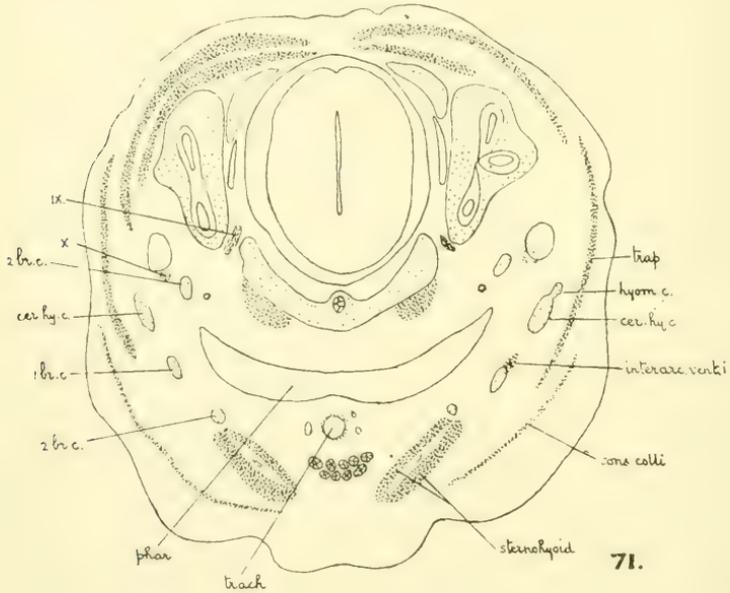
In view of the facts that in Scyllium the subspinalis and interbasales, developed from trunk-myotomes, are attached to the pharyngo-branchials, and that the trapezius is innervated only by the XIth—the most posterior of the vagus roots—even though a constituent from the glossopharyngeal (first branchial) segment takes part in its formation, it is probable that the absence of levatores and associated method of development of the trapezius in Scyllium, Sauropsida, and rabbit are secondary phenomena, and that the primary condition is a series of levatores formed from the uppermost portions of the branchial myotomes. This theory would also afford an explanation of the curious fact that whereas the

¹ In Teleostoman embryos the trapezius is developed from the upper edge of the levator, in Ceratodus and Amphibia from its external surface.

general development of the myotomes takes place from before backwards, the separation of the upper ends of the branchial myotomes, their backward growth, and fusion to form a trapezius, in *Scyllium*, *Chrysemys*, *Gallus*, and *Lepus*, take place from behind forwards—the process beginning in the last branchial myotome.

Adductors of the branchial bars are formed in *Scyllium* and in certain segments of some Teleostomi, on the inner

TEXT-FIG. 71.



Lacerta, embryo 12 mm., transverse section.

side of the branchial bars. The observations of Balfour¹ showed that the primary situation of the muscles is one external to the bars, so that the non-development of adductors in *Amphibia*, *Ceratodus*, and *Polypterus* would appear to represent a primitive condition.

It may be added that the adductors of the branchial bars are not serially homologous with the adductor mandibulæ, which is formed external to the mandibular arch.

¹ 'Comparative Embryology,' vol. ii, p. 471.

The simplest and probably primary condition of the muscles developed between the levatores above and the hypobranchial cranial muscles below is a series of *Mm. marginales*, as found in Anuran larvæ and *Ceratodus*. In Teleostomi these may unite with the (lateral halves of the) *transversi ventrales*. In *Scyllium* they undergo a quite special development, which is not found in any other group.

The above comparisons suggest that the probable primitive condition of each of the branchial myotomes was, from above downwards, a levator, a *M. marginales*, an *interarcualis ventrales*, and (the lateral half of a) *transversus ventralis*.

Œsophageal, Pharyngeal, and Laryngeal Muscles.—The term “pharynx” is employed by writers in two senses, either restricted in meaning to the branchial region of the alimentary canal, or including this and the next succeeding portion. In this paper it is used in the first sense.

The œsophagus is surrounded by a circular muscle, the constrictor, which is derived from cells given off from the splanchnic layer of the cœlomic epithelium.

No circular muscles are present in the branchial region of *Selachii*, *Acipenser*, and *Ceratodus*, but are present, in the form of *transversi dorsales*, in Teleostomi (*Vetter*), *Amia* (*Allis*), and *Lepidosteus* (*Wiedersheim*), and are formed by the constrictor of the œsophagus extending forwards, dorsally, into the branchial region. In *Polypterus senegalus* the fore part of the œsophageal constrictor slightly overlaps the branchial region dorsally, but the transversely directed fibres are not attached to any branchial bar. In *Lepidosteus* and *Amia*¹ the conditions are complicated by the presence of a dorsal larynx. The dorsal larynx of *Lepidosteus* is formed, in 8 mm. embryos, as a solid median upgrowth from the then solid œsophagus just behind the branchial region. The œsophageal constrictor (constrictor pharyngis of *Wiedersheim*) is formed from cells

¹ The adult condition of the dorsal larynx and its musculature has been fully described by *Wiedersheim*.

given off from the cœlomic epithelium; it spreads upwards round the œsophagus and dorsal larynx, forming the constrictor laryngis, and subsequently, in $9\frac{1}{2}$ mm. embryos, spreads forward to the branchial region and becomes attached to the os pharyngeum superior (of van Wijhe, the Pharyngealplatte of Wiedersheim) forming the transversus dorsalis and first obliquus dorsalis. The dilatator laryngis is formed from the dorsal part of the œsophageal constrictor (Text-fig. 32). The development of the retractor and protractor laryngis is described on pp. 238 and 267.

The development of the dorsal larynx of *Amia* is similar to that of *Lepidosteus*. The forward extension of the œsophageal constrictor begins in $8\frac{1}{2}$ mm. embryos (Text-figs. 30, 31, 32); it forms the transversus anterior and posterior and first obliquus dorsalis (of Allis). In *Salmo fario* the transverse fibres become attached to the fifth cerato-branchial, the fourth pharyngo- and epi-branchial and the third pharyngo-branchial.

The ventral larynx and musculature of *Polypterus* have been described by Wiedersheim, who says that the muscles consist of a dilatator and sphincter glottidis, the latter of which is continuous below with a muscle-sheet surrounding the lungs. As stated above, the dilatator is the transversus ventralis IV. The sphincter glottidis is continuous above with the constrictor of the œsophagus, which is not attached laterally to the trunk muscles.

The development of the ventral larynx of *Ceratodus* has been described by Kellicott, and the adult condition by Wiedersheim. It may be added that the posterior edge of transversus ventralis V underlies the lung, and that, though there is no dorso-laryngeus (Wiedersheim), the lateral edges of the constrictor of the œsophagus are attached to the lower ends of the trunk-myotomes (Text-fig. 49).

It was supposed by Gegenbauer, Wilder, Göppert, and Drüner, that the cartilagine laryngei of Amphibia were branchial bars, and that the laryngeal muscles were derivatives of branchial muscles. Wilder stated that the dorso-

laryngei s. dorso-tracheales were serially homologous with the levatores arcuum branchialium (called by him "dorso-branchiales"), that the laryngei were homologous with the transversi ventrales (called by him "pharyngo-branchiales"), and that these muscles and the laryngeal cartilages belonged to the fifth branchial arch.

This theory of the nature of the laryngeal muscles was formed on the evidence of adult anatomy only, without knowledge of the method of development.

Wiedersheim stated that the fibro-cartilage present in association with the ventral larynx of *Protopterus* might be due to the remains of a sixth arch, "noch plausibler aber erscheint es mir die betreffenden Knorpel auf einen in der Raphé des pharyngealen Constrictors sich abspielenden Chondrificationsprocess, das heisst auf Muskelwirkung zurückzuführen"; also that "a priori steht der Annahme gewiss nichts in der Wege das sich auch bei Amphibien die primitive Cartilago lateralis als eine Sehnenverknorpelung bilden kann ohne das dabei phylogenetische Beziehung zu den Kiemenbogen angenommen werden müssen."

In *Necturus* the larynx is formed as a median ventral pouch of the pharynx in the hinder part of the fourth branchial segment in 13 mm. embryos (Text-figs. 53, 54). The cells which form the constrictor of the œsophagus are budded off from a thickened portion of the splanchnic cœlomic epithelium, and spread upwards round it (Text-fig. 54). A similar production of splanchnic mesoblast from a thickened portion of splanchnic cœlomic epithelium takes place in the fourth branchial segment (Text-fig. 52), and the cells spread upwards round the developing larynx and pharynx. In 14½ mm. embryos this splanchnic mesoderm spreads upwards on either side of the pharynx internal to the fourth levatores arcuum branchialium, and forms the Anlagen of the dorso-laryngei and of the pharyngeal constrictor (Text-fig. 56). The splanchnic mesoderm ventral to the pharynx develops into the laryngei muscles, and, next the larynx, the laryngeal cartilages.

A similar development of the laryngeal muscles takes place in Triton and in Rana.

If the method of development of the laryngeal muscles be compared with that of the branchial muscles it is seen that they are very different. The branchial muscles are developed from the branchial myotomes, the laryngeal muscles are differentiations of an œsophageal or pharyngeal constrictor, which is formed from cells given off by the cœlomic epithelium. In *Polypterus* and *Ceratodus* the larynx is developed just behind the branchial region, in Amphibia in the last, i. e. fourth branchial, segment. In *Polypterus* the œsophageal constrictor is not attached to the trunk-myotomes; in *Ceratodus* it is attached, though no dorso-laryngei are developed; in Amphibia dorso-laryngei are developed. In *Ceratodus* there are no intrinsic laryngeal muscles; in *Polypterus* there is a sphincter glottidis, which remains in continuity with the constrictor of the œsophagus; in Amphibia there are laryngei or a sphincter laryngis, which is not continuous with the constrictor of the pharynx. In *Polypterus* and *Ceratodus* there are no skeletal structures; in Amphibia laryngeal cartilages are developed—later than the branchial bars and ventral, not lateral to the pharynx. These similarities and differences suggest that the conditions found in *Polypterus* and *Ceratodus* preserve stages in the phylogenetic development of the laryngeal structures of Amphibia. They tend to confirm the theory of Wiedersheim. The relations of the transversus ventralis of the last branchial segment (fourth in *Polypterus* and Amphibia, fifth in *Ceratodus*) to the laryngeal structures have been described above.

In 5 mm. embryos of *Chrysemys* the opening of the larynx is in the fourth branchial segment; the cœlomic epithelium in the third and fourth branchial segments is thickened and proliferating mesoblast cells, which surround the pharynx and developing larynx. In 8 mm. embryos the opening of the larynx is in the third and fourth branchial segments; in the fourth a constrictor pharyngis is formed from the splanchnic mesoblast; in the third and fourth the Anlagen of

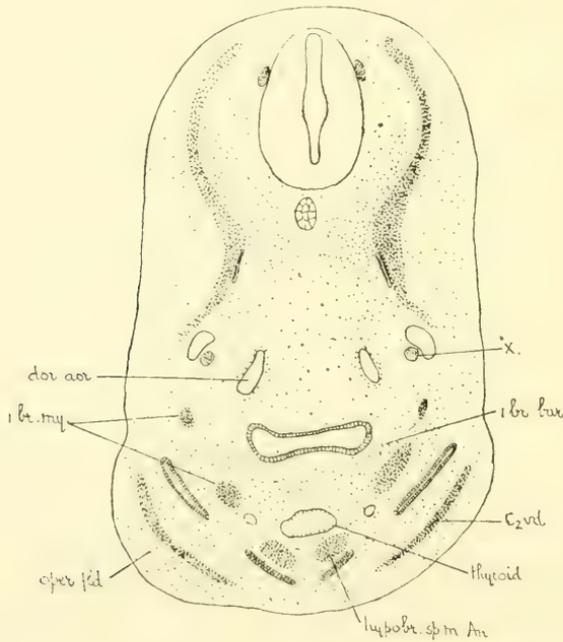
the sphincter laryngis and dilatator laryngis (Text-fig. 69) are formed, the latter having a vertical position and passing up outside the vagus. The dilatator has lost this position in 15 mm. embryos, and its hind end is attached to the cricoid. This supports the theory of Göppert that the dilatator laryngis of Sauropsida is the homologue of the dorso-laryngeus, and the sphincter the homologue of the sphincter and laryngei of Amphibia.

In 3 mm. embryos of the rabbit, cells are proliferated from the coelomic epithelium in the three branchial segments (Text-fig. 80); they spread upwards internal to the branchial aortic arches. In 5 mm. embryos they have spread a little round the pharynx in the second and third branchial segments (Text-figs. 85, 86), but in the first they exist only lateral to it. In 6 mm. embryos the splanchnic mesoblast forms a continuous sheet dorsal to the pharynx in the second and third segments, but in the first it is still only lateral to it. In 7 mm. embryos it is present there also, more probably as a result of forward extension from the second branchial segment than of upward extension in the first segment. This continuous splanchnic mesoderm sheet is continuous behind with that round the œsophagus (Text-fig. 90), which is formed in a similar way.

The stylo-pharyngeus is formed in the splanchnic mesoblast of the first branchial segment; it is first visible in $7\frac{1}{2}$ mm. embryos and gains an attachment to the hyoid bar (Text-fig. 93). The pharyngeal constrictor is also formed in the three branchial segments, though, perhaps, *vide supra*, derived from cells of the second and third segments only. The Anlage of the laryngeal muscles is also visible in $7\frac{1}{2}$ mm. embryos in the third branchial segment. The later development of the laryngeal muscles, in man, has been traced by Frazer, who says that they are developed in the third branchial segment from the ventral part of the layer of cells round the pharynx and larynx; of this laryngeal portion "the formation of the arytenoid seems to convert the dorsal hinder part into the crico-arytenoideus posticus, the upper

part into the arytenoideus, and the ventral part into the thyro-arytenoideus and lateral crico-arytenoid." According to Göppert, the crico-arytenoideus posticus is the homologue of the dorso-laryngeus of Amphibia, the other intrinsic muscles representing the sphincter and laryngei dorsalis and ventralis of Amphibia.

TEXT-FIG. 72.



72.

Text-figs. 72, 73.—Gallus, embryo four days. Text-fig. 72 through the first branchial, Text-fig. 73 through the second branchial segment.

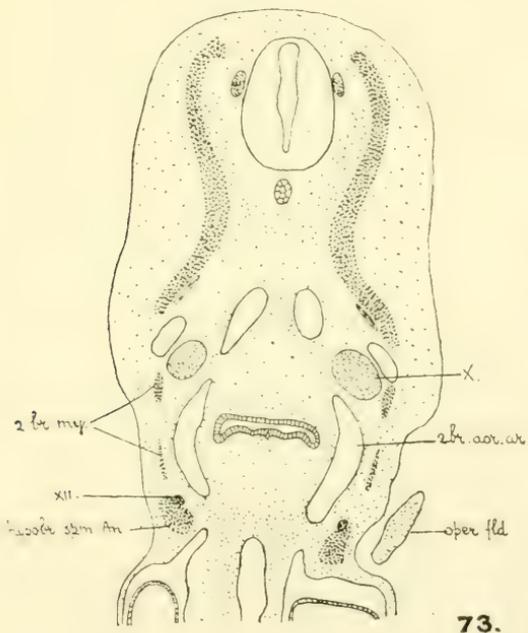
Frazer states that in man the thyroid cartilage is developed solely in the second branchial segment, i. e. it is the second branchial bar only, whereas in *Echidna* (Göppert) a third branchial bar is also formed.

The crico-thyroides, according to Frazer, is developed

from the second branchial pharyngeal constrictor; this confirms the opinion of Fürbringer.

The pharyngeal musculature extends forwards into the mandibular segment in 13 mm. embryos (Text-fig. 94); in 14 mm. embryos the anterior end becomes separated, forming the tensor palati, and in 16 mm. embryos the next succeeding portion forms the levator palati.

TEXT-FIG. 73.



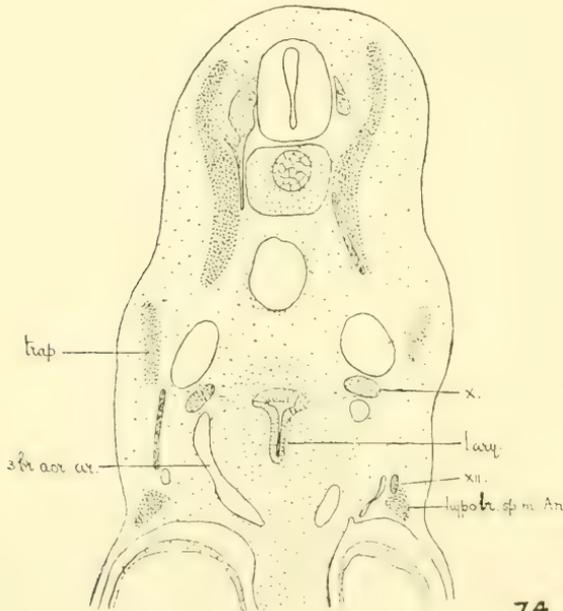
According to Futamura, as stated above, the Anlage of the palatal muscles of man is at first one with that of the tensor tympani, and is derived from the platysma faciei. I have failed in verifying this in the rabbit; the platysma faciei in that animal remains superficial, and the Anlage of the palatal muscles when first visible was quite distinct from that of the tensor tympani, which is cut off from the inner limb of mandibular myotome.

These phenomena afford an explanation of the innervation of the palatal muscles by the XIth medullary.

MUSCLES DERIVED FROM TRUNK-MYOTOMES PASSING TO THE UPPER ENDS OF THE BRANCHIAL BARS.

Vetter described a subspinalis and interarcuales muscles in

TEXT-FIG. 74.



74.

Gallus, embryo $4\frac{1}{2}$ days, transverse section.

Selachii. Fürbringer subsequently showed that the subspinalis and upper interarcuales (which he re-named "interbasales"), were innervated by the spino-occipital nerves, and he classed them together as "epibranchial spinal muscles." The lower interarcuales (second and third interarcuales of Vetter), he called "arcuales dorsales."

Observation of the development of these muscles confirms Fürbringer's theory of their nature. The subspinalis and

¹ Beavor and Horsley.

first, second, and third¹ interbasales of *Scyllium* are developed in 23 mm. embryos from the first, second, third, and fourth spinal myotomes (Text-figs. 12, 13, 14), by growths from their ventral edges internal to the ganglia of the ninth and tenth nerves.

A retractor arcuum branchialium dorsalis is found in some Teleostei (Vetter), e. g. *Perca* and *Cyprinus*, in *Amia* (Allis, Wiedersheim²), and in *Lepidosteus* (Wiedersheim²), but is not present in *Polypterus* (Pollard), or in *Salmo fario*. In *Amia* the muscle, which is inserted into the third infrapharyngo-branchial, arises according to Allis from the third and fourth, according to Wiedersheim from the seventh and eighth, vertebral bodies. In *Lepidosteus* the muscle is inserted into the third infrapharyngo-branchial, and arises from the lateral surface of the third and fourth vertebral bodies (Wiedersheim). The retractor was supposed by Allis and by Wiedersheim to belong to the system of the levatores arcuum branchialium.

The muscle is developed in *Amia* from downgrowths from the lower surface and lower part of the internal surface of the sixth to thirteenth trunk-myotomes in 8½ mm. embryos (Text-fig. 33); these downgrowths form a longitudinal muscle, the anterior end of which grows forward to the third bar. Its development in *Lepidosteus* is similar, taking place, in 12 mm. embryos, from the third to ninth trunk-myotomes.

HYPOBRANCHIAL SPINAL MUSCLES.³

In *Scyllium*, van Wijhe stated that the coraco-hyoideus was developed from ventral prolongations "sowohl des hintersten Kopfmotomes als die der vorderen Rumpfmotome," and that the coraco-branchiales and coraco-mandibularis were formed from the walls of the "unpaaren vorderen

¹ The third interbasalis, developed in the embryo, is not described in the adult by Fürbringer.

² Retractor laryngis of Wiedersheim.

³ This name is used in the sense stated above.

Verlängerung des Pericardiums." Neal, in *Squalus acanthias*, found that the Anlage of the hypoglossus musculature was formed from the fourth to the eighth post-otic myotomes by buds which separate and come to lie ventral to the branchial basket; they do not fuse into a common cell mass, but show their primary metamerism, the bud from the fourth myotome coming to lie between the hyoid and mandibular cartilages and forming "in part the Anlage of the proper tongue muscles," whilst "the four following myotomic buds come to lie between the hyoid and procoracoid."

I find that in *Scyllium* the initial stages of the development of the coraco-mandibularis and coraco-hyoideus are similar to those of *Squalus acanthias*, as stated by Neal. This stage is completed in 16 mm. embryos, and is immediately followed by one (17 mm.), in which the hind end of the primitive genio-hyoideus, which does not become affixed to the hyoid bar, grows backwards along the median edge of the coraco-hyoideus towards the shoulder-girdle—forming the coraco-mandibularis (Text-figs. 11, 12, 13).

The coraco-hyoideus of *Salmo salar* (Harrison) is developed from ventral downgrowths of the second, third, and fourth trunk myotomes, which bend round the pharyngeal region, and form a longitudinal column, the anterior edge of which extends forwards to the hyoid bar. A similar development of the hypobranchial spinal muscles takes place in *Acipenser*, *Lepidosteus*, *Amia*, and *Salmo*, occurring in 8 mm., 8 mm., 7 mm., and 5 mm. embryos respectively, and in each case from the second, third, and fourth trunk myotomes. In *Salmo fario* and in *Lepidosteus* the forward growth of the anterior end reaches the hyoid bar only, so that only a coracohyoid is formed. In *Acipenser* and *Amia* it extends further, to the symphysis, reaching this in 8½ mm. embryos of *Acipenser* (Text-figs. 21, 22), and in 8 mm. embryos in *Amia*. The long column then divides at the level of the hyoid bar into an anterior and a posterior group—the genio-hyoid¹

¹ Branchio-mandibularis of Vetter and Allis.

and coraco-hyoid.¹ The posterior end of the genio-hyoid grows backwards (Text-figs. 28, 29, 30), and becomes attached, in *Acipenser* to the third hypobranchial, and in *Amia* by two tendons, to the second and third hypobranchials and to a median aponeurosis between the two coraco-hyoidei (Y-shaped tendon of Allis).

In *Polypterus*,? species, Pollard described the hypobranchial spinal muscles as consisting of a brachio-mandibularis s. genio-hyoideus extending from the symphysis of the lower jaw to the first basi-branchial, and of a coraco-hyoideus which had an additional tendon attached to the first cerato-branchial. Fürbringer described the muscles as consisting of a coraco-mandibularis extending from the symphysis to the shoulder-girdle, and of a coraco-hyoideus.

In *Polypterus senegalus* (Text-figs. 35, 36, 37), the muscles consist of a genio-hyoideus and a coraco-hyoideus; the former extends from the symphysis backwards to the level of the third branchial bar, where it ends by being attached to the third cerato-branchial and by a tendon which passes downwards and is attached to a little median ossicle lying between the two coraco-hyoidei. The coraco-hyoideus extends from the cerato-hyal backwards to the shoulder-girdle, and has no tendon passing to the first cerato-branchial.

Greil stated that the "hypobranchial musculature" of *Ceratodus* was developed from ventral downgrowths of the third and fourth myotomes. He apparently included the coraco-branchiales as well as the coraco-mandibularis and coraco-hyoideus under this head, as the first-named were not described as developing in the branchial region. It has been stated above (p. 234) that the coraco-branchiales are developed from the lower ends of the branchial myotomes. The hypobranchial spinal muscle Anlage spreads forwards (Text-fig. 39) reaching the anterior extremity of Meckel's cartilage in stage 43. The portion in front of the hyoid bar separates

¹ Main portion of coraco-arcualis anterior (Vetter) in *Acipenser*; hypopectoralis (McMurrich), sterno-hyoideus (Allis) in *Amia*; the term used above is that of Fürbringer.

from that behind, and its hind end grows backwards below the coraco-hyoideus (Text-figs. 45, 46, 47) to the shoulder-girdle, forming the coraco-mandibularis. The portion behind the hyoid bar forms the coraco-hyoideus; in stage 63 it is partially separated into the coraco-hyoideus and abdomino-hyoideus of the adult, of which the latter is continuous with the trunk muscles behind the shoulder-girdle.

In *Necturus* (Miss Platt) the hypobranchial spinal muscles are developed from ventral downgrowths of the third, fourth, and fifth post-otic somites, joined by a few scattered cells from the second somite; the genio-hyoideus is formed from the third, the sterno-hyoideus from the fourth and fifth.

In *Triton* there is a similar development from the third, fourth, and fifth trunk myotomes in $6\frac{1}{2}$ mm. embryos.

The hypobranchial spinal muscles of *Rana* are developed from downgrowths of the first and second trunk myotomes in 6 mm. embryos (Text-fig. 57), which bend round the branchial region, forming a longitudinal column which reaches the inferior labial cartilage in 8 mm. embryos. It divides opposite the third branchial bar into genio-hyoid and sterno-hyoid.¹ The front end of the former is attached to the inferior labial cartilage (Text-figs. 60, 62), and its hind end to the hypobranchial plate as far back as the antero-posterior level of the third branchial bar. In 12 mm. embryos the internal portion of the genio-hyoid is proliferated from the median edge of the original muscle (Text-fig. 59). At metamorphosis the inferior labial cartilage forms the anterior end of the lower jaw, and the muscle so retains its primitive attachments. The front end of the sterno-hyoid becomes attached to the third cerato-branchial, and the muscle extends back to the diaphragm. Towards metamorphosis the shoulder-girdle is developed and the sterno-hyoid becomes attached to it, and a little later the omo-hyoid is separated from its external edge.

In *Alytes*, *Bufo lentiginosus* and *Pelobates* there is no

¹ Genio-hypobranchialis and diaphragmato-branchialis medialis of Schultze.

proliferation of an internal portion of the genio-hyoid; otherwise the condition in the larvæ is the same. In *Alytes* the hind end of the genio-hyoid is attached to the third cerato-branchial.

In the rabbit the hypobranchial spinal muscles are formed from downgrowths of the first three trunk myotomes in 4 mm. embryos (Text-fig. 82). These have separated in 4½ mm. embryos, and form a longitudinal column which extends forwards dorsal to the interhyoideus and intermandibularis, reaching the anterior extremity of Meckel's cartilage in 8 mm. embryos, and backwards, reaching the area of the anterior limb in 7 mm. embryos. In 13 mm. embryos it has divided into genio-hyoid and (primitive) sterno-hyoid, the adjacent ends of which are attached to the first branchial bar. In 17 mm. embryos the primitive sterno-hyoid has divided into the sterno-hyoid, sterno-thyroid, thyrohyoid, and omo-hyoid. The first trunk myotome, from which the most anterior of the downgrowths above mentioned takes place, atrophies in 7½ mm. embryos, the second and third in 9 mm. embryos.

The Homologies of the Hypobranchial Spinal Muscles.—In Amphibia, Sauropsida, and rabbit, the Anlage of the hypobranchial spinal muscles divides into anterior and posterior portions—the genio-hyoid and sterno-hyoid. The former extends from the symphysis of the lower jaws to the basi-branchial or some branchial bar, the latter extending thence to the shoulder-girdle or sternum. The division takes place in the neighbourhood of the first branchial bar in Urodela, Sauropsida, and rabbit; in Anuran larvæ it is at the level of the third branchial bar.

In Scyllium, Teleostomi, and *Ceratodus*, a similar division of the Anlage of the hypobranchial spinal muscles takes place at the level of the hyoid bar; the hind end of the anterior portion, which does not gain any temporary insertion to the hyoid bar, then grows backwards ventral or ventro-lateral to the posterior portion (coraco-hyoideus) and becomes attached to the first (*Polypterus*, ? species, described by Pollard), or to the second and third (*Amia*), or third (*Polypterus sene-*

galus, *Acipenser*) branchial bar, or to the shoulder-girdle, forming a coraco-mandibularis (*Scyllium*, *Ceratodus*, *Polypterus*? species, described by Fürbringer).

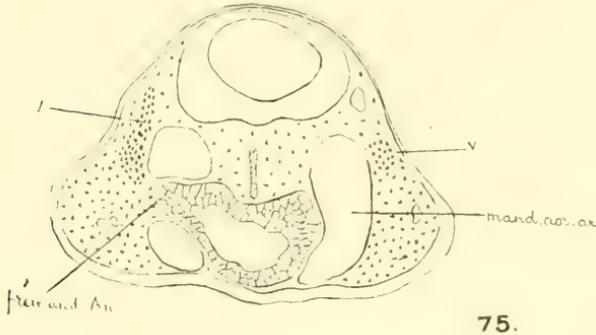
The anterior attachment of the genio-hyoid and coraco-mandibularis is to the front end of Meckel's cartilage except in *Anuran* larvæ, where it is to the inferior labial cartilage. In *Acanthias*, where there is an inferior labial cartilage (Gaupp), the coraco-mandibularis is not attached to this but to Meckel's cartilage. In *Callorhynchus* (Fürbringer) there is a coraco-præmandibularis developed, attached anteriorly to the inferior labial cartilage.

Fürbringer homologised the genio-hyoideus with the coraco-mandibularis of *Selachii*, and supposed that the former was derived from the latter, by giving up its attachment to the shoulder-girdle, and gaining a new one to (more rostrally lying) portions of the hyobranchial skeleton. Such a deduction was a legitimate one from the evidence of adult anatomy only, though the alternative was possible, and the embryological history of the muscles shows that it is this alternative which occurs; the condition in *Teleostomi*, *Elasmobranchs*, and *Ceratodus* is a secondary one.

The method of development of the hypobranchial spinal muscles in *Scyllium* lends additional interest to, and receives corroboration from, some anatomical facts described by Vetter and Fürbringer. The degree of backward extension of the coraco-mandibularis towards the shoulder-girdle varies, even amongst the *Selachii*. Thus in *Heptanchus* and *Scyllium* it does not reach the coracoid, whereas in *Læmargus* and *Prionodon* it does. Further, the coraco-mandibularis is not crossed by tendinous inscriptions, in this forming a marked contrast to the coraco-hyoideus, alongside of which it lies. The only possible exception to this among the forms depicted by Fürbringer is *Cestracion*, and this is probably an apparent one only; it is possible that the tendinous inscription really separates the coraco-hyoideus from the coraco-mandibularis, which only reaches the coracoid by its median edge. Similarly, according to Fürbringer, there are three tendinous

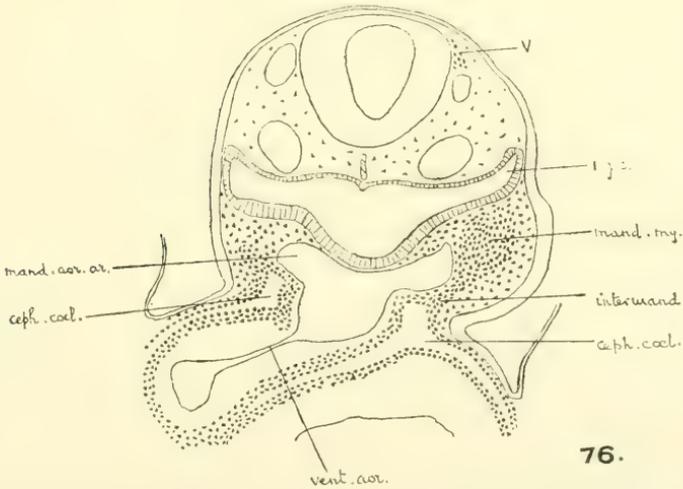
inscriptions in the cerato-hyoideus of *Ceratodus*, whilst there is only one doubtful one in the coraco-mandibularis;¹ in *Protopterus* there are two in the coraco-hyoideus, none in

TEXT-FIG. 75.



75.

TEXT-FIG. 76.



76.

Text figs. 75-77.—Rabbit, embryo 3 mm. Text-fig. 75 is through the premandibular Anlage, Text-fig. 76 through the mandibular segment, Text-fig. 77 through the hyoid segment.

the coraco-mandibularis; in *Polypterus* ? species there are two in the coraco-hyoideus, none in the coraco-mandibularis.

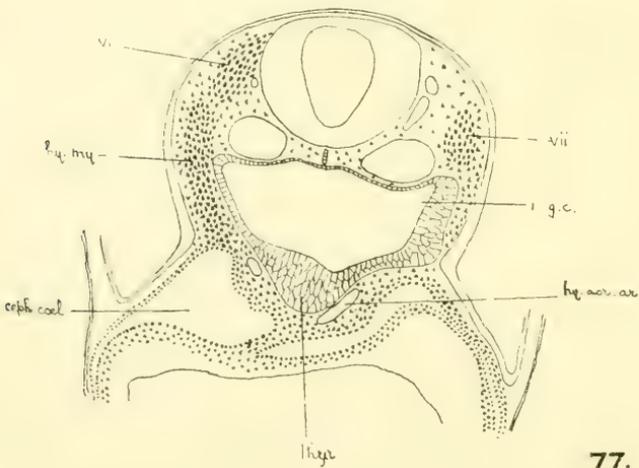
¹ It was not apparent in stage 63 (vide Text-fig. 45).

Again, there are two inscriptions in the coraco-hyoid of *Amia* (Allis), and *Acipenser* (Vetter), none in the genio-hyoid.

The non-development of a genio-hyoid, even as an atrophying Anlage, in *Lepidosteus* and *Salmo* would appear, on comparison with other forms, to be a secondary condition.

The developmental history of the hypobranchial spinal muscles suggests that their Anlage was primitively an undivided column, extending forward to the symphysis of

TEXT-FIG. 77.



77.

the jaws, an anterior prolongation of the rectus system of the trunk into the head segments. This divided into genio-hyoid and coraco-hyoid. In fishes the genio-hyoid secondarily extended backwards, overlapping the coraco-hyoid in varying degrees.

The Anlage of the hypobranchial spinal muscles is formed from downgrowths of several—two or more—trunk myotomes, that from the foremost myotome taking part in its formation extending furthest forward, that from the next succeeding it, and so on. There is a certain correlation between the number of trunk myotomes taking part in the

formation of the hypobranchial spinal muscles and the number of trunk-segments included in the skull (vide table, p. 299). In general, the less the number of trunk-segments included in the skull, the greater tendency there is for the most anterior trunk myotomes to take part in the formation of the hypobranchial spinal muscles, and the less the number of anterior trunk myotomes atrophying without taking part in their formation. The table also shows that the hypobranchial spinal muscles are derived from a variable number of trunk myotomes. The number varies from two (*Rana*) to five (*Scyllium*). Derivation from the smallest number is probably the most primitive condition. The number does not vary with that of the trunk-segments included in the skull, nor with the number of head-segments.

LINGUAL MUSCLES.

The researches of Gegenbaur and Kallius in *Salamandrina* have shown that the genio-glossus is developed from the genio-hyoideus and the sterno-glossus from the sterno-hyoideus; the former ends between the lingual glands which form the fore part of the tongue, and is "also ein Drüsen Muskel" (Gegenbaur); the latter becomes attached to a plate of dense connective tissue which probably develops at the site of fusion of the primitive tongue with the glandular portion. Kallius has also shown that in the *Anura* the genio- and hyo-glossus are developed from the genio-hyoid. The genio-glossus grows towards the subsequently glandular, precopular field, and later, the hyo-glossus fibres cross those of the genio-glossus.

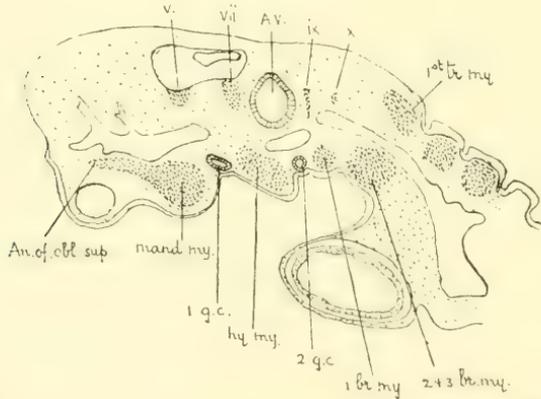
To this may be added that in *Rana* the genio- and hyo-glossus are developed from the inner division of the genio-hyoid, which (vide p. 270) is proliferated from the inner side of the primitive muscle.

In 27 mm. larvæ of *Alytes* the genio-glossus ends free beneath the mucous membrane of the precopular field, and the hyo-glossus passes forward at first below and then lateral

to the long forward projecting basihyal (Text-fig. 63), and is attached to it near its anterior end. In larvæ of *Bufo*, *Rana*, and *Pelobates* the hyo-glossus is not attached to the rudimentary basihyal.

The development of the lingual muscles of *Lacerta muralis* has also been investigated by Kallius. The development of the lingual muscles in other groups of *Sauropsida* (loc. cit.) showed that the primitive condition of the lingual muscles is a genio- and hyo-glossus, both developed from

TEXT-FIG. 78.



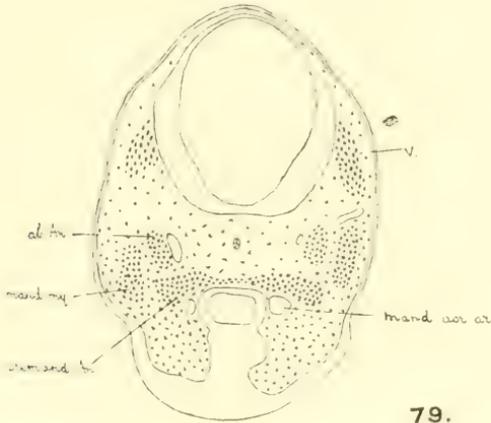
78.

Rabbit, embryo 3½ mm., longitudinal vertical section.

the genio-hyoid, attached to the long basihyal, the former to its front end, the latter to its side; and that extension into the tongue is a secondary phenomenon.

The condition of the hyo-glossus of *Alytes* is of interest when considered in relation to that of the lingual muscles of *Sauropsida*. Gegenbaur was of opinion that "die Muscularisierung scheint vorwiegend im Dienste der Drüsen zu stehen." An alternative hypothesis suggested by the condition in *Alytes* larvæ would be that the condition in *Sauropsida* is the primary one, and that their functions as glandular muscles in *Amphibia* is correlated with the absence (e.g.

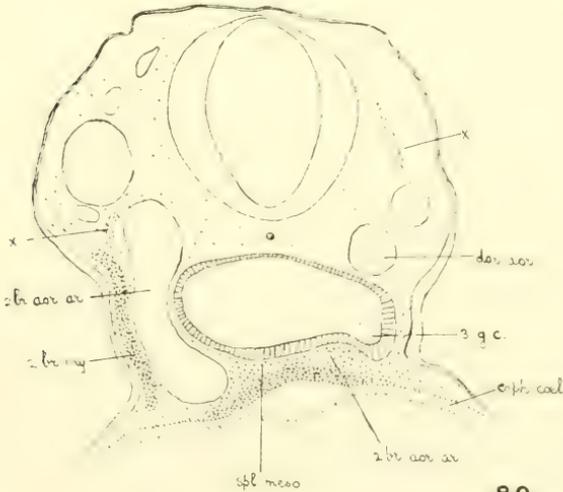
TEXT-FIG. 79.



79.

Rabbit embryo 3 $\frac{1}{4}$ mm. The upper part of the section is posterior to the lower.

TEXT-FIG. 80.



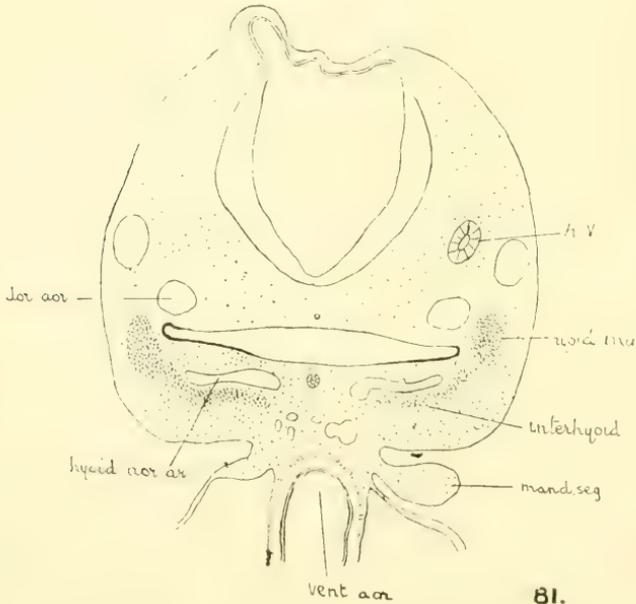
80.

Rabbit, embryo 3 $\frac{1}{4}$ mm. The right side of the section is a little anterior to the left, which passes through the second branchial myotome.

Triton) or rudimentary condition (e.g. *Rana*, *Pelobates*, *Bufo*) of the basihyal.

In the rabbit the Anlagen of the lingual muscles are formed from the anterior part of the Anlage of the hypobranchial spinal muscles, i.e. from the future genio-hyoid, in 9 mm. embryos, that of the genio-glossus and lingualis by upward growth, that of the hyo-glossus and stylo-glossus by an out-

TEXT-FIG. 81.



81.

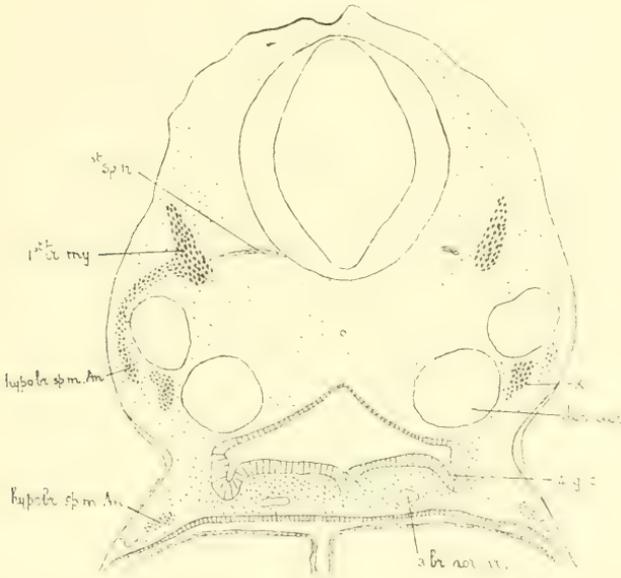
Text-figs. 81-83.—Rabbit, embryo 4 mm. Text fig. 81 is through the hyoid segment, Text-fig. 82 through the fourth gill-cleft, Text-fig. 83 through the third branchial gill-cleft.

growth directed upwards and laterally (Text-fig. 91). In 13 mm. embryos these muscles have separated from the genio-hyoid, and become distinct (Text-figs. 94, 95).

SOME PHYLOGENETIC CONSIDERATIONS.

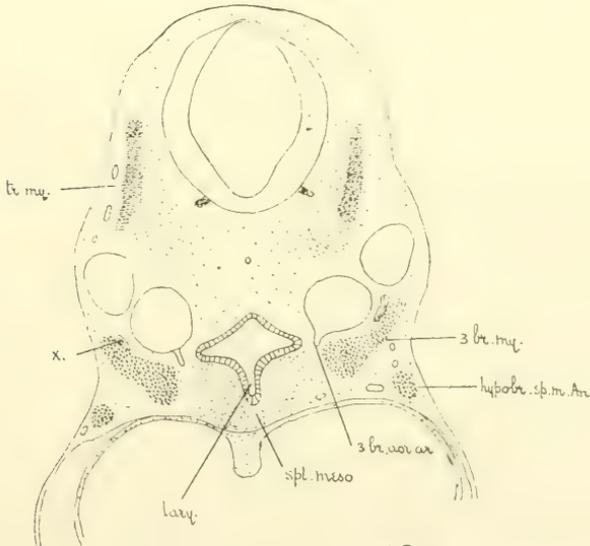
The probable phylogenetic relationships of the various Vertebrate groups are determined by the total morphological

TEXT-FIG. 82.



82.

TEXT-FIG. 83.



83.

evidence available. The cranial muscles form one item only of such evidence, but it is of interest to inquire how far their morphology falls in with generally received opinion, and in what direction it points in cases where opinions vary.

Such an inquiry is beset by the difficulties which arise from—(A) secondary innervation of muscles; (B) development of similar changes in various groups; (C) atrophy, and non-development of muscles.

(A) Comparison of the development and innervation of the cranial muscles shows that although, in general, a muscle is innervated by the nerve corresponding to its segment of origin, yet this is not invariably the case. Thus:

(1) The posterior part of the intermandibularis of Triton is innervated by the VIIth (Drüner).¹

(2) The intermandibularis of Selachians is, in part, e.g. Scyllium (Vetter), or wholly, e.g. Acanthias (Vetter), innervated by the VIIth.

(3) The intermandibularis anterior and posterior (the latter called "inferior genio-hyoid" by Allis) of *Amia* are innervated by both the Vth and VIIth (Allis).

(4) The hyo-maxillaris of Teleostomi, developed in the hyoid segment, is in some, e.g. *Menidia* (Herrick), wholly innervated by the VIIth; whereas in others, e.g. *Esox* (Vetter), *Salmo*, its hinder part is innervated by the VIIth and its fore part by the Vth; and in *Amia* (Allis) it is innervated by the Vth and VIIth.

(5) The anterior digastric of man, pig (*Futamara*), and rabbit, developed in the hyoid segment, is innervated by the Vth.

(6) The cerato-hyoideus externus of Urodela, developed in the hyoid segment, is innervated either by the VIIth, e.g. *Necturus* (Miss Platt), or by the IXth, e.g. Triton (Drüner).

(7) The interarcuales ventrales of Urodela are innervated both by the nerves corresponding to their segments of origin and also by those of the next anterior segments (Drüner).

(8) In some Teleostomi, e.g. *Polypterus senegalus*,

¹ Roman numerals denote cranial nerves.

Esox (Vetter), *Menidia* (Herrick), *Amia* (Allis), *Lepidosteus*; the only coraco-branchialis present, developed from the most posterior branchial segment (fourth or fifth), is innervated by the Xth; whereas in others, e. g. *Amieurus* (Wright), *Salmo* (Harrison), it is innervated by the spino-occipital nerves.

(9) The coraco-branchiales of *Acipenser*, *Ceratodus*, and *Seyllium*, developed in branchial segments, are innervated by spino-occipital nerves (Vetter, Fürbringer). The spino-occipital nerves also innervate the four coraco-branchiales of *Polypterus* (?) species, described by Fürbringer.

(10) The capito-dorso-clavicularis of *Lacerta agilis*, developed from the primitive trapezius, i. e. from branchial segments, is innervated by spinal nerves (Fürbringer).

(11) The cucullaris, i. e. trapezius, of *Gallus*, developed from branchial segments, is innervated both by the XIth and by spinal nerves (Fürbringer).

(12) The trapezius and sterno-mastoid of the rabbit, developed from branchial segments, is innervated both by the XIth and by spinal nerves.

(13) The retractor arcuum branchialium dorsalis of *Amia* and *Lepidosteus*, developed from trunk myotomes, is innervated by the Xth (Allis, Wiedersheim).

(14) The hinder part of the hypobranchial spinal muscles of the rabbit, which are developed from the first three spinal myotomes, are innervated by more posterior spinal nerves.

(15) The interarcualis ventralis I, i. e. branchio-hyoideus or branchio-mandibularis of *Sauropsida*, is innervated by the XIIth.

Fürbringer held that "Die Innervirung der Muskeln durch bestimmte Nerven ist das wichtigste Moment für die Vergleichung." In criticism of this theory, Cunningham gave instances from the myology of the trunk and limbs in which this criterion failed, and concluded that the nerve supply is "not an infallible guide" for determination of the homology of a muscle. The above-cited observations show that developmental phenomena should be taken into consideration.

The first fourteen of the phenomena recorded appear to be referable to a common cause; if a muscle spreads into one or more neighbouring segments, that portion tends to be innervated by the corresponding nerve or nerves. The backward extension of the origin of the XIth appears to be referable to the same cause.

It is not yet known what happens within the central nervous system—whether there is a corresponding migration of motor neuroblasts or whether new ones are locally formed.

The cause of the phenomenon cited under (15) above is much more obscure. The muscle is the interarcualis ventralis of the first branchial segment, and is homologous with the similarly developed muscle of Amphibia, some Teleostomi, and some Mammalia, and yet, unlike them, it is innervated by spino-occipital nerves and not by the IXth, just as if it were a coraco-branchialis I.

(B) The possibility of the independent development of similar secondary changes in the various groups arises in the case of the hypobranchial spinal muscles, the hypobranchial cranial muscles, the levatores arcuum branchialium, and trapezius, the hyoid bar and related muscles, the adductor mandibulæ.

In *Ceratodus* and in *Scyllium* the hind end of the genio-hyoid secondarily extends backwards to the shoulder-girdle. The question arises whether this feature is inherited from a common ancestor or whether it has been independently acquired. In favour of the second view are the facts that within the group of the Teleostomi all conditions exist between that of a genio-hyoid which has slightly extended backwards and a coraco-mandibularis.

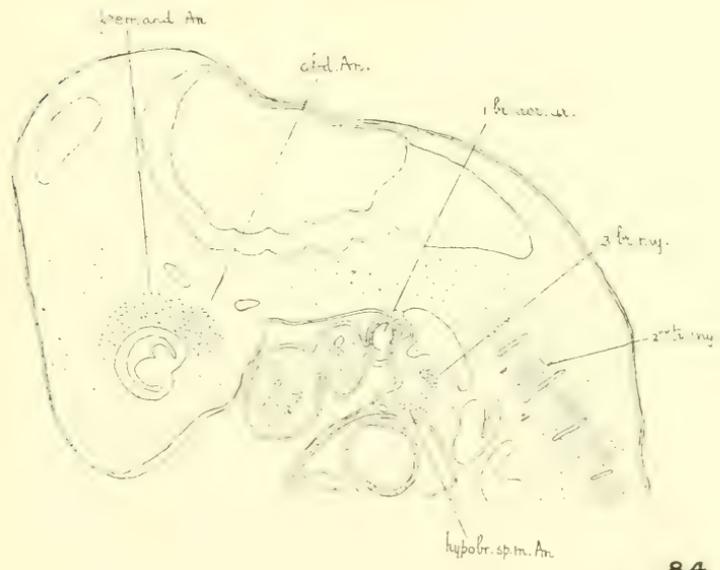
A similar question arises in regard to the formation of coraco-branchiales in *Ceratodus* and *Scyllium*. Again, within the group of the Teleostomi all variations exist between inter-arcuales ventrales and their homologues, coraco-branchiales.

These secondary modifications in the hypobranchial-spinal and hypobranchial-cranial muscles appear to be morphological expressions of an increased need of tying the

mandibulo-hyo-branchial skeleton to the shoulder-girdle, and the change of function of the latter group of muscles to one similar to that of the former tends to bring about a secondary innervation from spinal nerves.

A similar question arises in connection with the presence and absence of levatores arcuum branchialium. It has been suggested above that their absence and the related method of formation of the trapezius are secondary phenomena

TEXT-FIG. 84.



84.

Rabbit, embryo 4 mm., longitudinal vertical section.

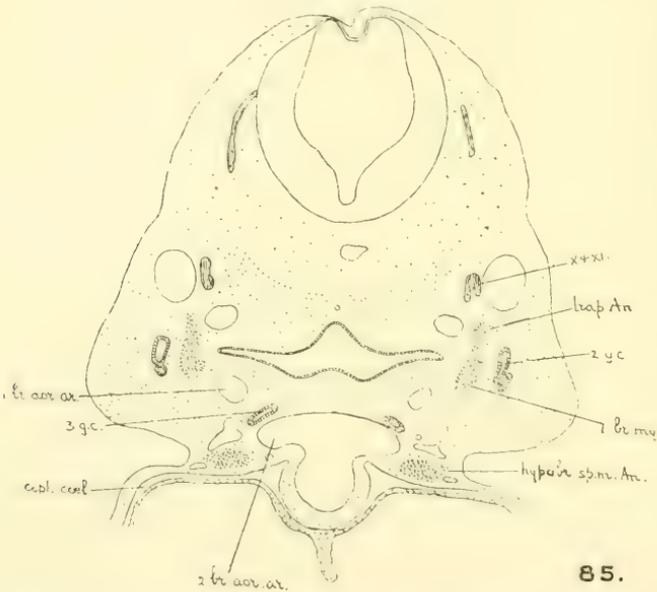
(p. 257). If so, it is possible that this has been independently acquired in Scyllium, Sauropsida, and rabbit.

In Scyllium and the Teleostomi a stage of development occurs in which there is a short hyoid bar like that of Amphibia with a levator hyoidei, which is succeeded by one in which the bar extends up to the periotic capsule. The relationship of the muscles and of the facial nerve to the later formed portion of the bar are so different in Scyllium

and Teleostomi that possibly the only common feature is the above-mentioned first stage. In *Ceratodus*, *Sauropsida*, and rabbit the hyoid myotome is external to the upper part of the hyoid bar, as in *Scyllium*.

In *Sauropsida* and certain *Teleostomi* the adductor mandibulæ divides into internal and external portions, but in *Teleostomi* there is no uniform upgrowth of the external

TEXT-FIG. 85.

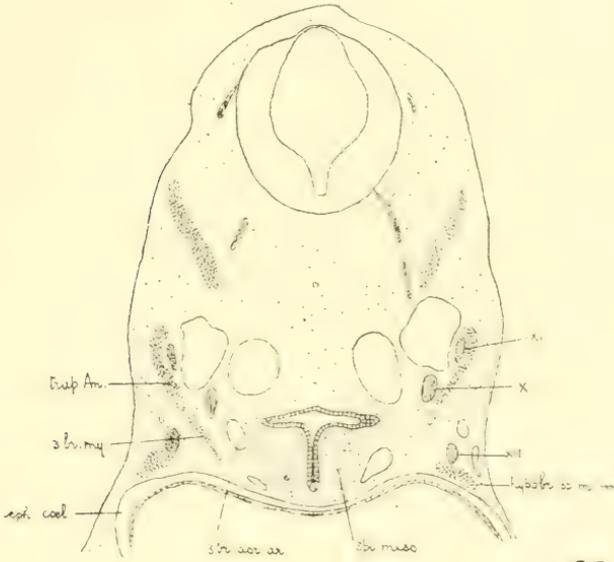


Text-figs. 85-87.—Rabbit, embryo 5 mm. ; Text-fig. 85 is through the first branchial segment, Text-fig. 86 through the third branchial segment, Text-fig. 87 just behind this.

portion to the skull as in *Sauropsida*. Both division and upgrowth have been independent occurrences in these two phyla.

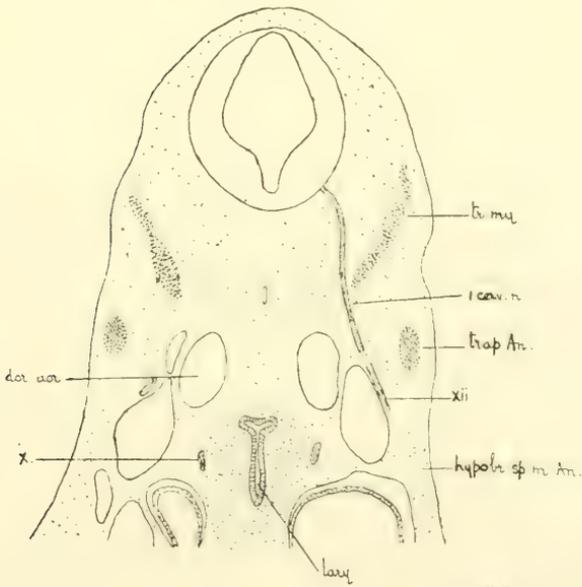
c. Amongst the animals investigated there are but few in which muscle-Anlagen are developed and then atrophy. The *Mm. marginales* and *interarcuales ventrales* of the larva of *Rana*, certain muscles of metamorphosing *Urodela* described by Drüner, the *levator maxillæ superioris* of *Chelone* and

TEXT-FIG. 86.



86.

TEXT-FIG. 87.



87.

Alligator, and the genio-hyoid of Gallus, were the only ones found. Otherwise if a muscle is not present in the adult it is not formed during development.

There are certain instances in which comparative evidence suggests that ancestors probably possessed muscles which are now no longer developed, even as Anlagen. Such are the genio-hyoid of *Lepidosteus* and *Salmo*, certain *Mm. transversi ventrales* in *Amphibia* and *Teleostomi*, the first two *obliqui ventrales* in *Polypterus senegalus*, the *hyo-maxillaris* in *Selachii* and *Sauropsida*, the *levator arcuum branchialis* in *Selachii*, *Sauropsida*, and *Mammalia*.

Consideration of the changes which take place in the Anlagen of the cranial muscles in the various Vertebrate groups suggests that the most important are those occurring in the myotome of the mandibular segment. In *Amphibia* and *Ceratodus* it does not, whilst in *Teleostomi*, *Selachii*, and *Sauropsida* it does divide into parts above and below the palato-pterygoid or pterygoid process of the quadrate. It has been stated above that the embryological phenomena support the view that the second condition has been derived from the first. In the rabbit the quadrate (*incus*) has no pterygoid process, and the myotome—as in *Amphibia* and *Ceratodus*—does not divide into upper and lower parts.

Changes take place in the Anuran tadpole, in the form of the palato-quadrate bar and in certain muscles in association with the development of a suctorial mouth, i.e. the backward elongation of the mandibular muscles, the development of a *submentalis* and *mandibulo-labialis*, the origin of the *orbito-hyoideus*, or of this and the *suspensorio-hyoideus*, and the partial origin of the first branchial levator from the palato-quadrate bar, the division of the *hyo-maxillaris* and attachment of one or two of its parts to the palato-quadrate bar. As the condition before these events takes place is very like that of an embryo of *Ceratodus* or an *Urodelan*, it would appear probable that the changes are secondary larval ones and not ancestral.¹

¹ The difficult question as to the origin and nature of the larval

On the other hand, the existence of a hyo-maxillaris and of *Mm. marginales*, the insertion of the orbito-hyoideus or of this and the suspensorio-hyoideus to the cerato-hyal, and the origin of the trapezius from the skull, are primitive features which are not developed or soon modified in Urodelan development.

In the Urodela the insertion of the levator hyoidei is transferred, wholly or partially, from the hyoid bar to Meckel's cartilage early in development, and the hyo-maxillaris Anlage forms a ligament. The development of gill-muscles from Anlagen which are homologous with those which give rise to the *Mm. marginales* of Anuran larvæ and *Ceratodus*, and of a cerato-hyoideus externus, are features peculiar to Urodela.

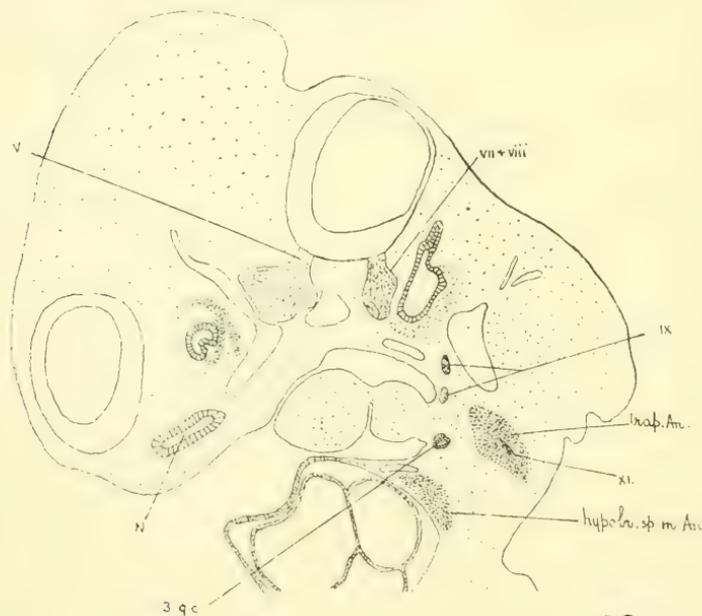
Ceratodus resembles *Selachii* and some *Teleostomi*, and differs from *Amphibia* in the backward growth of the genio-hyoid to the shoulder-girdle, and in the formation of coraco-branchiales. *Ceratodus* resembles *Selachii* and *Teleostoman* embryos, and differs from *Amphibia* in the backward growth of both hyoid myotome and interhyoideus, resulting in the formation of a continuous dorso-ventral sheet, C_2vd , behind the hyoid bar. *Ceratodus* resembles *Teleostomi* and *Amphibia*, and differs from *Selachii* in the formation of levatores arcuum branchialium and in the development of the trapezius from a levator. *Ceratodus* resembles *Amphibia*, and differs from *Selachii* and *Teleostomi* in the non-division of the mandibular myotome into upper and lower portions. *Ceratodus* resembles Anuran larvæ in the simple condition of the *Mm. marginales*, and Urodela in the ligamentous condition of the hyo-maxillaris.

According to K. Fürbringer, "Wenn wir somit keine bestimmte Ordnung der *Amphibia* von den *Dipnoern* ableiten können, so ergibt sich daraus kein Einwand gegen eine Abstammung von den *Dipnoern* über-
condition of the suctorial mouth and jaws in Anuran larvæ was discussed by Balfour and by Gaupp, though without reference to the muscles.

haupt. . . ." The development, however, in *Ceratodus*, of a coraco-mandibularis, of coraco-branchiales, of a hyomandibula, and of a dorso-ventral sheet C_2 vd behind the hyoid bar, are all secondary to more primitive conditions present in Amphibia.

Goodrich was of opinion that "the Dipnoi are probably a specialised offshoot from the Teleostoman stem which

TEXT-FIG. 88.



88.

Rabbit, embryo 7 mm., longitudinal vertical section.

acquired an autostylic structure before the hyomandibula had become very large and before the hyostylism had become fully established." The non-division of the mandibular myotome and the persistence of the dorso-ventral sheet C_2 vd are, however, more primitive features than exist in Teleostomi; and in the embryo of *Ceratodus* there is a hyomandibula, the relations of which are different from those occurring in Teleostomi.

Graham Kerr's opinion was that "the Teleostomes the Dipnoans and the Amphibians have arisen in phylogeny from a common stem . . ."

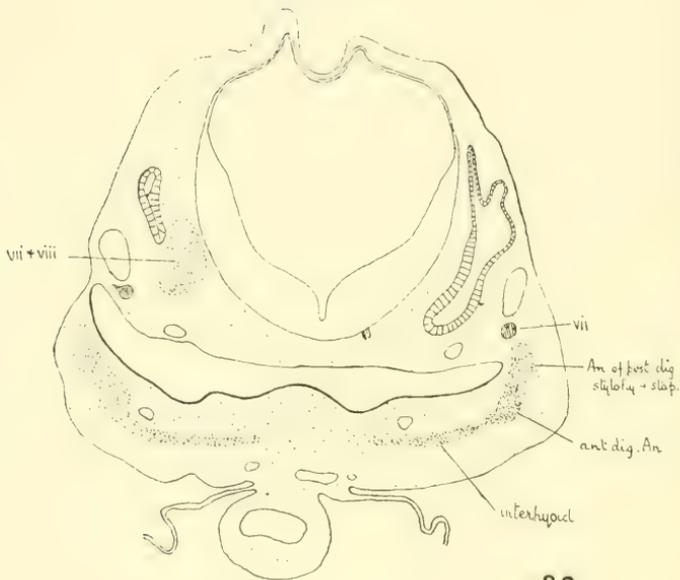
Kellicott's statements that "the resemblances in the vascular system between *Ceratodus* (the most primitive of the living Dipnoi) and the Amphibia, especially Urodela, are numerous and fundamental and cannot be explained as parallelisms," and that "most of the Elasmobranch characters are parallelisms, some of them actually being preceded by Amphibian conditions (e.g. the carotid arteries)" are also true of the cranial muscles.

Consideration of the common features in the cranial muscles of Teleostoman embryos leads to the probability that some remote ancestors possessed—a mandibular myotome divided into upper and lower parts¹; a levator hyoidei, which, owing to the upgrowth of the hyoid bar to the periotic capsule, was inserted into the inner or posterior surface of a hyomandibula; a dorso-ventral sheet in the opercular fold, divided into a *M. opercularis* and a constrictor operculi; a series of levatores arcuum branchialium; a trapezius developed from the fourth levator; a series of *Mm. marginales* not fused with the transversi ventrales; a series of hypobranchial-cranial muscles consisting of interarcuales ventrales and of a coraco-branchialis attached to the last branchial bar; hypobranchial-spinal muscles, consisting of a coraco-hyoideus, and of a genio-hyoid, the hind end of which had grown back to some more posterior branchial bar overlapping the coraco-hyoideus.

All these features, with five exceptions, may be supposed to have characterised primitive Amphibia; and these exceptions, viz. division of the mandibular myotome, formation of a *M. opercularis*, and of a coraco-branchialis, backward growth of the genio-hyoid, upward extension of the hyoid

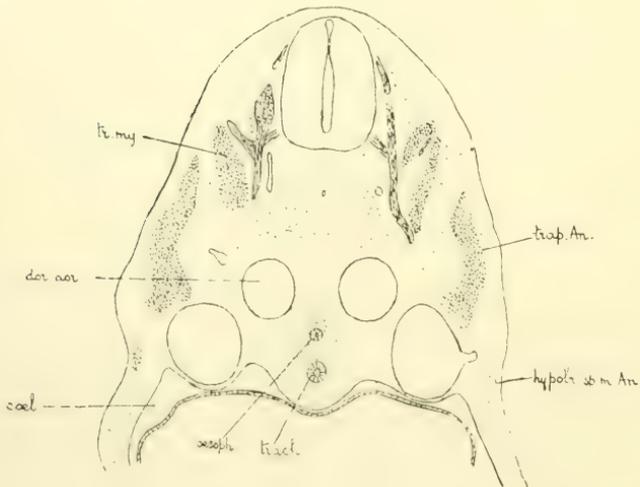
¹ On the supposition that the protractor hyomandibularis of *Acipenser* is a case of atavism in its non-division into levator arcus palatius and dilatator operculi, this division of the levator maxillæ superioris would have once characterised the whole group.

TEXT-FIG. 89.



89.

TEXT-FIG. 90



90.

Text-figs. 89 and 90.—Rabbit, embryo $7\frac{1}{2}$ mm.; Text-fig. 89 through the hyoid segment, Text-fig. 90 through the neck.

bar to the periotic capsule—are, as shown by their development, modifications of more primitive features existing in Amphibia.

These phenomena may be considered as additional arguments in favour of the theory of a descent of Teleostei, as advocated by Assheton, from a proto-amphibian stock; and of Teleostomi in general, as advocated by Graham Kerr, from a stem common to the Teleostomi, Dipnoi, and Amphibia.

In the condition of the cranial muscles Teleostei do not show any closer resemblances to Amphibia than do other groups of the Teleostomi.

The curious fact that the trapezius is developed from the fourth levator arcuum branchialium in *Acipenser*, *Lepidosteus*, *Amia*, and *Salmo*, though there are five branchial segments, suggests that ancestors of the Teleostomi may have had, like Amphibia, only four branchial segments, and that an increase to five took place within the group.

In the possession of only four branchial segments, of interarcuales ventrales I, II, and III, of obliqui ventrales not fused with transversi ventrales, and of very primitive laryngeal muscles, *Polypterus senegalus* shows closer resemblances to Amphibia than do the other Teleostomi examined.

The main characteristics of the cranial muscles of Selachii are: (1) Division of the mandibular myotome into levator maxillæ superioris and adductor mandibulæ; (2) great backward extension of the intermandibularis below the interhyoideus; (3) non-formation of an opercular fold; (4) upgrowth of the hyoid bar internal to the hyoid myotome, which, originally forming a levator hyoidei, becomes inserted into its external surface (hyomandibula, or this and ceratohyal); (5) non-formation of a hyo-maxillaris; (6) extension backwards of hyoid myotome and interhyoideus forming a dorso-ventral sheet C_3vd behind the hyoid bar, though not in an opercular fold; (7) non-formation of levatores arcuum branchialium; (8) formation of a trapezius from the upper ends of all the branchial myotomes; (9)

formation of subspinalis and interbasales from anterior trunk myotomes; (10) formation of coraco-branchiales; (11) formation of adductors from the portions of the branchial myotomes which lie internal to the branchial bars; (12) formation of arcuales dorsales, interbranchials, and superficial constrictors from the portions of the branchial myotomes which lie external to the branchial bars; (13) non-formation of transversi ventrales; (14) extension backward of the genio-hyoid, forming a coraco-mandibularis. Of these features, (3) (9) and (12) occur in Selachii and them only.

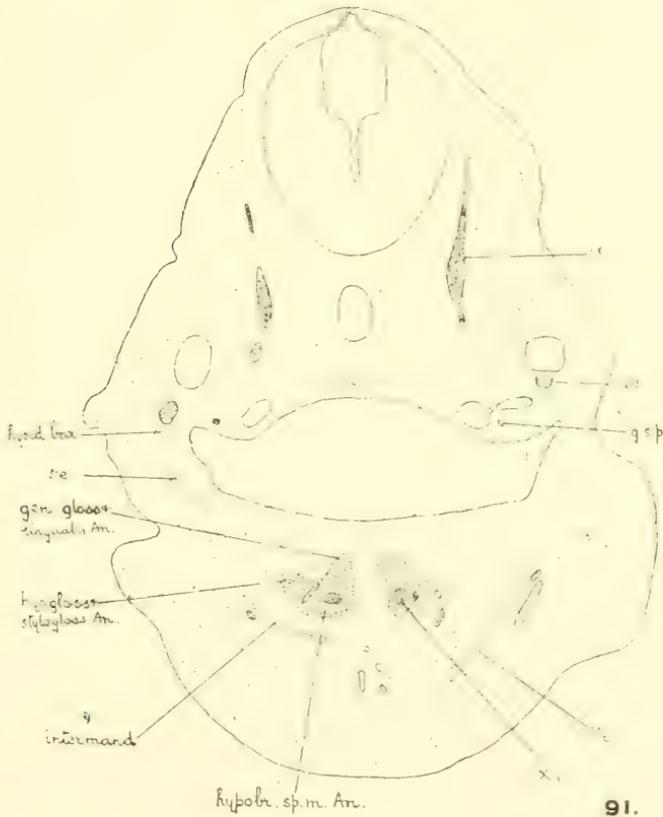
The great development of the branchial musculature, external to the branchial bars, is correlated with the absence, probably the loss, even in developmental stages, of an opercular fold. It is of interest to note that in *Chimæra* (Vetter) (1) a hyo-maxillaris (hyoideus inferior) is present; (2) the dorso-ventral sheet C_2vd is situated in an opercular fold; (3) the branchial musculature, external to the bars, consists of simple vertical muscles ("interbranchials" of Vetter), which are similar to the *Mm. marginales* of Anuran larvæ and *Ceratodus*, and to the dorsal portions of the *obliqui ventrales* of Teleostomi.

According to Graham Kerr, "the Teleostomes, the Dipnoans, and the Amphibians have probably arisen in phylogeny from a common stem, which would in turn probably have diverged from the ancestral Selachian stock." Fürbringer's theories in regard to the hypobranchial muscles and the neocranium, and Ruge's respecting the facial muscles, are also based on a similar theory.

Consideration of the morphology of the cranial muscles leads to some doubt on this question. The embryology of each group of cranial muscles, mandibular, hyoid, branchial, hypobranchial-cranial, and hypobranchial-spinal, suggests that the conditions found in Selachii are secondary to those which may be supposed to have characterised Amphibian ancestors—are modifications of a proto-amphibian type. Certain of these modifications occur in other groups also: thus division of the mandibular myotome into upper and lower

parts also occurs in Teleostomi and Sauropsida; backward extension of both hyoid myotome and interhyoideus to form a dorso-ventral sheet also occurs in Ceratodus and Teleostomi (though in these, in an opercular fold) formation of

TEXT-FIG. 91.



91.

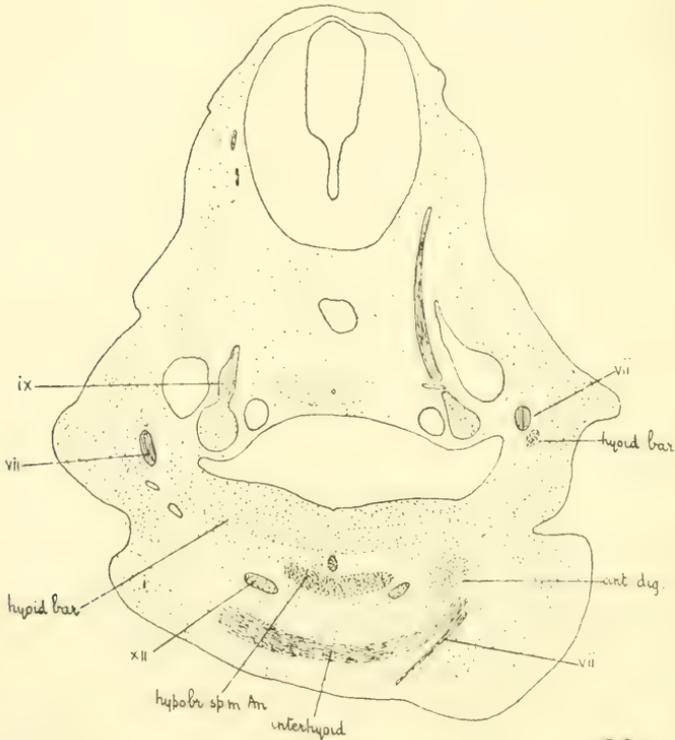
Text-figs. 91-93.—Rabbit, embryo 9 mm.; Text-fig. 91 through the mandibular segment, Text-figs. 92 and 93 through the hyoid segment.

coraco-branchiales and of a coraco-mandibularis also occurs in Ceratodus and some Teleostomi; non-formation of levatores arcuum branchialium, and the associated method of development of the trapezius occurs in Sauropsida and rabbit.

The significance of such resemblances from a phylogenetic point of view is doubtful, though probably the first two named are by far the most important.

The ancestry of Mammals has been the subject of inquiry and speculation for many years. Two theories have been

TEXT-FIG. 92.



92.

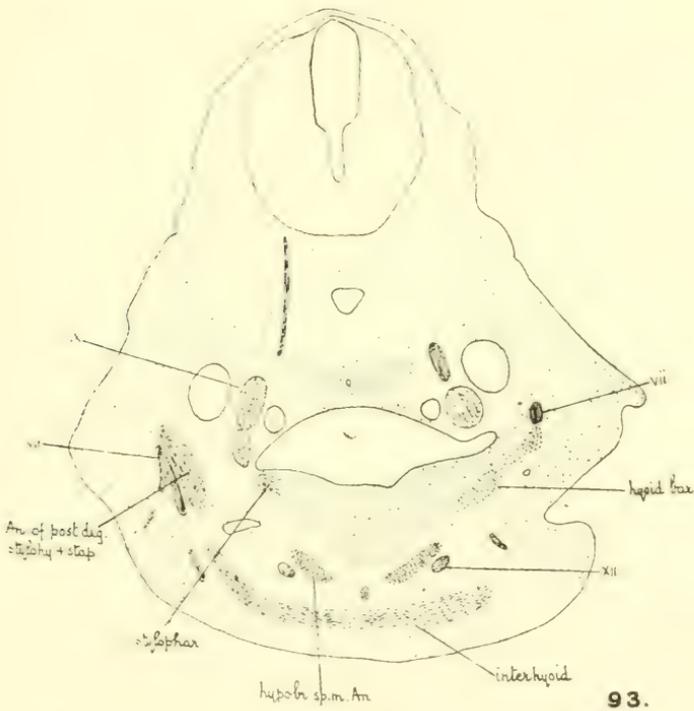
held—one, that Mammals are descended from Sauropsida, the other, that they are descended from Amphibia.

As regards the cranial muscles, Mammals resemble Amphibia, and differ from Sauropsida in the following particulars: non-division of the mandibular myotome into dorsal and ventral parts, formation of a hyo-maxillaris (anterior digastric), non-formation of a dorso-ventral sheet

C₂yd in the hyoid segment, innervation of the interarcualis ventralis I (branchio-hyoideus) by the IXth.

On the other hand, Mammals resemble Sauropsida, and differ from Amphibia, in the non-formation of levatores arcuum branchialium, and the associated development of the

TEXT-FIG. 93.

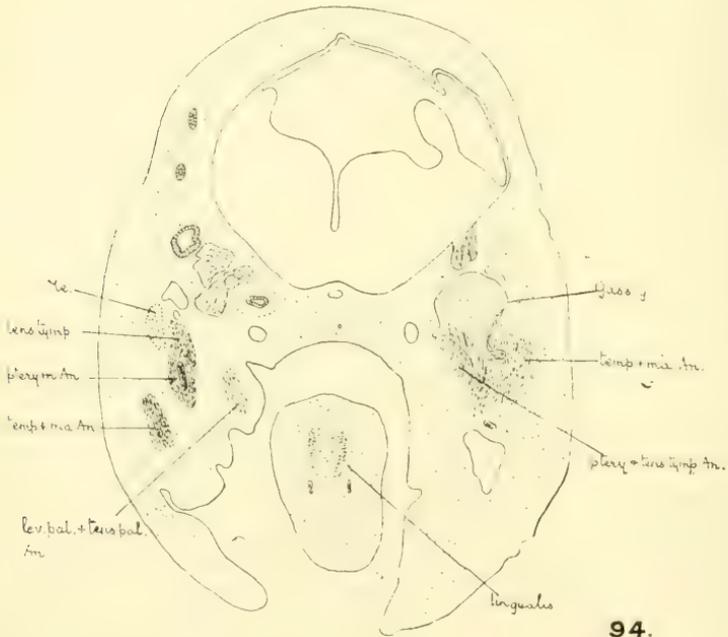


trapezius from the upper ends of all the branchial myotomes, disappearance of the branchial myotomes (after formation of trapezius and interarcuales ventrales from their upper and lower ends), non-formation of transversi ventrales.

It has been suggested above in discussing individual groups of muscles that all the first-named features are primary ones, and that all the second-named features are secondary phenomena. It is possible that secondary features may have

been independently acquired; thus the absence of levatores arcuum branchialium and method of formation of the trapezius also occurs in Selachii. The morphology of the cranial muscles is thus in favour of an Amphibian ancestry of Mammals. In the attachment of the posterior digastric to the hyoid bar, and not to the lower jaw, some Mammals

TEXT-FIG. 94.



94.

Text-figs. 94 and 95.—Rabbit, embryo 13 mm.; transverse sections through the mandibular segment. Text-fig. 94 is the more anterior.

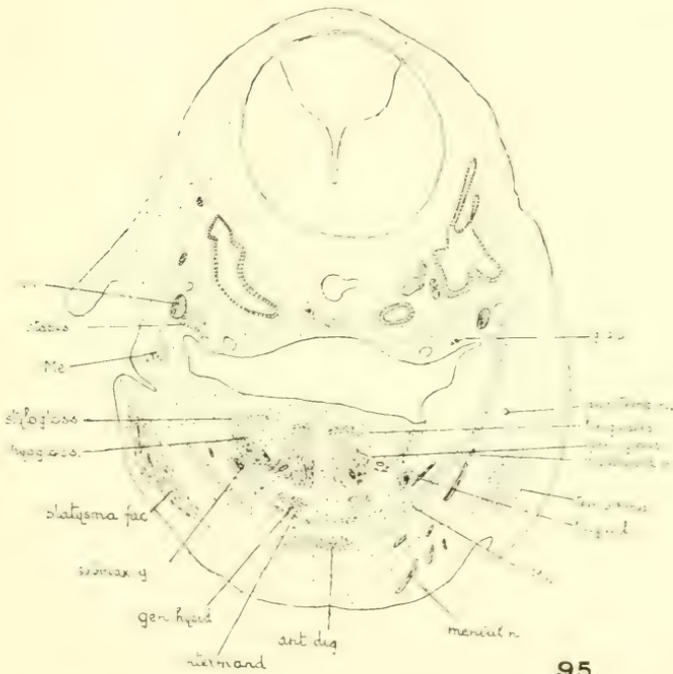
present a more primitive feature than is found in any adult Amphibia. A descent from a proto-amphibian stock is thus suggested.

The ancestry of Sauropsida has been the subject of but few speculations. Fürbringer was of opinion that "Die strepto-stylen Pro-reptilia aber haben sich neben den strepto-stylen Pro-mammalia auf tiefer stehenden streptostylen

Thieren entwickelt welche im Grade ihrer Ausbildung amphibienartige Thiere gleichzusetzen sind. . . ." Graham Kerr's opinion was that "the ancestors of the Amniota probably diverged about one or several points from the region of the stem common to Dipnoi and Amphibia."

As regards the cranial muscles, the differences between

TEXT-FIG. 95.



95.

Sauropsida and Amphibia have been mentioned above. In the division of the mandibular myotome into upper and lower portions, and in the formation of a ventro-dorsal sheet, C_2 vd, in the hyoid segment, Sauropsida resemble Selachii and Teleostomi, and also as regards C_2 vd, Dipnoi. The shifting of insertion of the levator hyoidei from cerato-hyal to Meckel's cartilage and the morphologically primitive condition of the hypobranchial spinal muscles are common to both Sauropsida and Amphibia.

ON FÜRBRINGER'S THEORY OF THE SKULL.

It is of interest to inquire whether the above suggestions as to the phylogeny of various groups of Vertebrates receive any support from the morphology of the skull.

According to Fürbringer's theory the portion of the cranium in front of the exit of the vagus is the original cranium—the palæocranium. The neocranium has been formed by the addition of spinal skeletal elements, which originally were free. This took place in several stages; in the first a protometamer neocranium is formed—present in Selachii and Amphibia. The union of further additional elements brings about the auximetamer condition of the neocranium, found in higher fishes and Amniota.

The added spinal nerves—spino-occipital nerves—can be divided into two categories, the “occipital,” brought in with the protometamer neocranium, and the “occipito-spinal,” additionally added with the auximetamer neocranium. The varying number of spino-occipital nerves is due to the varying position of the cranio-vertebral junction.

The assimilated occipital nerves are indicated by the terminal letters of the alphabet, the assimilated occipito-spinal nerves by the initial letters. Their corresponding myotomes are given corresponding (larger) letters. By this method it is possible to express either or both of two possibilities—the reduction of more anterior or the addition of more posterior nerves.

The following table, which is taken mostly from Gaupp, shows the results of the investigation of various vertebrates, and a column has been added showing the number of myotomes taking part in the formation of the hypobranchial muscles.

A spinal segment is typically indicated by a somite or myotome, anterior nerve root, and posterior nerve root. The researches of Fürbringer and other observers have shown that as segments are assimilated their nerve roots tend either not

MORPHOLOGY OF CRANIAL MUSCLES IN SOME VERTEBRATES. 299

	No. of trunk segments taken up into the skull.	Nature of neocranium according to Fürbringer's theory.	Taking part in the formation of hypobranchial spinal muscles.
Anura—			
Pelobates (Sewertzoff) . . .	3	Protometamer	—
Rana (Miss Elliott) . . .	3	„	1st, 2nd.
Urodela—			
Siredon (Sewertzoff) . . .	2	„	—
Necturus (Miss Platt) . . .	3	„	2nd (few cells), 3rd, 4th, 5th (Miss Platt).
Triton	—	—	—
Dipnoi—			
Ceratodus (K. Fürbringer) . . .	5	Auximetamer (Gaupp)	2nd 3rd (Greil).
Protopterus (Agar) . . .	3	Protometamer (Agar)	2nd, 3rd, 4th (Agar).
Lepidosiren (Agar) . . .	3	Ditto	2nd, 3rd, 4th (Agar).
Mammals—			
Sheep, calf (Frobiep) . . .	3	Auximetamer	—
Rabbit	—	„	1st, 2nd, 3rd.
Reptilia—			
Ascalobates (Sewertzoff) . . .	4	„	—
Lacerta (Hoffman) . . .	5	„	2nd, 3rd, 4th, 5th (Hoffman)
Lacerta (Chiarugi and v. Bemmelen).	4	„	—
Birds—			
Timunculus (Suschkin) . . .	4	„	—
Gallus	—	„	1st (few cells), 2nd, 3rd, 4th, 5th.
Teleostomi—			
Amia (Schreiner) . . .	4	„	2nd, 3rd, 4th.
Salmo salar (Wilcox) . . .	5	„	2nd, 3rd, 4th (Harrison).
Trutta fario (Wilcox) . . .	5	„	—
Lepidosteus (Schreiner) . . .	7	„	2nd, 3rd, 4th.
Acipenser (Sewertzoff) . . .	7	„	2nd, 3rd, 4th.
Selachii (Gaupp)—			
Squalus acanthias . . .	—	Protometamer	—
Seyllium canicula . . .	—	„	4th, 5th, 6th, 7th, 8th (Neal).
			4th, 5th, 6th, 7th, 8th.

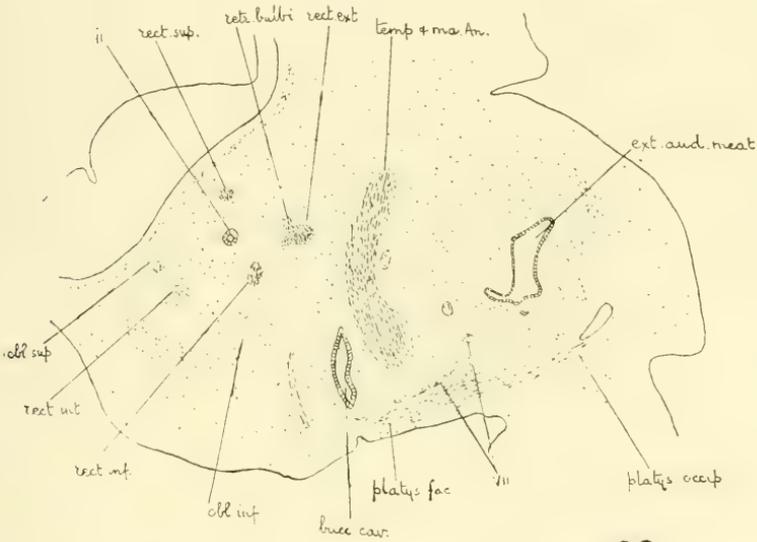
to be developed, or after development to atrophy, and that this takes place from before backwards. The non-development or atrophy affects dorsal more readily than ventral roots. Reduction, i. e. atrophy after development, of somites

or myotomes comes last. This general rule leads to hesitation in accepting the existence of anterior nerve roots without corresponding somites or myotomes as evidence of assimilated segments, e.g. deductions from the observations of Chiarugi and Martin in Mammals.

The theory of Fürbringer is based on the probability of the primitive nature of the conditions found in Selachians. But from the foregoing table of the observed number of assimilated spinal segments in various Vertebrates it would appear that the descriptive adjectives applied to some neocrania are not deserved. As determined by the number of assimilated spinal segments the Amphibian neocranium is shorter than that of Selachians. It was therefore maintained that the occipital region of Amphibians corresponds to a multiplum of spinal segments. The difficulty of doing so is emphasised by the absence of any direct evidence in its favour. If the muscles of the head in Amphibians and Selachians be compared it is clear that the condition in the former is far more primitive than in the latter, and that many cranial muscles of *Scyllium* pass through what may be regarded as an Amphibian stage during development; and if the observed facts in regard to the number of assimilated spinal segments be taken sans parti pris the condition of the skull tells the same tale. Fürbringer states that the junction of the skull and vertebral column is at the same place in Sauropsida and Mammalia; hence the five occipital nerves in Reptilian embryos are called v, w, x, y, z; and the three in Mammals x, y, z, so that the last assimilated nerve is the same—z. But in Mammals there appear to be only three assimilated somites, in Reptiles four or five. The argument drawn from the existence of a pro-atlas is probably of no great weight in determining the limits of the skull and vertebral column, for in *Sphenodon* (*loc. cit.*) that structure is the persisting costal process of the last coalescing vertebra, and the same may be true in Mammals without there being any but a serial homology between these last coalescing vertebræ.

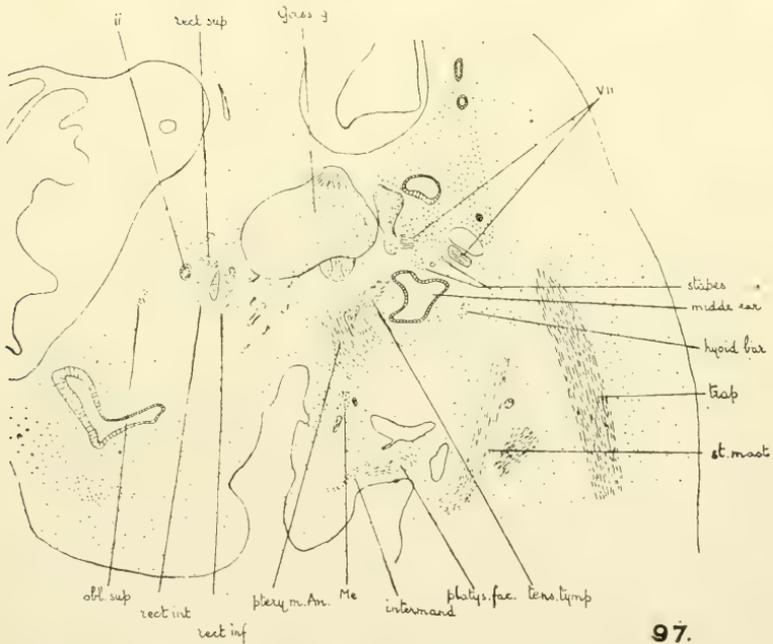
The conclusion which might be drawn from the number of

TEXT-FIG. 96.



96.

TEXT-FIG. 97.



97.

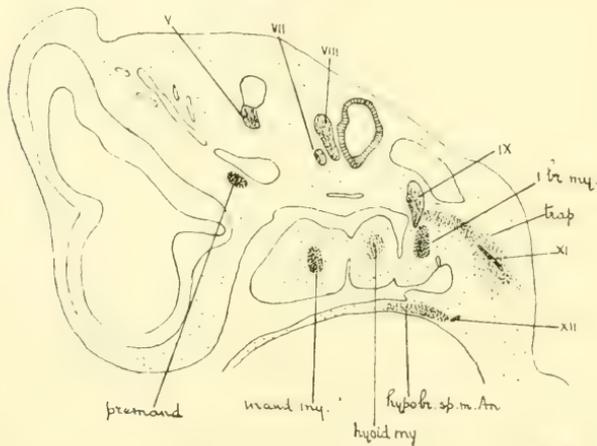
Text-figs. 96 and 97.—Rabbit, embryo 13 mm., longitudinal vertical sections. Text-fig. 96 is the more external.

coalescing spinal segments in Amphibia, Sauropsida, and Mammalia—viz. 2 or 3, 4 or 5, and 3—harmonises with the evidence of the cranial muscles, in which there is a closer similarity between Mammalia and Amphibia than between Mammalia and Sauropsida.

A SUGGESTED MORPHOLOGICAL CLASSIFICATION OF THE MOTOR CENTRES OF THE MID- AND HIND-BRAIN IN MAN.

Gaskell divided the motor centres of the cranial nerves

TEXT-FIG. 98.



98.

Fig, embryo 8 mm., longitudinal vertical section.

into two categories: (1) Somatic, a continuation of the anterior column of the spinal cord, innervating somatic muscles—IIIrd (external ocular muscles), IVth, VIth, VIIth (part which arises from the VIth nucleus), XIIth. (2A) Non-ganglionated splanchnic, a continuation of the lateral column of the spinal cord, innervating voluntary splanchnic muscles—Vth (motor descending root), Vth (motor), VIIth, IXth, Xth, XIth (part which arises from lateral horn). (2B) Ganglionated splanchnic, a continuation of Clarke's column—IIIrd (G. ciliare), VIIth (N.

intermedius with gang. genic.), IXth (gang. petros.), Xth, XIth (gang. trunci vagi), XIIth (gang. hypoglossi).

This classification of the motor centres, as regards those of voluntary muscles, followed v. Wijhe's theory of the morphology of the cranial muscles. It was also adopted by Strong and by Herrick.

According to Streeter the motor nucleus of the Vth nerve in man is developed in the lateral plate, and the nucleus ambiguus of the VIIth, IXth, and Xth in the basal plate.

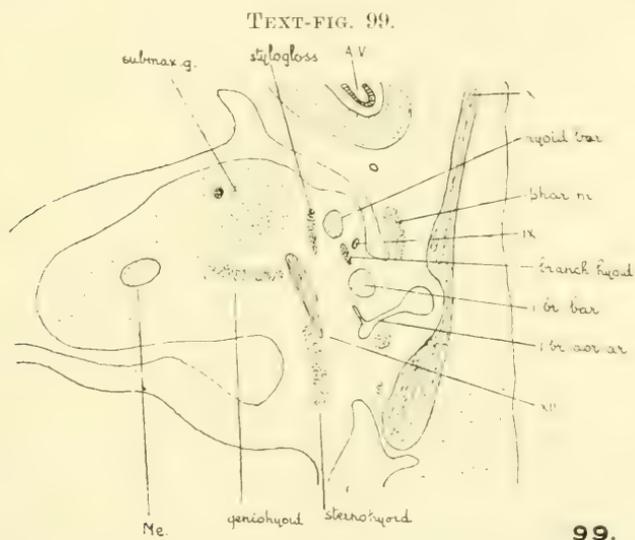


Fig. embryo 15 mm., portion of longitudinal vertical section.

The issuing fibres of the Vth pass straight outwards like those of the dorsal efferent fibres of the IXth, Xth, and XIth (medullary); whilst those of the VIIth, IXth, and Xth, arising from the nucleus ambiguus, have a characteristic curved path. The motor nucleus of the Vth is a hypertrophied representative of the dorsal motor nuclei of the IXth, Xth, and XIth (medullary), or the latter is represented in the mesencephalic root of the Vth.

Kappers showed that the original position of the VIIth, IXth, and Xth motor nuclei is medio-dorsal, and that the

ventral position of the nucleus ambiguus is only found in Mammals, where the importance of the ventral tegmentum is increased by the pyramidal tract, whilst a part keeps its original position near the mid-dorsal line because not very much influenced by the long descending tracts of the frontal parts of the brain.

It would result from a comparison of these researches that the ventral position of the VIIIth nucleus, and of the nucleus ambiguus of the IXth and Xth, is a secondary one, the curved path of their issuing fibres representing a phylogenetic descent of the whole or part of their nuclei; whilst the motor nucleus of the Vth has preserved its original position. This position is a dorso-median one. The nucleus of the XIth spinal occupies a more or less lateral position in the cervical cord, but, as shown by the development of the muscles it innervates, the nerve is a specialised branch of the Xth, the nucleus of which has extended backwards into the spinal cord.

The following classification of the motor nuclei of the cranial nerves is a repetition from a neurological point of view of the theory which has been advanced above concerning the morphology of the cranial muscles, and consequently stands or falls with it.

Somatic, innervating muscles derived from the myotomes of the cerebral and three anterior body segments; IIIrd (external ocular muscles), IVth (superior oblique), VIth (external rectus), Vth (temporal, masseter, pterygoids, tensor tympani, anterior digastric), VIIth (posterior digastric, stylohyoid, stapedius), IXth (interarcualis ventralis I s. branchiohyoideus, when present), Xth and XIth medullary (interarcualis ventralis III s. interthyroideus, in *Ornithodelphia*), XIth spinal (sterno-mastoid and trapezius), XIIth (hypo-branchial spinal muscles, and lingual muscles derived from the genio-hyoid). Splanchnic, innervating muscles derived directly or indirectly from the walls of the cephalic cœlon, i. e. part of motor nucleus of Vth, which innervates mylohyoid; part of motor nucleus of VIIth, which innervates facial and platysma muscles; part of motor nuclei of IXth, Xth, and

XIth medullary, which innervates tensor and levator palati, palato-glossus, stylopharyngeus, pharyngeal constrictor, laryngeal muscles, crico-thyroid.

The primary cranial nerves are the IIIrd, Vth, VIIth, IXth, and Xth; the Xth innervating in the rabbit two myotomes (second and third branchial), the others one each. The primary dorsal position of their motor nuclei (other than that of the IIIrd), the dorso-lateral emergence of their motor with their sensory fibres, and the relationship—external—of the issuing nerves to the corresponding myotomes, are related phenomena. If Balfour's theory, that the head and trunk became "differentiated from each other at a stage when mixed dorsal and sensory posterior roots were the only roots present," be associated with Fürbringer's theory that the myotomes primitively lay exclusively lateral to the notochord, it would follow that in the body region anterior nerve roots were secondarily developed in correlation with the upgrowth of the myotomes to the mid-dorsal line, and the posterior roots became exclusively, or almost exclusively, sensory. In the head, where this upgrowth does not take place, or to a very limited extent, a more primitive condition persists both in the position of the motor nuclei and the emergence of their efferent fibres.

A further, probable, distinction between the somatic muscles of the body and those of the head is that ganglionated muscle-sensory nerve-fibres pass to the former but not to the latter.¹

The position of the nucleus of the IIIrd nerve and the path of its nerve-fibres may be associated with the loss of cutaneous sensory fibres. Evidence of such loss and of a primitive dorso-lateral emergence of its nerve-fibres is found in the observation of Neumeyer that in the twenty-nine and forty-three hours old chick "der Nerven vom dorsalen Theile des Mittelhirns, also in der Gegend der Ganglionleiste seinen Ursprung nehmen, sich also sekundär mit seinem definitiven Abgangsort vereinigen."

¹ I hope to give the evidence for this in a future paper.

The Anlagen of the superior oblique and external rectus are developed from forward extensions of the upper ends of the mandibular and hyoid myotomes, and the IVth and VIth nerves may be regarded as, phylogenetically, late formations.

There do not appear to be any investigations on the existence of cell-groups in the Vth motor nucleus, which might correspond to the somatic and splanchnic muscles innervated. The nucleus contains a centre for the anterior digastric, but it is not known whether this migrates, during development, from the facial nucleus, or whether it is locally developed. The fibres of the Vth mesencephalic root join the motor root (Cajal), but it does not appear certain what structures it innervates.

The motor nucleus of the VIIth nerve consists, according to van Gehuchten and Marinesco, of four cell groups, three ventral and one dorsal: of these, the internal ventral is the centre for the stapedius, the middle for the auricular muscles, the external for the inferior facial muscles, and the dorsal nucleus for the superior facial muscles (*frontalis*, *corrugator supercilii*, and *orbicularis palpebrarum*). According to this account there is no special cell-group for the posterior digastric and stylohyoid, which seems unlikely. More recently, Kosaka has stated that the dorsal cell group in the fowl is the motor nucleus for the digastricus. The subject evidently needs further investigation.

The glosso-pharyngeal nucleus, according to v. Gehuchten, consists of a ventral cell-group only; according to Streeter it has a dorsal nucleus as well as a nucleus ambiguus. In the monkey (Beever and Horsley) it innervates the stylo-pharyngeus and (?) the middle constrictor of the pharynx. It is not known whether there is a separate cell-group for the branchio-hyoid in animals, e. g. pig, dog, where this muscle exists.

The Xth and XIth medullary are primitively, in the rabbit, the nerves of the second and third branchia¹ segments. The Xth efferent fibres arise from dorsal and ventral motor nuclei, those of the XIth medullary from a dorsal nucleus only (v.

Gehuchten). As all the fibres of the XIth medullary join the Xth, and all the fibres of the XIth spinal pass to the trapezius and sterno-mastoid,¹ it is a little questionable whether the old distinction of the two parts of the accessorius is worth preserving. The term XIth or accessorius might well be limited to what is now known as the XIth spinal. In a Mammal like the rabbit, where the whole of the second and third branchial myotomes (other than their dorsal ends which take part in forming the trapezius and sterno-mastoid) disappear during development, the Xth and XIth medullary motor centres contain none of the original somatic efferent fibres or cell-groups, and their new centres are those innervating muscles derived from cells proliferated from the wall of the cephalic cœlom. They also contain motor centres for certain visceral muscles which are developed in the body region.

The Xth and XIth medullary centres overlap antero-posteriorly the hypoglossal nucleus, probably owing to their backward extension into the first three segments of the spinal cord.

The XIth spinal is, as emphasised by Fürbringer, a true cerebral and not a spinal nerve. It innervates a special group of muscles which, in the rabbit, are derived from the upper ends of the three branchial myotomes. Its nucleus of origin is, from a phylogenetic point of view, a backward extension into the spinal cord of the (dorsal) nucleus of the XIth medullary, but it is not known what happens in embryonic development.

The hypoglossal nucleus is the motor centre of the hypobranchial spinal muscles, of the rectus system, developed from the first three body myotomes. Cell-groups corresponding to the upper, atrophying portions of these myotomes have been lost. It is not known whether the subdivision of the nucleus into the parts with large and with moderate-sized cells corresponds with individual muscles or muscle-groups. The hinder part of the hypobranchial spinal muscles has a

¹ In dog (*loc. cit.*) and man (Streeter).

secondary innervation from cervical segments—first, second, and third in man, first and second in the dog—but it is not known whether this is due to backward migration or to local development of motor neuroblasts.

ON THE SIZE OF THE MEDULLATED NERVE-FIBRES PASSING TO CRANIAL MUSCLES.

Gaskell stated that in the dog large fibres, 14·4 to 18 μ in diameter, were present in the IIIrd (external ocular muscles), IVth, VIth, VIIth (destination not traced), and XIIth. The corresponding muscles were considered to be somatic. Nerve-fibres not exceeding 10·8 μ in diameter were found in VIIIth (facial muscles), pharyngeal nerves, and recurrent laryngeal; and the corresponding muscles were considered to be splanchnic. Apparently he did not take the size of the nerve-fibres as the sole criterion of the somatic or splanchnic nature of a muscle, for the sterno-mastoid and trapezius were considered to be splanchnic, though the nerve (spinal XIth), showed the larger size of nerve-fibres. A further analysis (*loc. cit.*) of the size of nerve-fibres passing to cranial muscles in the dog shows that: (1) In any individual nerve, fibres are found of all sizes up to the largest present; (2) the nerve-fibres taper very slightly as they pass from the central nervous system to the muscles; (3) if comparison be made between the maximum size of the nerve-fibres and the morphological nature of the muscles to which they pass, the following results appear: (A) Nerve-fibres of the greatest size (17·6 μ in diameter,¹ in some dogs only 16 μ), are found in the nerves of the external ocular muscles, temporal, pterygoids, tensor tympani, digastric (both from Vth and VIIth), stylo-hyoid, branchio-hyoid, trapezius, sterno-mastoid, genio-hyoid, sterno-hyoid, sterno-thyroid, thyro-hyoid, and omohyoid—all of which, according to the theory

¹ This is also the maximum size of the nerve-fibres in the anterior roots of the non-limb portions of the spinal cord. In the limb areas it is slightly greater.

advanced above, are somatic in origin. (B) Nerve-fibres of a less maximum diameter ($12\cdot8 \mu$, in some dogs only $11\cdot2 \mu$), are found in the nerves of the mylohyoid, facial and platysma muscles, palatal, pharyngeal, and laryngeal muscles and cricothyroid — all of which, according to the theory advanced above, are splanchnic in origin; and also in the nerves of the lingual muscles, which are developed from the geniohyoid—a somatic muscle.

Herrick stated that the nerve-fibres of the branchial muscles of *Menidia* were characterised by their large size, and supposed—on the theory that these muscles were of splanchnic origin—that they had acquired this somatic feature. On the theory advanced above, however, the branchial muscles are somatic in origin.

The small size of the nerve-fibres of the lingual muscles is curious, but the muscles, though somatic in origin, have intimate relations to a splanchnic epithelium. This suggestion is supported by the measurements of the nerve-fibres passing to the geniohyoid and lingual muscles of *Lacerta viridis* and *Testudo mauritania*; in the former animal the maximum diameters found are $11\cdot6$ and $9\cdot6 \mu$ respectively, whereas in the latter animal both maxima are the same, viz. $7\cdot5 \mu$.

I have, in conclusion, to express many thanks to Prof. Salensky for embryos of *Acipenser*; to Prof. Bashford Dean for embryos and for the loan of sections of *Ceratodus*; to Prof. Graham Kerr for specimens of *Polypterus senegalus*; and to Prof. Fawcett for the loan of sections of the pig; also to the last-named for much kindness shown to me during many years in his laboratory.

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EXPLANATION OF REFERENCE LETTERS ON THE
TEXT-FIGURES.

abd. An. Abducens Anlage. *An. of sup. obl.* Anlage of obliquus superior. *abd. hyoid.* M. abdomino-hyoideus. *add. mand. M.* adductor mandibule. *add. mand. ext. M.* adductor mandibule externus. *add. mand. int. M.* adductor mandibule internus. *ant. dig. M.* digastricus

anterior. *arcualis dors.* M. arcualis dorsalis. *aur. temp. n.* Auriculo-temporal nerve. *br. add.* M. adductor arcus branchialis. *br. aor. ar.* Branchial aortic arch. *br. bar.* Branchial bar. *br. my.* Branchial myotome. *branch. hyoid.* M. branchio-hyoideus. *bucc. cav.* Buccal cavity. *ceph. cæl.* Cephalic cœlom. *cer. br.* Cerato-branchial cartilage. *cer. hy. ang.* M. cerato-hyoideus angularis. *cer. hyal. c.* Cerato-hyal cartilage. *first cerv. n.* First cervical nerve. *cæl.* Cœlom. *cons. colli.* M. constrictor colli. *cons. opere.* M. constrictor operculi. *cor. branch.* M. coraco-branchialis. *cor. hyoid.* M. coraco-hyoideus. *cor. mand.* M. coraco-mandibularis. *c₂vd.* Dorso-ventral muscular sheet in hyoid segment. *dil. lary.* M. dilatator laryngis. *dilat. opere.* M. dilator operculi. *dor. aor.* Dorsal aorta. *dorso-lary.* M. dorso-laryngeus. *epibr.* Epibranchial cartilage. *ext. aud. meat.* External auditory meatus. *extra-temp.* M. extra-temporalis. *Gass. g.* Gasserian ganglion. *gen. glossus.* M. genio-glossus. *gen. glossus and lingualis an.* Anlage of M. genio-glossus and lingualis. *gen. hyoid.* Genio-hyoid. *gill. m. An.* Anlage of muscles of external gill. *g.-c.* Gill-cleft. *hy. ceph. cæl.* Hyoid section of cephalic cœlom. *hyogloss.* M. hyoglossus. *hyogloss. and stylogloss. An.* Anlage of M. hyoglossus and M. styloglossus. *hyohy. inf.* M. hyo-hyoideus inferior. *Hyohy. sup.* M. hyo-hyoideus superior. *hyoid bar.* Hyoid bar. *hyoid my.* Myotome of hyoid segment. *hyoid aor. ar.* Hyoid aortic arch. *hyomax.* M. hyomaxillaris. *hyomax. lig.* Hyomaxillaris ligament. *hyomand. c.* Hyomandibular cartilage. *hypobr. c.* Hypobranchial cartilage. *hypohyal.* Hypohyal cartilage. *hypobr. sp. m. An.* Anlage of hypobranchial spinal muscles. *inf. lab. cart.* Inferior labial cartilage. *interarc. vent.* M. interarcualis ventralis. *interbas.* M. interbasalis. *interhyal.* Interhyal cartilage. *interhyoid.* M. interhyoideus. *intermand.* M. intermandibularis. *lary.* Larynx. *lev. br.* M. levator arcus branchialis. *lev. hyoid.* M. levator hyoidei. *lev. lab. sup. An.* Anlage of M. levator labii superioris. *lev. max. sup.* M. levator maxillæ superioris. *lev. pal. and tens. pal. An.* Anlage of levator and tensor palatini. *lingualis.* M. lingualis. *M. marg.* M. marginalis. *mand. aor. ar.* Mandibular aortic arch. *mand. ceph. cæl.* Mandibular section of cephalic cœlom. *mand. lab.* M. mandibulo-labialis. *mand. my.* Myotome of mandibular segment. *mand. seg.* Mandibular segment. *mass.* M. massetericus. *mylohyoid n.* Mylohyoid nerve. *Me.* Meckel's cartilage. *mental n.* Mental nerve. *N.* Olfactory epithelium. *nictat. m. An.* Anlage of nictating muscles. *obliq. dors.* M. obliquus dorsalis. *obliq. inf.* M. obliquus inferior. *obliq. sup.* M. obliquus superior. *obliq. vent.* M. obliquus ventralis. *œsoph. const.* Constrictor of œsophagus. *oper. fld.* Opercular fold. *orb. hyoid.* M. orbito-hyoideus. *pal. pr. of quad.* Palatine process of quadrate. *pal. quad.* Palato-quadrate. *pal. quad. Me.* palato-quadrate-mandibular arch. *phar.* Pharynx. *phar. br.* Pharyngo-branchial cartilage. *phar. clav. ext.* M. pharyngo-clavicularis externus. *phar. clav. int.* M. pharyngo-clavicularis

internus. *phar. m.* Pharyngeal muscles. *platys. colli.* Platysma colli. *platysma fac.* Platysma faciei. *platys. occip.* Platysma occipitalis. *premand. An.* Anlage of premandibular muscles. *post. dig., stylohy. and stap. An.* Anlage of posterior digastric stylohyoid and stapedius muscles. *proc. asc.* Processus ascendens of quadrate. *proc. bas.* Processus basalis of quadrate. *protr. hyom. M.* protractor hyomandibularis. *ptery. M.* pterygoideus. *quad.* Quadrate. *quad. ang. M.* quadrato-angularis. *rec. lary. n.* Recurrent laryngeal nerve. *rect. ext. M.* rectus externus. *rect. inf. M.* rectus inferior. *rect. int. M.* rectus interior. *rect. sup. M.* rectus superior. *retr. arc. br. M.* retractor arcuum branchialium. *retr. bulbi. M.* retractor bulbi. *retr. hyom. M.* retractor hyomandibularis. *retr. hyom. et operc. M.* retractor hyomandibularis et opercularis. *scap.* Scapula. *sh. girdle.* Shoulder girdle. *spl. meso.* Splanchnic mesoderm. *st. mast. M.* sterno-mastoideus. *sterno-hyoid. M.* sterno-hyoideus. *stylogloss. M.* styloglossus. *stylophary. M.* stylopharyngeus. *submax. M.* submaxillaris. *submax. g.* submaxillary gland. *subment. M.* submentalis. *suborb. c.* Suborbital cartilage. *subtemp. M.* subtemporalis. *susp. ang. M.* suspensorio-angularis. *temp. M.* temporalis. *temp. and mass. An.* Anlage of M. temporalis external pterygoid masseter. *tensor tympan. M.* tensor tympani. *trach.* Trachea. *trap. M.* trapezius. *tr. my.* Trunk myotome. *trans. vent. M.* transversus ventralis. *vent. aor.* Ventral aorta. *Roman numerals.* Cranial nerves.

A Monograph of the Tape-worms of the Sub-family Avitellininæ, being a Revision of the Genus Stilesia, and an Account of the Histology of Avitellina centripunctata (Riv.).

By

Lewis Henry Gough, Ph.D.,

From the Zoological Laboratories of the Universities of Basel, Switzerland, and of Leeds; England.

With Plates 12-14 and 6 Text-figures.

THE following paper was commenced at Leeds; it was originally intended only to give an account of the anatomy of *Stilesia hepatica*, Wolffhügel, which has been very imperfectly known until now, as much of the original description is not only incorrect but actually misleading. That section of this paper treating of *Stilesia hepatica*, Wolffhügel, was prepared at Leeds. I am much indebted to Prof. Garstang for his hospitality in placing his laboratory at my disposal and for the encouragement I received from him, and desire to express my thanks to him for it here.

As I was spending the winter at Basel, Switzerland, Prof. Zschokke kindly offered me a table in his laboratory, and suggested extending the scope of the paper I had commenced at Leeds so as to cover all the known species of the genus *Stilesia*; he also helped me to bring together the material required in order to make the paper complete. Its scope was again extended to include an account of the histology of *Avitellina* (*Tænia*) *centripunctata* (Riv.), on account of several histological peculiarities of the worm, which seem to throw a new light on the problems connected

with the structure of the Cestodes, and because my material was in a much better state of preservation than is usually seen. Excepting the account of the histology and anatomy of *Stilesia hepatica*, Wolffhügel, the rest of this paper has been prepared at Basel.

I feel great pleasure in expressing my thanks to Prof. Zschokke for his hospitality and assistance.

My thanks are also due to Prof. Railliet, of Alfort, who placed not only the original material of *Stilesia vittata*, Railliet, at my disposal, but also more recent specimens of both that species and of *Stilesia globipunctata* (Rivolta). I must also thank Prof. Colin, of the Natural History Museum of Berlin, for the loan of the type-specimens of *Stilesia hepatica*, Wolffhügel, and Prof. Fuhrmann, of Neuchâtel, for having kindly re-examined his specimens of *Stilesia sjöstedti* at my request and for the loan of his type-specimens, thus enabling me to fix its true systematic position, and for procuring material of *Dibothriocephalus* and *Trienophorus* for me.

To my friend Dr. O. Huber I am indebted for the delineation of figs. 5, 6, 7, and 12, and desire to express my thanks to him here.

I have divided this paper into two chapters. The first deals with the systematic revision of the genus *Stilesia*; the second is an account of the histology of *Avitellina centripunctata* (Riv.).

REVISION OF THE GENUS *STILESIA*, RAILLIET.

The material employed in connection with this revision of the genus is derived from the following sources:

Stilesia globipunctata (Rivolta).

(1) From the small intestine of a goat, collected in British India by Leese, from Prof. Railliet's collection (No. P196^b).

(2) From the small intestine of a sheep, collected in France, from Prof. Railliet's collection (No. P191).

Stilesia vittata, Railliet.

(1) From the small intestines of a dromedary, collected at Alfort, April 22nd, 1896, from Prof. Railliet's collection (No. P196). Type.

(2) From the small intestines of a dromedary, collected at Alfort, May 27th, 1906, from Prof. Railliet's collection (No. P192).

(3) From the small intestine of a dromedary, collected in British India by Leese, 1909, from Prof. Railliet's collection (No. P713⁵⁰).

Stilesia hepatica, Wolffhügel.

(1) From the bile-ducts of sheep and goats, collected in German East Africa, belonging to the Natural History Museum, Berlin. Type.

(2) From the bile-ducts of sheep, collected in the Lydenburg District, Transvaal, belonging to the Natural History Museum, Berlin. Co-type.

(3) From the bile-ducts of sheep, collected at Pretoria, Transvaal, 1909 (author's collection).

Stilesia Sjöstedti, Fuhrmann.

(1) From the bile-ducts of *Hippotragus equinus*, collected in North-east Rhodesia (author's collection).

(2) From *Tragelaphus silvaticus mernensis*, collected by the Sjöstedt expedition (Fuhrmann's type).

Avitellina centripunctata (Rivolta).

(1) From the small intestines of sheep, collected at Pretoria, Transvaal, 1909 (author's collection).

HISTORY OF THE GENUS *STILESIA*.

The genus *Stilesia* was proposed by Railliet (1893) to include two species of tape-worm from the small intestines of sheep, which had been described by Rivolta in 1874 as *Tænia globipunctata* and *Tænia centripunctata*.

The best description of these two species available hitherto was by Stiles (1893), who also revised the generic diagnosis, basing his revision on the then known data, though evidently not feeling quite sure as to the desirability of leaving both species in one genus.

In 1896 Railliet described a new species, *Stilesia vittata*, from the intestines of a dromedary; he considered this species to be very closely allied to *Stilesia globipunctata* (Riv.), and, perhaps, only to be a variety.

In 1903 another new species, closely related to *Stilesia globipunctata* (Riv.), was described by Wolffhügel, from the bile-ducts of sheep and goats in South and East Africa, as *Stilesia hepatica*.

In 1906 Tempère briefly refers to *Stilesia centripunctata* and figures its scolex, apparently only quoting from Railliet (1893) and Neumann (1893).

In 1908 Gough states briefly that *Stilesia hepatica*, Wolffhügel, is usually not double-pored.

In 1909 Fuhrmann places *Stilesia* and *Thysanosoma* in a new sub-family, the *Thysanosominae*.

In 1909 Fuhrmann describes a new species from *Tragelaphus sylvaticus mernensis*, collected by Dr. Sjöstedt on the Masai steppes, as *Stilesia sjöstedti*.

In 1909 Gough gives a full description of the anatomy of *Stilesia centripunctata* (Rivolta), with remarks on *Stilesia hepatica*, Wolffhügel.

At present, therefore, the genus contains the following five species:

Stilesia centripunctata (Rivolta), 1874; *Stilesia globipunctata* (Rivolta), 1874; *Stilesia vittata*, Railliet, 1896; *Stilesia hepatica*, Wolffhügel, 1903; *Stilesia sjöstedti*, Fuhrmann, 1909.

The last four species agree very closely in their anatomy; the first differs from all the others in several important respects of generic value. A new genus will therefore have to be proposed for *Stilesia centripunctata*.

Stilesia globipunctata (Riv.) is the type species of the

genus *Stilesia*; this species and *Stilesia vittata*, Railliet, were described as having irregularly alternating genital pores. *Stilesia hepatica*, Wolffhügel, and *Stilesia sjöstedti*, Fuhrmann, have been described as double-pored; they do not, however, differ from the type species in this respect, all four being without doubt single-pored.

The generic diagnosis, as revised by Stiles (1893), reads:

“*Stilesia*, Railliet, 1893. Type species, *S. globipunctata* (Riv.), Railliet, 1893. Head with four suckers, but no hooks. Strobila thin and narrow. Genital pores irregularly alternate. Segments broader than long. Two distinct sets of testicles present in each segment, one on each side, but no testicles in the median line. Eggs very small and with but one shell.

“The following points, which may prove to be of generic value, have been established only for *S. globipunctata*: Genital canals pass dorsally of nerve and ventral canal, but ventrally of dorsal canal. Egg-shell with two conical projections at opposite poles.

“Habitat: Intestine of sheep. Development unknown.”

The generic description can now be amplified to some extent and also altered in some respects.

Stilesia, Railliet, 1893. Type species, *Stilesia globipunctata* (Rivolta), Railliet, 1893. Head with four suckers, but without hooks. Strobila thin and narrow. Genital pores irregularly alternate. Segments broader than long. Two distinct sets of testicles present in each segment, one on each side, but no testicles in the median line. Ovary on the pore side. No vitelline gland, no shell-gland. Uterus double, finally void of eggs, which are contained in egg-pouches (paruterine organ). The genital canals pass dorsally of the nerve and of the ventral canal, and ventrally of the dorsal canal. Eggs with two envelopes. Habitat: Intestine of sheep, goat, and dromedary, and bile-ducts of sheep, goat, and South African wild antelopes (Africa, India, Italy, France).

In the genus as thus restricted, only *St. globipunctata*

(Riv.), *vittata*, Railliet, *hepatica*, Wolffhügel, and *sjöstedti*, Fuhrmann, remain. For *Tænia centripunctata*, Rivolta, a new genus must be erected, for which I propose the name *Avitellina* (to denote the absence of a vitelline gland).

Avitellina, nov. gen. Type species, *Avitellina centripunctata* (Rivolta). Head with four suckers, but without hooks. Strobila thin and narrow. Segments broader than long, flat in the proximal portion of the strobila, nearly cylindrical in the posterior portion. Genital pores irregularly alternate. Four distinct sets of testicles in each segment, one right and one left of each longitudinal canal, but no testicles in the middle field. Ovary nearer the pore side; no vitelline gland, no shell gland; a single uterus. Eggs finally enclosed in egg-pouches (paruterine organ). The genital canals pass dorsally of the nerve and longitudinal canals. Eggs with two envelopes. Habitat: Intestine of sheep, Africa, Italy.

The genera and the hitherto described species of *Stilesia* and *Avitellina* can be recognised by the following key:

(1) Uterus single; a single paruterine organ; testicles in four groups; the genital canals pass dorsally of the dorsal canal. *Avitellina*, 4.

Uterus double; two paruterine organs; testicles in two groups; the genital canals pass ventrally of the dorsal canal. *Stilesia*, 2.

(2) Testicles all lateral to the ventral canal. 3.
Testicles mostly median or dorsal to the ventral canal.

St. hepatica, Wolffhügel.

(3) The vas deferens forms a dense packet of convolutions (functionally a vesicula seminalis) between nerve and ventral canal before reaching the cirrus pouch; inhabits the small intestines of the dromedary. *St. vittata*, Railliet.

The vas deferens forms at the most three or four loose convolutions between the nerve and the ventral canal before reaching the cirrus pouch; inhabits the intestines of sheep and goat. *St. globipunctata* (Rivolta).

(4) The vas deferens runs its entire length dorsal to the

testicles; length two to three metres; inhabits the intestine of sheep. Only known species: *A. centripunctata* (Rivolta).

STILESIA HEPATICA, WOLFFHÜGEL, 1903. Figs. 14-16;
Text-fig. 1.

Synonymy.

Stilesia hepatica, Wolffhügel, 1903.
Stilesia hepatica, Wolffhügel, Gough, 1908.
Stilesia sjöstedti, Fuhrmann, 1909.

Literature.

Wolffhügel.—“*Stilesia hepatica* nov. spec. ein Bandwurm aus den Gallengängen von Schafen und Ziegen Ostafrikas,” ‘Berliner Tierärztlichen Zeitschrift,’ 1903, No. 43.

Gough.—“Notes on South African Parasites,” ‘S.A.A.A.S.,’ Grahamstown, 1908.

Gough.—“The Anatomy of *Stilesia centripunctata* (Rivolta),” ‘The Veterinary Bacteriological Laboratories of the Transvaal,’ Pretoria, 1909.

Fuhrmann.—“Cestodes,” ‘Schwedische Expedition nach dem Kili-mandjaro,’ 1909.

Habitat.—Bile-ducts of sheep, goats, and wild ruminants in South, East, and Central Africa.

[Note.—Although *Stilesia hepatica*, Wolffhügel, is not the type species of the genus, I propose to consider it first, as its anatomy is very much better known than that of *Stilesia globipunctata* (Rivolta), the type species; the anatomy of all known species of *Stilesia* is, as far as yet worked out, very constant, only differing in minor points. As a description at full length is necessary only for one of the species, only the points in which the other three differ will be found under their respective headings.]

Stilesia hepatica was described in 1903 by Wolffhügel as being double-pored, and as differing chiefly in that respect from *Stilesia globipunctata* (Rivolta).

When working in the Transvaal I repeatedly had to deal with a *Stilesia* infesting the bile-ducts of sheep, which I identified with *Stilesia hepatica*, Wolffhügel, although

all the specimens that passed through my hands were invariably single-pored. In 1908, in a paper read before the South African Association for the Advancement of Science, at Grahamstown, C.C., I stated that *Stilesia hepatica*, Wolffhügel, was single-pored, and that the original description given by the author was at fault. Since then, by the kindness of Prof. Colin, I have been able to examine the type specimens of *Stilesia hepatica*, Wolffhügel. There is no possible doubt; the type specimens are certainly single-pored, with irregularly alternating pores. The anatomy of the worm differs considerably also in other respects from the data given by Wolffhügel. In the following the anatomy of the worm is given entirely on my own observations on fresh material, supplemented by re-examination of the type.

The worm invariably inhabits the bile-ducts, never the intestine. It occurs in sheep, goats, duiker (*Cephalopus*), roan antelope (*Hippotragus equinus*), *Hippotragus sylvaticus mernensis*, fide Fuhrmann, and various other wild ruminants occurring in South, East, and Central Africa. The scolex is almost invariably lodged in the peripheral capillaries of the bile-ducts. The parasites are often present in large numbers, dilating the bile-ducts; their presence does not cause calcification of the ducts, as *Distomum hepaticum*, L., does, but only a thickening of the tissues of the ducts. They appear to do otherwise but little injury to the host; almost all adult sheep in the Transvaal are affected.

Stilesia hepatica, Wolffhügel, is probably primarily parasitic in wild ruminants, and can be supposed to have adapted itself secondarily to sheep. The absence of records of the conspicuous parasite from other parts of the world, its occurrence in the wild antelopes, which are so characteristic of the Ethiopian region, and the wide range in its choice of hosts, would seem to speak for the probability of its not being originally a parasite of sheep.

Stilesia hepatica, Wolffhügel, is extremely contractile, more so than most other cestodes I have handled hitherto.

As one very rarely succeeds in extracting a worm entire its total length is very difficult to estimate, but it is probably between twenty and fifty centimetres. In life, when expanded, it is thin, gelatinous in appearance, semi-transparent, the edges of the strobila being serrated on account of the projection of the posterior angles of the segments. Against a black surface, the middle field appears clear, the lateral fields more or less opaque. In older segments in the posterior portion of the strobila, the uteri and paruterine organs show up as an opaque spot on each side of each proglottid; when contracted, the worm is thick, with frilled edges, and more or less opaque.

The scolex has four suckers, directed outwards and forwards. Very frequently the head is followed by what appears to be a thick "neck," 2 mm. in length, as broad as, or even slightly broader, than the scolex; behind this "neck" the strobila suddenly narrows to half the width. Examinations of the "neck" (in sections), however, reveals the fact that it is composed of young segments, and consequently belongs to the strobila and not to the scolex. The contraction of the first two millimetres of the strobila is of extremely regular occurrence, so much so as to cause remark, when one, as occasionally happens, comes across a worm not contracted in this way. As the scolex is usually lodged in a capillary of the bile-duct the swelling of the anterior portion of the strobila can be of use to the worm as an aid to the suckers, helping to anchor the worm by gripping the sides of the duct. Wolffhügel figures a scolex in his paper, which he states may belong to *Stilesia hepatica*; although the scolex in question is not followed by the contraction of the anterior portion of the strobila, I see no reason to doubt its belonging to this species.

The swelling of the portion of the strobila directly posterior to the scolex in *Stilesia hepatica*, Wolffhügel, on account of its probable function, can probably be compared to the pseudo-scolices of *Idiogenes* and *Fimbriaria*, and be considered as representing the first step towards the

acquisition of a pseudo-scolex. A fundamental difference is, however, that in *Stilesia* all segments must have passed through the pseudo-neck during the course of their development, whereas it is usually accepted that a true pseudo-scolex is formed after the fertile segments have been produced, and that the segments composing a pseudo-scolex remain sterile. The habit of contracting the youngest segments appears to be an old acquisition in the genus *Stilesia*; a scolex of *Stilesia globipunctata* (Rivolta), is illustrated in fig. 12, showing a similar contraction of the anterior portion of the strobila, though in a less degree.

The segments are much shorter than wide, and about twice as wide as thick. The width of the strobila varies from one to two or three millimetres. The posterior segments are longer than the anterior. The posterior margin of each segment surrounds, collar-like, the anterior end of the following, except at the middle of the segment. Segmentation is quite distinct (without sectioning) at 2.8 mm. from the scolex; the genital anlagen appear already at 9 mm.

The genital pores open near the middle of the lateral margin of the segment; they are single and irregularly alternate.

The cuticula does not appear smooth (as that of *Avitellina centripunctata* [Rivolta]), but is villous (in sections).

The longitudinal canals are both well developed. The lumen of the dorsal canal does not become obliterated. The ventral canal is situated lateral and ventral to the dorsal canal. At the posterior end of each segment transverse canals connect the ventral canals, forming a transverse ring, the dorsal and ventral branches forming a few anastomoses near the middle of the segment. The transverse canals arise from more than two, usually three or four openings in the ventral canal on its median side, and two or three lateral to the ventral canal; these last usually meet and form a lateral loop. The histology of the transverse canals is the same as of the ventral canal. They both have a thin membrane, produced by flat epithelial cells, surrounding the canal; these

but one pore, the pores being irregularly alternate. There is also only a single ovary to each segment, not two; and finally the arrangement of the testicles and vas deferens is quite different from what Wolffhügel described.

There are ten to twelve testicles on each side of the segment (fig. 13 and Text-fig. 1); they lie dorsally between the ventral and the dorsal canals, and dorsal to the ventral canal. Seen from the dorsal side of a total mount, the testicles lie in two or three rows of about four or five (sometimes six) in a row. On transverse sections (Text-fig. 1), one only sees a single row. The diameter of a testicle is about 50 to 55 μ . The vasa efferentia arise on the dorsal side of the testicles, as do the vasa deferentia. The vas deferens of the testicles on the right side of the proglottid runs from right to left, the vas deferens collecting from the left group of testicles from left to right; at the middle of the proglottid the left and right branches meet and join to form a common vas deferens which bends ventrally, and, having passed into the depth of the proglottid past the testicles, turns towards the pore side of the segment. It passes the dorsal canal ventrally, the ventral canal and nerve dorsally. Before reaching the cirrus pouch it forms a number of twists, whose function is that of a vesicula seminalis; these lie above the ventral canal. The cirrus pouch (fig. 16) is oblong, measuring 83 μ by 50 μ , the diameter of the cirrus 16.5 μ . Cirrus and vagina open into a wide and deep genital cloaca, whose aperture is situated near the middle of the segment.

The female organs also differ considerably from Wolffhügel's statements (fig. 13). There is but one ovarium, lying on the pore side, between the ventral and dorsal canals, nearer to the ventral than to the dorsal canal. The uterus is double, one uterus lying close to the ovarium, the other on the other side of the proglottid in the corresponding position. The two uteri are connected by a duct, the inter-uterine duct, which, however, may be morphologically but the median portion of the uterus. This duct crosses the ventral field ventral to the dorsal canal. The ovarium contains very few, at

the most fifty, eggs, measuring $14\ \mu$ in diameter; it atrophies very rapidly after the appearance of the uterus. (Wolffhügel's figure only shows the uteri, which have been erroneously labelled ovarium by him.) The ovarium measures $86\ \mu$ in diameter.

The oviduct, the uterine duct and the canalis seminalis meet a short distance from the receptaculum seminis, as in *Avitellina*. There is no vitelline gland, nor shell-gland. The function of the missing yolk-cells is exercised by abortive eggs in the ovarium (ovarial nutritive cells, see p. 371), and by cells derived from the uterine walls (uterine nutritive cells, see p. 375). The eggs in the uterus are firmly embedded in the uterine nutritive cells, as has also been observed by Fuhrmann (1909), who already suggested that their function is probably nutritive; however, contrary to his supposition, the uterus is originally hollow. The uterus measures $50\ \mu$ to $86\ \mu$; the eggs are finally enclosed in paruterine organs similar to the paruterine organ described as occurring in *Avitellina* (see p. 375). The paruterine organ arises within the uterus; each segment contains two paruterine organs, one within each uterus. They measure $50\ \mu$ to $86\ \mu$. The uteri and later on the paruterine organs are connected by a band of fibrous tissue, which covers the uterus anteriorly, passes through into the median field and tapers off towards the middle of the segment, the two halves of the band meeting in the middle. Their course across the segment is not quite direct, the middle portion drooping towards the posterior end of the proglottid. The eggs are enclosed in two envelopes, the outer of which invariably appears wrinkled whilst the inner is always smooth and rounded. The inner envelope seems to possess a prolongation at each pole (perhaps due to optical delusion and not existent; it is almost impossible to get rid of the outer envelope so as to examine the inner properly). The eggs, measured over the outer shell, are $26\ \mu$ long by 16 to $19\ \mu$ broad, the embryo 15 to $16\ \mu$.

Wolffhügel states the size of the eggs as $26\ \mu \times 16\ \mu$, Fuhrmann as $16\ \mu$ (evidently only the embryo)!

Calcareous bodies are frequent in this species, as also in *Stilesia globipunctata* (Rivolta), and *Stilesia vittata*, Railliet; they measure on an average 10μ in diameter, and are most frequent in the axis of the scolex, and at the posterior end of the segments. No calcareous corpuscles were observed in the type of *Stilesia sjöstedti*, Wolffhügel, but here, as elsewhere, this is probably only due to individual variation.

STILESIA GLOBIPUNCTATA (RIVOLTA), 1874. Figs. 10, 11, 12.

Synonymy.

- Tænia globipunctata*, Rivolta, 1874.
Tænia ovipunctata, Rivolta, 1874.
Stilesia globipunctata (Rivolta), Railliet, 1893.
Stilesia globipunctata (Rivolta), Stiles, 1893.

Literature.

- Rivolta.—‘Sopra alcune specie di *Tænia* della Pecora,’ Pisa, 1874.
 Perroncito.—‘I Parassiti dell’Uomo e degli Animali Utili,’ Milano, 1882.
 Perroncito.—‘Trattato teorico-pratico sulle malattie piu comuni degli Animali domestici,’ Torino, 1886.
 Railliet.—‘Elements de Zoologie Médicale et Agricole,’ Paris, 1886.
 Neumann.—‘Traité des Maladies parasitaires non-microbiennes des Animaux domestiques,’ Paris, 1888 (1st edition).
 Neumann.—“Observations sur les *Ténias* du Mouton.” ‘C. R. Soc. Hist. Nat., Toulouse,’ 1891.
 Neumann.—‘Traité,’ 2nd edit., 1892.
 Stiles.—“Bemerkungen über Parasiten 17. Über die topographische Anatomie des Gefäss-systems in der Familie *Tæniadae*,” ‘Centralblatt für Parasitologie,’ 1893.
 Stiles.—‘Adult Cestodes of Cattle, Sheep, and allied Animals,’ Washington, 1893.
 Railliet.—‘Elements,’ 2nd edit.
 Railliet.—“Sur quelques parasites du Dromadaire,” ‘C. R. Soc. Biol.,’ 1896.
 Perroncito.—‘Trattato teorico pratico,’ 2nd edit., 1902.
 Wolffhügel.—“*Stilesia hepatica* nov. spec. ein Bandwurm aus den Gallengängen von Schafen und Ziegen Ostafrikas,” ‘Berliner Tierärztlichen Wochenschr.,’ 1903, No. 43.
 Fuhrmann.—“Cestodes,” ‘Wissenschaftliche Ergebnisse der Schwe-

dischen zoologischen Expedition nach dem Kilimandjaro, dem Meru und den Umgebenden Masaisteppen deutsch Ostafrikas, Stockholm, 1909.

Habitat.—Small intestine of sheep and goats. (Linstow's record from cattle in 'Compendium der Helminthologie' is a printer's error, as he is there citing Rivolta, who described the worm from sheep.

Geographical Distribution.—Italy, Rivolta, 1874; India (Giles fide Stiles, 1903—also specimens in Railliet's collection examined by the present author); France, Railliet, 1896.

For the following description I have had to rely considerably on Stiles (1893), the material at my disposal being rather badly macerated.

The worms are stated to be transparent, gelatinous in appearance when living, resembling *Stilesia hepatica*, Wolffhügel, in this respect.

The length varies from 45 to 60 cm., Railliet, 1896. The widest segments are 2.5 m.m. broad, the anterior and posterior being much narrower.

The scolex is square when viewed en face; it measures 0.768–0.9 mm. in diameter. The suckers (fig. 12) are directed anteriorly and diagonally; their opening is round or oval. The anterior portion of the strobila is sometimes contracted, as is more frequently the case in *Stilesia hepatica*, Wolffhügel.

The proglottids are always much broader than long, but in the posterior portion of the strobila are comparatively longer than in the middle of the worm. The middle portion is very frequently much contracted, the outline becoming crenate and twisted.

There are four to seven testicles on each side, lying lateral to the ventral canal, median to the nerve (fig. 10). The ovarium lies on the pore side, just median to the ventral canal; the uterus is double, one lying dorsal to the ovarium, the other close to the ventral canal of the other side of the body. The vagina lies dorsal to the cirrus pouch; it crosses the ventral canal dorsally; median to the ventral canal it increases

in size and forms a receptaculum seminis. The median end of the receptaculum seminis forms two branches, one of which, the oviduct, goes to the ovarium, the other, the uterine duct, goes to the uterus. (Do these two ducts arise directly from the receptaculum or from a canalis seminalis as in the other members of the group?) The uteri of both sides are probably connected as in *St. hepatica*, Wolffhügel, by an interuterine duct. The cirrus pouch is pyriform, 56μ long by 40μ broad, the cirrus 50 to 60μ long. Cirrus and vagina open into a large and wide cloaca, which is directed diagonally lateral and forwards, opening near the anterior angle of the segment. Stiles observed the vas deferens to run from the cirrus-pouch anteriorly of the testicles of the pore side, dorsally of the ventral canal and female organs, ventrally of the dorsal canal, then through the median field, lying anterior and dorsal to the transverse canal; it is further stated to cross the dorsal canal (of the opposite side) ventrally, the ventral canal dorsally, and to be finally lost in the testicles. Should this last be correct, it would be a totally different course to that of the vas deferens in *Stilesia hepatica*, Wolffhügel; fresh material will have to decide this point.

The ovary contains but few eggs; there is no vitelline gland, and no shell-gland. The eggs enter the uterus fertilised; in the uterus they are surrounded by nutritive cells, as in *St. hepatica*, Wolffhügel. The eggs are finally enclosed in a paruterine organ. They have two envelopes, an outer wrinkled fusiform and an oval inner one; the inner one is devoid of spines (fig. 11). The spines in Stiles's figure are probably the shrivelled outer envelope. The eggs measure $56 \mu \times 27 \mu$ over the outer, $27 \mu \times 22 \mu$ over the inner envelope, 14μ across the embryo.

The uteri of both sides, and later on the paruterine organs, are in contact with a band of fibrous tissue, which "extends partially around the uterus, crosses the dorsal canal ventrally, and tapers off into a fine point, which runs through the median field to meet," and is continuous with, the corresponding organ of the other side (Stiles, 1893, p. 78).

STILESIA VITTATA, RAILLIET, 1896. Figs. 8, 9.

Synonymy.

Stilesia vittata, Railliet, 1896.

Literature.

Railliet.—“Sur quelques parasites du Dromadaire,” ‘C. R. Soc. Biol.,’ 1896, p. 491.

Habitat.—Small intestine of dromedary.

Geographical Distribution.—India. (Algiers? The type was collected in Alfort in a dromedary that died there, and a second batch was collected at the same place about two weeks after the first.)

Stilesia vittata, Railliet, so closely resembles *Stilesia globipunctata* (Rivolta), that Railliet, after describing the species, states that it may be only a variety of *Stilesia globipunctata* (Rivolta). However, certain constant differences can be found, if one may rely on Stiles’s description of *Stilesia globipunctata* (Rivolta), and there appears to me to be no reason to doubt the correctness of that most accurate observer.

The worm has the same appearance (judging from formalin material) as *Stilesia hepatica*, Wolffhügel, and as *Stilesia globipunctata* (Rivolta). Its length is stated as being 18 to 23 cm., its breadth 1 mm. to 1.3 mm. In shape the scolex is similar to that of *Stilesia globipunctata* (Rivolta); however, when viewed en face the breadth (latero-lateral measurement) is somewhat greater than its thickness (dorso-ventral measurement). Its length is shorter than the breadth or thickness.

Three scolices measured were :

Broad .	(1) 0.60 mm.	. (2) 0.54 mm.	. (3) 0.55 mm.
Thick .	(1) 0.525 mm.	. (2) 0.48 mm.	. (3) 0.48 mm.
Long .	(1) 0.55 mm.	. (2) 0.375 mm.	. (3) 0.48 mm.

The shape of the proglottids is similar to that of the other two species, the posterior border of each segment overlapping the anterior end of the next proglottid in the same way.

There are five to seven testicles on each side, lying lateral to the ventral canal. The entire course of the vas deferens has not been made out, but the outer half of it runs ventral to the dorsal canal, and dorsal to the ventral canal and nerve. Between ventral canal and nerve the vas deferens forms a number of very close and densely packed convolutions, whose function is without doubt that of a vesicula seminalis (fig. 8, *v. s.*). In a "teased" specimen this packet of convolutions comes away entire. It appears almost to be enclosed in a membrane, but the material was too macerated to make quite sure.

The cirrus pouch measures 75μ in length; it opens into a genital cloaca, which is directed laterally and anteriorly, and opens near the anterior angle of the proglottid.

The position of the female organs and their arrangement appears to be the same as in *Stilesia globipunctata* (Rivolta), the band of fibrous tissue between the uteri is, however, somewhat more strongly developed.

The muscles are arranged in two layers, the inner being composed of bundles of five to nine; the outer of only one to three.

The eggs have two envelopes, an outer shrivelled one and an inner oval or rounded one. They measure $38\mu \times 24\mu$ over the outer, 22μ over the inner envelope, the embryo measuring about 14μ (fig. 9). (Railliet's measurements were $14-17\mu \times 13-17\mu$.) The inner envelope is not provided with spines of any kind.

AVITELLINA CENTRIPUNCTATA (RIVOLTA), 1874. Figs. 1 to 9,
17 to 35, 37 to 65. Text-fig. 2.

Synonymy.

- Tania centripunctata*, Rivolta, 1874.
Stilesia centripunctata (Rivolta), Railliet, 1893.
Stilesia centripunctata (Rivolta), Stiles, 1893.
Stilesia centripunctata (Rivolta), Gough, 1909.

Literature.

- Rivolta.—'Sopra alcune specie di *Tania* della Pecora,' Pisa, 1874.
 Perroncito.—'I Parassiti dell'uomo e degli Animali Utili,' Milano, 1882.

Perroncito.—'Trattato teorico-pratico sulle malattie piu comuni degli Animali domestici.' Torino, 1886.

Railliet.—'Éléments de Zoologie Médicale et Agricole,' Paris, 1886 (1st edition).

Neumann.—'Traité des Maladies parasitaires non-microbiennes des Animaux domestiques,' Paris, 1888 (1st edition).

Neumann.—'Observations sur les Ténias du Mouton,' 'C. R. Soc. Hist. Nat., Toulouse,' 1891.

Neumann.—'Traité,' 1892 (2nd edition).

Railliet.—'Éléments,' 1893 (2nd edition).

Perroncito.—'Trattato teorico-pratico,' 1902 (2nd edition).

Tempère.—'Parasites internes de l'homme et des Animaux domestiques,' 'Micrographe Préparateur,' vol. xiv, 1906, p. 27.

Gough.—'Notes on some South African Parasites,' 'S.A.A.S.S.' Grahamstown, 1908.

Gough.—'The Anatomy of *Stilesia centripunctata* (Rivolta),' 'The Veterinary Bacteriological Laboratories of the Transvaal,' Pretoria, 1909.

Habitat.—Small intestine of sheep.

Geographical Distribution.—Italy (Rivolta, 1874); Algiers (Railliet, 1891); South Africa (Gough, 1908).

In life the worm has a gelatinous, semi-transparent appearance. The strobila from 10 cm. from the scolex on appears longitudinally; there is a median opaque line, flanked on either side by a very transparent line (caused by the enormous ventral canals); laterally on each side the worm is again somewhat less transparent.

Avitellina centripunctata (Rivolta) reaches 202 cm. to 285 cm. in length. The greatest breadth is frequently, but not always, near the scolex. The breadth varies from 1 mm. to 3 mm. (or even 4 mm., Railliet). The anterior and middle of the strobila is flat, the posterior end is round or elliptical on section.

The scolex is large (fig. 7); in my specimens the suckers are invariably directed diagonally outwards and forwards. Railliet and Tempère, however, figure it with the suckers directed anteriorly. The scolex is usually, but not always, broader than long; it measures from 1.5 mm. to 2.8 mm. broad by 1.5 mm. to 3.1 mm. long.

The segments are always much broader than long, and usually also much broader than thick (except in the posterior portion of the strobila). The extreme brevity of the segments causes the genitalia at male maturity all to lie in one plane, single transverse sections 4μ thick then often presenting the whole anatomy, as in a diagram. When the paruterine organ develops in the terminal portion of the strobila, the anterior and posterior surfaces of the segments are no longer flat, but are arched above the paruterine organ, bulging thus into the segments nearest in front and behind, and receiving depressions from the pressure of the paruterine organs of the segments anterior and posterior to it (fig. 3). Except at the posterior end of the strobila, the hind end of a proglottid does not surround the anterior end of the next.

The genital pores alternate irregularly; they are very slightly developed as compared to those of the *Stilesiæ*.

Calcareous corpuscles are extremely rare; two only have been observed in over one hundred series of sections.

The longitudinal muscles are apparently arranged in bundles of twenty-four or more, and a few solitary muscles are seen close to the subcuticula. The "bundles" are, however, not distinct on horizontal sections (see p. 352). The transverse and the dorso-ventral muscles are very weak.

The ventral canals are very strongly developed in the strobila; their diameter varies from 72μ at the apex of the scolex to $160 \times 240\mu$ at 70 cm. from the scolex. The dorsal canal measures 72μ at the apex, 32μ at the base of the scolex. Its lumen is almost obliterated at 40 cm. from the scolex.

The course of the canals in the scolex is described further on.

The first traces of the genital organs are seen at 1 cm. from the scolex. The testicles are recognisable at 12 cm., the ovarium appears at 40 cm., male sexual maturity is reached at 70 cm.; at this stage the uterus begins to develop. The paruterine organs commence to develop at 90 cm.

There are three to six testicles on each side of each of the ventral canals, leaving a great gap in the middle of the segment

without testicles (fig. 1 and Text-fig. 2). The testicles lie slightly dorsal to the transverse axis. The vas deferens

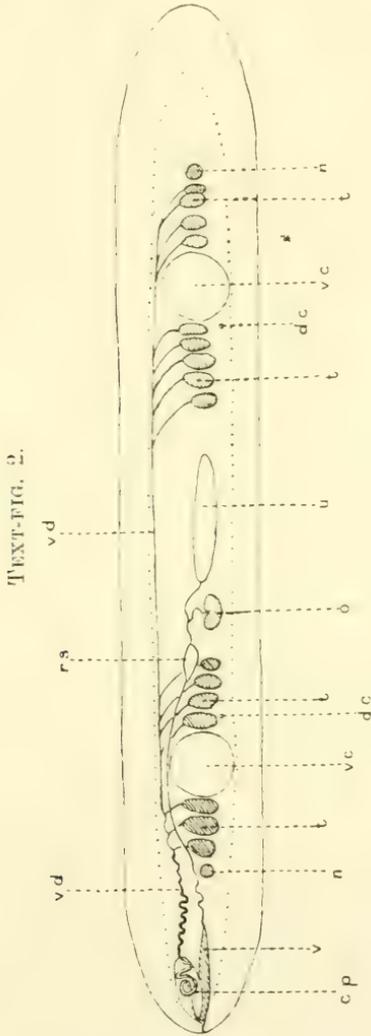


Diagram of *Avitellina centripunctata*. (List of abbreviations at end of paper.)

crosses the dorsal side of the median field, quite close to the transverse muscles, dorsal to the testicles, the nerve, the longitudinal canals, and to the female genitalia. Between the

ventral canal of the pore side and the cirrus-pouch it becomes distended with spermatozoa, and also slightly convoluted (vesicula seminalis). The cirrus pouch lies ventral or dorsal, anterior or posterior, to the vagina (figs. 4 and 43). The end of the cirrus is bent over and joins the vagina, it does not appear to be introduced into, but fused to the end of the vagina. There is a short and very narrow genital pore into which the vagina opens. The vagina runs straight to dorsal of the nerve and of the testicles; passing the ventral canal dorsally it widens median of the ventral canal (pore side) to form a receptaculum seminis. From the receptaculum seminis the canalis seminalis arises, which runs a short distance in the same direction as the axis of receptaculum would if lengthened; then it branches, one branch, the oviduct, turning ventrally towards the ovarium, the other, the uterine duct, also turning ventrally, leads to the uterus. The ovarium is bean-shaped, or kidney-shaped; it contains but few eggs. There is no vitelline gland nor shell-gland. The eggs pass through the oviduct into the uterine duct and then into the uterus, fertilisation taking place during the transit. The eggs receive nourishment from certain cells in the ovary and in the uterus (ovarial and uterine nutritive cells, pp. 371, 375). The eggs are finally enclosed in a paruterine organ, which arises within the uterus. Pads of fibrous tissue, lying anterior to the uteri, serve as support to the paruterine organs, and help to separate the genitalia of adjacent segments.

The eggs are enclosed in two spherical envelopes; the outer measures $40\ \mu$, the inner $23\ \mu$, the embryo $19\ \mu$ (fig. 2).

THE SYSTEMATIC POSITION OF THE GENERA STILESIA AND AVITELLINA.

Fuhrmann (1908) placed *Stilesia* together with *Thysanosoma* in a new sub-family which he called *Thysanosominae*.

Now that the anatomy of the species of *Stilesia* and *Avitellina* are so much better known than they used to be,

it becomes necessary to review their position, and to see how far they are related to *Thysanosoma*.

The points common to the three genera *Stilesia*, *Avitellina* and *Thysanosoma* are: the marginal arrangement of the testicles, the irregular alternation of the single genital pores (which does not hold good for *Thysanosoma*, double-pored specimens being frequently met with in South Africa) and the possession of a paruterine organ. They differ, however, in several very important points: *Avitellina* and *Stilesia* do not possess either a shell-gland or a vitelline gland; their eggs receive nourishment from nutritive cells in the ovarium and in the uterus.

The points in which the three genera agree are hardly of sufficient importance to weigh very heavily; the position of the testicles and of the genital pores is liable to vary considerably within a sub-family; the possession of a paruterine organ can, as shown by Fuhrmann (1908), be acquired independently by genera belonging to various sub-families.

The lack of a vitellogene gland and shell-gland and the results of their absence are, however, quite sufficient to separate the two genera from all other known cestodes. I therefore propose to separate the genera *Stilesia* and *Avitellina* from the *Thysanosominæ* and to place them in a new sub-family of the *Anoplocephalidæ*, calling the new sub-family *Avitellininæ*, after the genus *Avitellina*, which is certainly the better known of the two genera.

Diagnosis of the *Avitellininæ*.—Scolex without hooks with four suckers. Segments short, genital pores irregularly alternating, testicles in two or four groups, marginal, none in the middle field. A single ovarium, no vitelline gland, no shell gland; uterus single or double, eggs finally enclosed in a paruterine organ. Eggs in ovary and uterus surrounded (and nourished) by nutritive cells. Oncosphere with two envelopes. Type genus, *Avitellina*, Gough, 1910. All the known species inhabit Ruminants, development unknown.

AN ACCOUNT OF THE HISTOLOGY OF AVITELLINA CENTRIPUNCTATA (RIVOLTA).

Avitellina centripunctata (Rivolta) is on account of the large size of its histological elements and the looseness of their arrangement, exceptionally favourable for study.

The best results were obtained from worms fixed with Zenker's solution. I allow the solution to act for at least six hours, then I transfer the specimens to running water for twenty-four hours, after which they are carried through alcohol 25 per cent. and 50 per cent., remaining in each for at least three hours, being finally preserved in alcohol 75 per cent.

In order to obtain the worms as expanded as possible, I usually hold them up with a pincette, allowing them to hang free in the air; this almost instantly causes them to expand on account of their own weight, when I suddenly plunge them into the fluid, lifting them out at once; after letting them hang again for a few seconds I finally deposit them in the reagent. The worms treated in this way fix in a fairly expanded condition, and are not contorted or twisted. It is of great importance, however, to obtain the specimens alive, and they ought not to be washed previous to fixing. It is not necessary to use iodine to remove the last traces of sublimate from material treated in the manner described.

I have used sublimate, formalin, and silver nitrate, as well as Zenker, but no other reagent I know is to be compared with Zenker's solution for fixing cestodes. It is specially favourable for the study of the subcuticula and its connection with the muscles.

Staining was performed with Ehrlich's hæmatoxylin and orange G, which gives wonderfully clear pictures of the subcuticula and muscles. For the study of the eggs, and the changes taking place before maturation and fertilisation, I recommend iron-hæmatoxylin and eosin. This combination also presents the best results for the flame-cells and the nephridial cells surrounding the dorsal canal, and also shows

up the structure of the longitudinal muscles very distinctly. I have also employed hæmatein Apathy and Delafield's hæmatoxylin with good results. In using the Delafield one can obtain much the same results for the subcuticular cells as with Ehrlich's hæmatoxylin if, instead of differentiating with acidulated alcohol and blueing with ammoniated alcohol, one washes the specimens after staining in running water only. The nuclear structures do not stain as cleanly, however, as they do when using the stain in the ordinary way.

For specimens mounted in toto I use borax carmine; most of my material has been stained with borax carmine before cutting, being re-stained with hæmatoxylin after sectioning. I do not find that this spoils the final result; on the contrary, one often finds that the borax carmine helps to differentiate the nucleoli from the chromatin bodies in the nuclei.

My sections are invariably 4μ thick, which appears to be the best thickness for *Avitellina* material.

Almost all the drawings have been made with the Leitz oil-immersion $\frac{1}{12}$ and ocular 2, and are reproduced as far as possible at the same scale.

I have made sections of *Tænia serrata*, Goeze; *Anoplocephala magna* (Abilgaard); *Dipylidium caninum*, L., and *Stilesia hepatica*, Wolffhügel, in order to obtain comparative material fixed, hardened, and stained in the same way. It was unfortunately not possible to obtain *Ligula* material.

THE CUTICULA. Figs. 17-21.

The cuticula consists of the usual two layers, which seem to have been observed by all recent observers, namely, a thin outer layer (Comidien Schicht, Minckert, 1906) and a thick inner layer (Homogene Schicht, Minckert, 1906), within which lies the extremely thin basal membrane (Grenzstreifen, Minckert).

The outer layer, or comidial layer, stains very deeply with hæmatoxylin; it does not appear to be provided with fine

hairs or other such structures. A fine radial striation is, however, readily observable in favourable sections (fig. 17). The thickness of this layer is from $1\ \mu$ to $1.5\ \mu$. The comidial layer easily becomes detached, and is then sloughed.

The homogeneous layer is $3\ \mu$ thick on the average. Unlike the outer layer, it hardly stains with hæmatoxylin, but takes orange G or eosin readily. It appears (when fixed by "Zenker") to be quite structureless, except on the suckers, but impregnation with silver shows a definite structure also elsewhere. I have not been able to find any structures which can be compared to Minckert's (1906) trophopores, trophoporelles, neurophyses, or neuropores in the homogeneous layer of *Avitellina centripunctata* (Rivolta), nor any signs of pores running from surface to surface through this layer. However, in specimens impregnated with silver there are minute black granules scattered through the homogeneous layer, not quite evenly distributed, but more crowded towards the basal membrane, more scattered towards the comidial layer (fig. 18). I have observed a similar structure of the cuticula in *Dipylidium caninum*, L., fixed with Zenker, stained with iron-hæmatoxylin, and counter-stained with eosin; here the homogeneous layer presents exactly the same appearance as that of *Avitellina* when fixed with silver nitrate. The granules must therefore represent a finest structure of the cuticula, and are probably not merely artefacts (silver precipitates), as I was at first inclined to believe.

Certain modifications of the homogeneous layer are, however, invariably present in the cuticula of the suckers. These remain constant whether the specimen was fixed with Zenker, silver nitrate, or formalin. The comidial layer presents no contrasts to elsewhere, but the inner half of the homogeneous layer appears spongy or reticulated (fig. 17). The reticulations are formed by fibres running mostly at right angles to the homogeneous stratum, and forming numerous anastomoses amongst themselves. These fibres may be continuous with the parenchyma fibres of the suckers, which they much

resemble, especially in silvered specimens. The reticulations formed by the fibres enclose cavities which are probably in connection with each other. This structure has only been found in *Avitellina*; I have not observed anything similar in the other species I have examined.

Within the homogeneous layer and separating it from the subcuticular muscles lies an extremely thin membrane, the basal membrane, which can usually be quite readily demonstrated. The basal membrane is generally accepted as being derived from the parenchyma.

The formation of the cuticula has recently been studied by Young (1908) in *Cysticercus pisiformis*; according to him, "The cuticula of *Cysticercus pisiformis* is developed from a groundwork of simple parenchyma fibrillæ by a deposition among them of a cement substance. There are no specialised fibrillæ or cellular processes concerned in its development. The fact that in its development the processes of the subcuticular cells take part does not in any way detract from the above statement, since primitively the subcuticular cells themselves are undifferentiated parenchyma cells." Further (p. 288), "The cuticula is formed before the differentiation of the subcuticular cells."

Young's opinions are totally opposed to Blochmann's (1896) and his followers, who consider the cuticula to be mainly a product of the subcuticular cells.

It can, however, be proved from adult cestodes that the cuticula is not derived from the subcuticula. There is no doubt as to the presence of a cuticula consisting of comidial and homogeneous layers on the surface of the suckers, yet there are no subcuticular cells in the suckers. I have been able to convince myself of their absence in *Anoplocephala magna* (Abilgaard), *Tænia serrata*, Goeze, *Dipylidium caninum* (L.), *Stilesia hepatica* Wolffhügel. Young (p. 225) also states, "The subcuticula cells are lacking in the suckers." In all these species the cuticula above the suckers is similar to that elsewhere, differing somewhat in this respect from *Avitellina centripunctata* (Rivolta). There being

no subcuticula in the suckers, the cuticula of the suckers must arise independently of the subcuticula.

Unfortunately, neither Rössler (1902) nor Young (1908) has made any observations concerning the development of the suckers. Leuckart ('Parasiten des Menschen,' 2nd edition, vol. i, p. 445) states that their development commences by the formation of four hemispherical depressions of the cuticula in the substance of the "Kopfzapfen," representing the cavities of the suckers, and that the radial muscles arise out of the subcuticular cells. Gläser (1909) finds the suckers arising somewhat differently, already before the subcuticula is formed, at least he avoids using the term "subcuticula." Leuckart considers that the fact of the development of muscles out of the subcuticular cells of the suckers is evidence that the subcuticula has nearer affinity to the musculature than to the epidermoidal apparatus. In discussing the subcuticula I will have to show that the subcuticula of the proglottids stands in very close relationship to both the dorso-ventral and to the transverse muscles; Leuckart's observation of the development of the radial muscles out of subcuticular cells would contain nothing very remarkable, as the subcuticular cells are actually muscle-elements.

If, as according to Blochmann, the cuticula is a product of an epithelial layer, and the subcuticula is that layer, then no cuticula can exist where there is no subcuticula. There is, however, no subcuticula in the suckers, and yet the suckers are clothed with a cuticula; the subcuticula cannot therefore be the epithelium producing the cuticula.

Cuticula with no underlying subcuticula is also to be found in older portions of the strobila, in segments where the paruterine organ is fully developed; but here we have not to deal with primary but with secondary conditions. In the anterior portion of the strobila the dorsal and ventral surfaces of the segments are more or less parallel to each other (fig. 43); proceeding distally we find that the segments are broader at their posterior than at their anterior end, the strobila appearing serrated on longitudinal sections. Examina-

tion of a single section (fig. 21) shows that dorsally and ventrally the following changes have taken place. The original cuticula, together with the subcuticula, has shifted its position from parallel to the longitudinal axis to one at right angles to the long axis, pivoting on the posterior border of the segment, the margin originally anterior having become the outer margin. The space between the outer margin, the anterior margin and the posterior border of the segment has filled with parenchyma; the new surface from the anterior border to the outer margin is clothed with a thin cuticula, under which there is no subcuticula. This cuticula may have been derived by stretching of the already present cuticula, or it may be of new origin; the fact that the cuticula covering the subcuticula now on the posterior surface is enormously thickened (by contraction?) would suggest that the cuticula covering the new surface is of new origin. The new cuticula only measures 1.5μ to 1.7μ that on the posterior surface is much folded, and measures up to 9μ . The subcuticular muscles are also much more evident than usual on the posterior surface, the whole giving the impression that the cuticula and muscles have been compressed laterally (i.e. in the plane of the surface of the cuticula), thus causing the increase in thickness.

THE SUBCUTICULA. Figs. 19-21.

The subcuticula is present everywhere in a single layer close under the cuticula, except in the suckers and places mentioned above. It consists of a stratum of elongate cells, standing vertically to the cuticula. Its component cells are widely separated from each other, or densely congregated, according to the state of contraction of the worm. In shape they usually taper slowly towards the cuticula and are rounded or tapering towards the parenchyma (figs. 19, 20). Before reaching the cuticula several branches are usually formed, so that each cell touches the cuticula in more places than one. The outline of the cell is extremely distinct in properly fixed and hardened material; a thin membrane is

possibly present. The plasma is finely granular, staining readily with hæmatoxylin. The nucleus is oval, clear, surrounded by a distinct membrane, and contains a nucleolus and usually four chromatin bodies. The nucleolus stains differently to the chromatin bodies, my material being mostly stained in toto with borax carmine, and stained in section with Ehrlich's hæmatoxylin, counter-stained with orange G, the nucleolus staining reddish, the chromatin blue. Whether conical or rounded towards the parenchyma, each subcuticular cell gives off one or more fibrillar processes, which run inwards; the processes from several adjacent cells usually converge and collectively join the dorso-ventral muscles, where those muscles break through the longitudinal muscle (fig. 20). The subcuticular cells on the extreme lateral margin also behave in the same manner, only that they join the transverse muscle. Towards the lateral margins the connection between the dorsal or ventral subcuticular cells and the muscles is even more apparent, as the cells lie grouped around the base of the muscles, the subcuticular elements belonging to any one of the outer dorso-ventral muscles all lying lateral to their muscle, thus assuming a direction oblique to the cuticula.

I have been able to verify this connection between subcuticular cells and muscles in *Dipylidium*, *Tænia*, *Dibothriocephalus* and *Triænophorus*. The subcuticular cells consequently belong to the dorso-ventral and transverse muscle systems. In well preserved material it is possible to demonstrate this connection of muscles and subcuticula for every cell lying entirely in the plane of the section.

The question arises, Why have such processes not been observed before? In my opinion this is probably due to the methods used for fixing and hardening, and to the necessity to cut the sections in the plane of the cells. The subcuticular cells appear to be very difficult to fix and harden satisfactorily, Zenker's solution being the only one which has given me such results as yet. The silver impregnation method em-

ployed by Zernecke is probably not quite satisfactory, apart from its capriciousness and unreliability.

In older proglottids the subcuticular cells atrophy, their nuclei becoming smaller, their nucleolus and chromatin forming apparently a single mass filling the entire nucleus; and the outlines of the cells themselves become indistinct (fig. 21).

Having shown that the subcuticula belongs to the dorso-ventral and transverse muscle systems, and that the cuticula of the suckers arises independently of a subcuticula, and bearing in mind Young's observation of the development of the cuticula in *Cysticercus* prior to the differentiation of the subcuticular cells, it will be seen that serious doubts must again arise concerning the epithelial nature of the subcuticula. Balss (1908) also opposes the theory that the subcuticula is an ectodermal epithelium; he considers both cuticula and subcuticula to be of mesodermal origin, but he still admits that the cuticula is a product of the subcuticula. Of course the mere fact that the subcuticular cells form part of the dorso-ventral and transverse muscles would not induce me to deny their epithelial nature and their being the producers of the cuticula; remembering the epithelial muscles of hydroids, etc., it is mainly on account of the cuticula being formed in places where there is no subcuticula.

THE MUSCLES. Figs. 22-29.

The muscles in the strobila, scolex, and suckers can everywhere be divided into the two groups, subcuticular muscles and parenchyma muscles.

The subcuticular muscles form two layers, whose fibres run at right angles to each other; the outer of these is situated very close to the cuticula, and runs horizontally, the inner runs longitudinally.

The parenchyma muscles in the strobila form three distinct muscle systems—the dorso-ventral, the transverse, and the longitudinal muscle systems. The first two are much weaker than the last.

The muscles in the scolex are more complicated than those in the strobila, as their course is modified by the suckers, and their requirements (Lühe, 1894). The following systems of muscles have been made out in connection with the suckers of *Avitellina centripunctata* (Rivolta).

Commencing at the apex of the scolex, and proceeding towards the strobila, we meet :

(1) A diagonal cross-system anterior to the terminal loops of the excretory canals ; it covers the entire anterior surface of the scolex, passing from the front of the left ventral to the front of the right dorsal sucker, and from the front of the right ventral to the front of the left dorsal sucker.

(2) A second diagonal cross system, composed of four bundles of muscles, each consisting of only a few fibres, is situated just posterior to the terminal loops of the ventral canals ; it runs from sucker to sucker in such a way as to connect—

(A) The median face of the right ventral sucker with the lateral face of the left dorsal sucker.

(B) The lateral face of the right ventral sucker with the median face of the left dorsal sucker.

(C) The median face of the left ventral sucker with the lateral face of the right dorsal sucker.

(D) The lateral face of the left ventral sucker with the median face of the right dorsal sucker.

(3) An orthogonal cross-system, running dorso-ventral and transversely, is situated just behind the second diagonal cross system and between it and the great nerve commissures. It connects :

(A) The median faces of the two left suckers.

(B) The median faces of the two right suckers.

(C) The lateral faces of the two dorsal suckers.

(D) The lateral faces of the two ventral suckers.

(4) A second orthogonal cross-system is situated behind the nerve commissure, near the base of the suckers. Its insertions correspond to those of the previous orthogonal system.

(5) Near their base the suckers are connected by a system of muscles, which connects the two dorsal suckers with each other and the two ventral suckers with each other, but does not seem to connect the dorsal with the ventral suckers. Its fibres connect :

- (A) The right sides of the two dorsal suckers.
- (B) The left sides of the two dorsal suckers.
- (C) The right sides of the two ventral suckers.
- (D) The left sides of the two ventral suckers.

The histological elements composing the parenchyma muscles can be divided into the following groups :

- (1) Bipolar myoblasts with terminal fibrillæ.
- (2) Bipolar myoblasts with lateral fibrillæ.
- (3) Elongate bipolar myoblasts lying axially within the tubiform muscles of the longitudinal muscles.

The first group I have only found in the dorso-ventral muscle; the second forms the transverse muscle, and occasionally occurs in the dorso-ventral muscle; the third occurs in the longitudinal muscle.

(1) The bipolar myoblasts with terminal fibrillæ occur in all parts of the dorso-ventral muscle, and are its chief components. The cells are spindle-shaped, with an oval nucleus, whose long axis lies in the long axis of the cell (figs. 22, 23). The nucleus measures, on an average, $3.5\mu \times 4.25\mu$; it contains a nucleolus and two or more round chromatin bodies. The plasma stains deeply, and is sharply defined at the margins, as though enclosed by a membrane. At each end of these cells a fibrilla arises, which is several times longer than the cell itself; the fibrillæ are extremely thin, measuring only a fraction of a μ in diameter, they run in the general direction of the long axis of the cell; their course is, however, usually not quite straight, but sinuous or zig-zag. The fibrillæ may, perhaps, only act as tendons, in which case the cell would represent the contractile portion of the combination, but it is more probable that the fibrilla is itself contractile. These cells are usually found in rows, their fibrillæ lying in part apposed to each other, thus form-

ing the connection of the whole row to a muscle. The fibrillæ of the outermost myoblasts do not insert directly in the cuticula, but are in contact with the fibrillæ arising from the subcuticular cells.

Cells similar to these simplest myoblasts have been seen and described by Schiefferdecker (1874) in *Tænia saginata*, Küchenm.; he considered them to be connective elements; and by Hamann in *Tænia* (*Mesocestoides*) *lineata*, Rud., who described them as elements of the parenchyma.

(2) Bipolar myoblasts with lateral fibrillæ are, in *Avitellina*, chiefly found in the transverse muscle. They are the form of myoblast most frequently recorded by recent authors (e. g. Pinter, 1881; Hamann, 1882; Krämer, 1892; Will, 1893; Zerneck, 1895; Rössler, 1902; Young, 1908) in various genera and species. These muscle-cells differ from those described above inasmuch as the fibrilla runs continuously over one surface of the cell and does not originate only at the poles, otherwise they are very similar in appearance and dimensions (figs. 24, 25). Their nuclei measure $3.5 \mu \times 4.25 \mu$; their plasma is attached to the fibrilla, being widest at the middle and drawn out to a point at each pole. The plasma stains fairly deeply, and has a very distinct outline, probably being enclosed by a membrane. The fibrillæ are long, apparently homogeneous, and lie arranged parallel to each other. At the margins the fibrillæ of the transverse muscle spread fan-like towards the cuticula, as usual in other cestodes. Some of them appear to connect with the most lateral subcuticular cells. The fibrillæ are very thin, less than 1μ in diameter. Young (1908), describing such myoblasts, states that the myoblast is always connected to the muscle by fibrillæ, even when the cell remains very close to the muscle. I have not been able to verify this observation in my material.

(3) The elongate bipolar myoblasts, lying axially in the tubiform muscles of the longitudinal muscles, are more difficult to observe than the other two varieties of

myoblast of *Avitellina*. The longitudinal muscles differ at first sight from the dorso-ventral and transverse muscles even under a low power, by the great development of the muscle-fibres; the myoblasts themselves are hard to find on account of their great length, which reaches as much as $65\ \mu$ or more, and on account of the length of the muscle-fibres, which can often be followed continuously through more than nine segments. Owing to their elongation, it too frequently happens that the myoblasts are not entirely contained in a single section, and owing to the large number of fibres in the longitudinal muscle it is hopeless to look for the continuation of such myoblasts in the next section.

The muscles are thick, measuring up to $9\ \mu$ in diameter, they are round or oval or even polygonal with rounded angles on transverse sections. On thin transverse sections a large number of the muscles, especially those of the outer layers, appear annuliform, having a clear space in the middle. Towards the inner layers of the transverse muscle, all the fibres are found to be solid. Zernecké (1896) figures such annuliform sections of the longitudinal muscles of *Ligula*, and I have found similar muscles in *Dipylidium*. The solid muscles are probably derived from tubiform muscles for the following reason. In sections stained with iron-hæmatoxylin, if favourably differentiated, the solid longitudinal muscles are seen to consist of a dark (black) staining core surrounded by a light outer sheath. This peculiarity can be observed in sections passing transversely to the muscles as well as in such running in the direction of the muscle and containing uncut muscles. As we will see later on, the tubiform muscles contain a portion of the axial myoblast in their hollow. If the myoblast deposits or produces the muscle substance, then the centre or axis of the muscle represents the younger deposit, which again stains differently to the outer portion. The fact that the annuliform sections very frequently do not contain any portion of the myoblast is probably due to shrinkage or contraction during fixation. If the solid muscles are the older ones, the outside position

of the annuliform muscles would indicate that the young muscles develop on the outside of the transverse muscle.

The myoblasts are bipolar, spindle-shaped and very elongate (figs. 26, 27); their nuclei resemble in most respects the nuclei of the myoblasts of the other two systems, but they are usually somewhat larger. Those measured averaged $4.25\ \mu$ to $5\ \mu$ long by $2.5\ \mu$ to $4.25\ \mu$ broad. The plasma is granular; a membrane appears to be present. The ends of the cells are inserted in the tubular ends of the muscles. In some cases the two tubiform muscles appear to be connected by a strip of muscle-tissue passing along one side of the myoblast. Similar muscles were observed by Salensky (1874) in *Amphilina*, although not re-found by either Hein (1904) nor by Cohn (1904) in the same object.

The longitudinal muscles appear to me to be produced by these cells, which in that case are true myoblasts, the contractile substance being formed or deposited on the surface of the cell, the body of the cell penetrating the axis of the muscle for a long time; then, as the muscle develops, its cavity becomes filled with muscle substance. In the end the cell disappears from the axis of the muscle, and becomes displaced so as to lie laterally to the muscle. Nuclei lateral to the muscles are frequently observed.

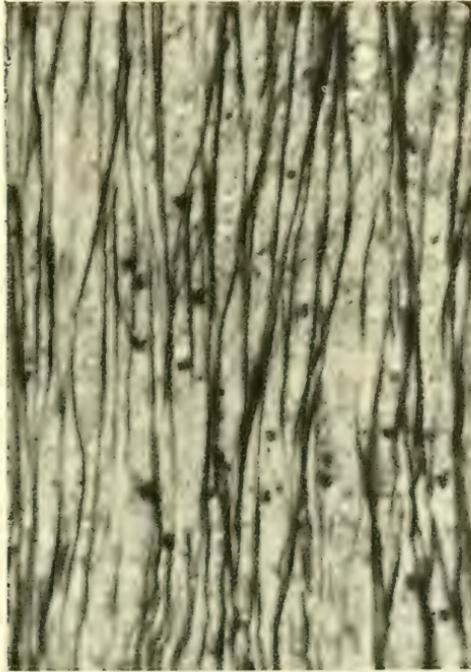
The muscle-fibres can be traced through a number of segments. Their course is not quite straight, but more or less sinuous. Text-fig. 3 is a photograph of a section cut parallel to the longitudinal axis of the worm, and represents a distance of about ten segments.

In transverse sections the muscles appear to be arranged in bundles, whose elements are more crowded towards the transverse muscle, more scattered towards the subcuticula. However, I distrust the appearance of the bundles, since it is impossible to re-discover them in horizontal sections (see Text-fig. 3). It will be seen that where a few muscles do group together, they receive fibres from the adjoining groups and give off fibres.

The schematic Text-figs. 4, 5, 6 will help to make my

meaning more clear. Fig. 4 shows the course of six muscles composed of bundles of four; one sees the sections of the dorso-ventral muscles separating the bundles. Text-figs. 5 and 6 have been drawn to show that it is possible to obtain transverse sections giving the appearance of bundles, but that

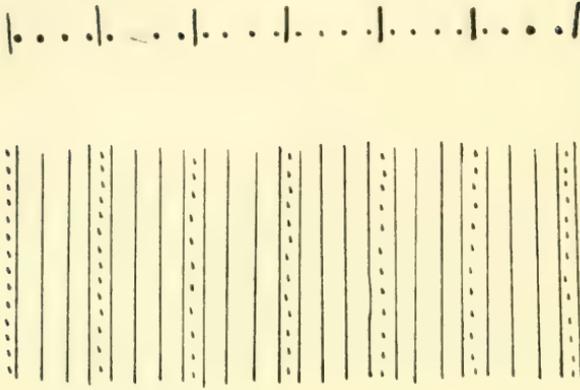
TEXT-FIG. 3.



Horizontal section through the longitudinal muscle. About nine or ten segments are included in the photograph.

in each case the bundles at the levels *a*, *b*, *c*, *d* are differently composed. Thus at level *a*, 1, 2, 3, 4 form the first, 5, 6, 7, 8 the second, 9, 10, 11, 12 the third bundle, etc. At level *b*, 2, 3, 4, 5 form the first, 6, 7, 8, 9 the second, 10, 11, 12, 13 the third bundle. By the time level *e* is reached not a single fibre forming the first bundle is the same as at level *a*. The

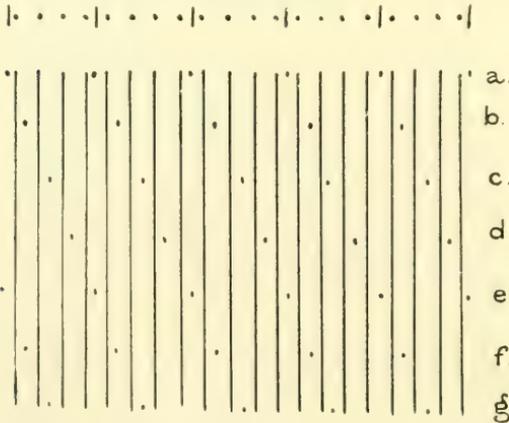
TEXT-FIG. 4.



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

The top row represents a transverse section, the lower part of the figure a horizontal section. The figure shows the sections of the dorso-ventral muscles separating the bundles. The bundles are real.

TEXT-FIG. 5.



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20.

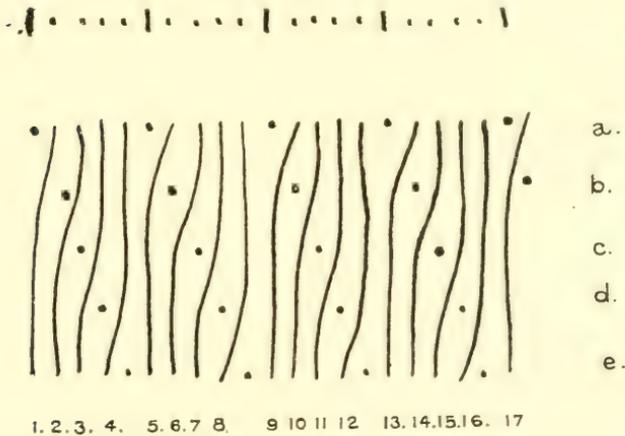
The top row represents a transverse section, the lower part of the figure a horizontal section. The figure shows the dorso-ventral muscles passing through between the longitudinal fibres. At the levels *a*, *b*, *c*, *d*, etc., one would find the muscles arranged in bundles of four, as in Text-fig. 4. The arrangement in bundles is only apparent.

number of fibres in a bundle and the number of bundles have remained the same, and if we were to judge entirely by transverse sections, we would have to conclude that the number of fibres in a bundle and the number of bundles is constant.

The fibres of the longitudinal muscle are apparently arranged in bundles of about twenty-four, with a few solitary muscles scattered near the subcuticula.

The subcuticular muscles arise, according to the usual view

TEXT-FIG. 6.



The figure represents a modification of the arrangement of the muscle-fibres shown in Text-fig. 5. The arrangement in bundles, as seen in transverse section, is only apparent.

as products of the "Sommer-Landois" cells, which are supposed to be myoblasts. Young (1908) opposes this view, as he finds the subcuticular muscles already formed before the "Sommer-Landois" cells are differentiated. Young consequently refuses to apply the term "myoblast" to them, and calls them "neuro-muscular" cells, because they send fibrillar processes into the muscles and because they are also connected with the nerves.

The "Sommer-Landois" cells of *Avitellina* are best examined in sections through the scolex. These cells send

one or two processes towards the cuticula to subcuticular muscles, and also one or two processes into the depth of the scolex to connect with the nerves (fig. 28). The nucleus is large, round, measuring 5μ in diameter; it contains a nucleolus and a few chromatin bodies. The plasma is fibrillar in appearance, several fibrillæ uniting to form the processes leading to the subcuticular muscles or to the nerves. The whole cell has much more resemblance to a nerve-element than to a myoblast; it differs from the myoblasts of the parenchyma muscles in the obvious connection with the central nervous system, in being connected to more than one muscle-fibre, and in being multipolar instead of bipolar.

Young (1908) finds that the "Sommer-Landois" cells originate from cells similar to those I have described as occurring in the dorso-ventral muscle, and he lays importance on the fact that the muscles are present before the "myoblast" differentiates. The nerve-connection was observed already by Zerneck (1908) and Blochmann (1896) before Young. I consider Young to have been quite justified in giving the name "neuro-muscular cell" to the Sommer-Landois cells, as the cells are certainly quite as much nerve-elements as muscle-elements.

The Muscles of the Suckers.—It is possible to distinguish between parenchyma and subcuticular muscles in the suckers in the same way as in the rest of the body, the myoblasts and "neuro-muscular" or "Sommer-Landois" cells giving the clue to the group to which the muscles belong.

The radial fibres belong to the parenchyma muscles, the muscles just under the cuticula running parallel to the cuticula to the subcuticular muscles.

In *Avitellina* the radial muscles have lost their myoblasts, but in other forms examined by me (*Tænia*, *Dipylidium*; *Cittotænia*) it has been possible to readily demonstrate the presence of lateral myoblasts.

In *Avitellina centripunctata* (Rivolta), and in *Anoplocephala magna* (Abilgaard), there is a layer of cells

close to the cuticula of the suckers, separated from the cuticula by the superficial layer of muscles. This layer of cells does not represent the subcuticula, but rather must be homologised with the "Sommer-Landois" cells of the subcuticular muscles of other parts of the worm. The cells are more or less flattened, and lie in a plane parallel to the cuticula—subcuticular cells are perpendicular to the cuticular; these cells are multipolar (fig. 29), each cell sending out several processes, which either terminate in connection with muscle-fibres, or connect with processes from similar adjacent cells. The cells further stand in contact with the ganglion cells of the sucker by means of neuro-fibrillæ coming from the nerve-cells. The nuclei of the neuro-muscular cells in the suckers are oblong, small, measuring only 3.5μ long. The points of resemblance between these cells and the "Sommer-Landois" cells are their connection with more than one muscle-fibre, their multipolarity, their connection with the central nervous system (of the sucker in this case).

Granted that these cells are really the homologa of the "Sommer-Landois" cells of the rest of the body, then the muscles they belong to must be subcuticular muscles, as "Sommer-Landois" cells have hitherto only been found connected to subcuticular cells.

The arrangement of the muscles in the suckers follows roughly the arrangement in the rest of the body; subcuticular muscles, in two layers, occupy the space directly under the cuticula, and are parallel to the cuticula; parenchyma muscles run perpendicular to the cuticula, and, in deeper layers, parallel to the cuticula.

The suckers are separated from the scolex by a delimiting membrane; directly within this membrane are two sets of fibres running parallel to it. The first set forms concentric rings, as can be seen on sections touching the base of the sucker; the second set radiates, the centre of the radiation being at approximately the same spot as the innermost of the concentric rings. These two sets lie close to each other, and remind one of the subcuticular muscles, which they possibly

are. Next to the subcuticula one also finds two sets of muscles; the outer is also composed of circular, concentric muscles, the inner of radial fibres. These two sets are without doubt subcuticular muscles; the "Sommer-Landois" cells mentioned above stand in contact with them. Between the cuticula and delimiting membrane are the radial fibres; in the middle of the thickness of the sucker there are other muscles, running at right angles to the radial fibres, whose course is possibly circular. These two last sets are without doubt parenchyma muscles.

Around the margin of the opening of the suckers the subcuticular circular muscles are much developed, forming a kind of sphincter.

It is worthy of remark that the subcuticular muscles of the suckers are much more developed in *Avitellina* and *Anoplocephala* than in *Tænia* and *Dipylidium*.

THE EXCRETORY SYSTEM. Figs. 5, 6, 30-37.

The excretory system consists of the usual two pairs of longitudinal canals, the secondary transverse commissures, and of the flame cells and their capillaries.

In the strobila the ventral canal is enormously developed; it lies lateral to the dorsal canal, whose lumen almost atrophies in the posterior portion of the strobila.

Transverse commissures connect the ventral canals; they are not very readily observed in *Avitellina centripunctata* (Rivolta), but are wide and conspicuous in *Stilesia hepatica*, Wolffhügel, *Stilesia vittata*, Railliet, and *Stilesia globipunctata* (Rivolta), where they form a more or less complicated network at the posterior end of each segment.

The flame cells are very frequent in the medullary zone of *Avitellina centripunctata* (Rivolta), but extremely rare exterior to the transverse muscle. This is worth notice, as in other cestodes (*Tænia serrata*, Goeze, *Dipylidium caninum* [L.], and *Ligula* [fide Blochmann]) they seem to be very frequent among the subcuticular cells.

The course of the canals in the scolex is somewhat complicated, but can be reduced to quite a simple scheme. If it is remembered that the right and left halves only communicate by a single loop situated at the apex of the scolex, and that the dorsal and ventral canals of each side, after mounting through the scolex, meet and join each other just at the apical connecting loop, it will be seen that the whole can be reduced to the scheme given by v. Janicki for *Inermicepsifer* in a paper to be published shortly. The actual plan of the canals in the scolex is, however, complicated by the fact that the ventral canal forms a large number of loops and turns before reaching the apex. The course of the dorsal canals, on the other hand, is quite straight.

Before commencing the description of the course of the ventral canal it is necessary to explain the terminology to be used. I must distinguish between dorsal and ventral suckers, also between right and left suckers; the median face and the lateral face, the outer angle formed by two suckers and the inner angle formed by two suckers, and the base and apex of a sucker.

Dorsal and ventral require no further explanation; the median face of a sucker is that side which faces a line dividing the right dorsal and ventral suckers from the left dorsal and ventral suckers; similarly the lateral face is that side which looks towards the line separating the two dorsal from the two ventral suckers. The outer angle formed by two suckers is the angular space between the suckers near the subcuticula, the inner angle the corresponding space or angle opening on the medullary zone of the scolex. The base is the convex end, turned towards the strobila, and the apex the end of the sucker containing the mouth of the sucker (see diagram).

It has already been stated that the ventral canal in the proglottids lies lateral to the dorsal canal. Following the canals from the strobila into the scolex, we find that their relative positions change close to the scolex, the ventral canals lying internal to the dorsal canals.

The left ventral canal (fig. 5), on entering the scolex, turns

at right angles to its course, running horizontally towards the base of the dorsal sucker, then rising vertically it proceeds up the lateral surface of the sucker to the level of the bottom of the hollow of the sucker; it then turns over and proceeds downwards parallel to its ascent. Arriving near the base of the sucker it gives off a short branch, which runs towards the median outer surface of the sucker and terminates at the level of the bottom of the hollow of the sucker. The main canal re-crosses the base of the scolex horizontally, internal to and parallel to its first horizontal portion, but external to the dorsal canal, until it reaches the base of the left ventral sucker. It then ascends the median side of the left ventral sucker, almost to the mouth of the sucker, then turns again, running parallel to its former course until the base of the sucker is once more reached; it then passes around the external surface of the base of the sucker, forming a few spiral loops, until the outer lateral angle of the left suckers is reached. It then ascends the outer lateral angle, giving off a short blind branch towards the dorsal sucker at the level of the nerve commissure. Arrived near the summit of the scolex, it bends over and runs down the inner lateral angle of the left sucker until the level of the cross nerve commissure is reached; here it bends inwards, and then upwards past the nerve commissure, running ventral to and parallel to the dorsal canal until the apex of the scolex is reached. Here it bends over dorsally and fuses with (or is continued into) the dorsal canal. At the point of junction of the two canals the commissure connecting the right and left halves of the excretory canal system inserts. The dorsal canal is straight throughout its entire length.

The right ventral canal runs in a similar manner, at first ventral to and parallel to the right dorsal canal (fig. 6). Near the base of the suckers it also bends over dorsally, and, passing the dorsal canal externally, runs horizontally to the base of the right dorsal sucker, following the outer face of the sucker, which it ascends almost to its mouth, veering during this ascent more and more to the right.

Having ascended thus far, it again turns and descends parallel to its ascent; on reaching the base of the sucker it re-crosses the base of the scolex exterior to the dorsal canal, but interior to its first horizontal stretch. Arrived at the median internal side of the base of the right ventral sucker, it ascends the median ventral side of the sucker to the height of its mouth, then turns back parallel to the ascending portion of the canal. Passing around the base of the right ventral sucker internally, it ascends the outer angle of the two right suckers, sending off two blind branches towards the outer surfaces of the suckers at the level of the nerve commissure. The main canal continues its course parallel to the long axis of the worm to near the apex of the scolex; then it bends over, descends the inner angle of the right suckers until the nerve commissure is reached, when it bends over again, proceeding past the nerve commissure ventral to and parallel to the dorsal canal. Arrived at the summit of the scolex, it unites with the dorsal canal and with the connecting commissure in the same way as the left canal does. The right dorsal canal is straight throughout, agreeing in this respect with the left dorsal canal.

The "blind" branches given off by the ventral canals I have not been able to follow further than stated; of course, they may be much longer than their observed length. Bearing in mind the difficulty of tracing the transverse commissures in the proglottids of *Avitellina centripunctata* (Rivolta) for any length (I had not seen them at all in 1909), I certainly hesitate to say that a ring-canal is not formed; these branches may represent portions of a ring-canal, which, in that case, would be a transverse canal in the scolex homologous to the transverse commissures in the proglottids.

It is probable that the various loops change their course considerably according to the state of contraction of the scolex. *Stilesia hepatica*, Wolffhügel, also invariably shows a similar dorsal bend of the ventral canal at the base of the scolex; the entire course, however, I have not been able to study.

The ventral and dorsal canals are equal in diameter at the apex of the scolex, where they both measure $72\ \mu$; proceeding away from the apex, or from the scolex, the ventral canal increases in diameter and the dorsal canal decreases. At the base of the scolex the ventral canal measures $128\ \mu$ in diameter, the dorsal $32\ \mu$. At 10 cm. from the scolex the ventral canal was found to be $32\ \mu$ by $80\ \mu$, the dorsal $16\ \mu$ by $32\ \mu$; at 20 cm. the ventral canal measured $96\ \mu$, the dorsal $28\ \mu$ by $20\ \mu$; at 30 cm. the ventral had increased to $112\ \mu$ by $72\ \mu$, the dorsal decreased to $4\ \mu$. After this the dorsal canal becomes almost obliterated, whilst the ventral canal goes on increasing until it measures $160\ \mu$ by $240\ \mu$, this size being reached at 70 cm. from the scolex.

The dimensions quoted show that the ventral canal is not always round on section; its shape varies considerably according to the state of contraction of the proglottid from round or oval to angular, square or polygonal sections being quite frequent in histologically perfect material. The ventral canal often occupies almost the entire thickness of the medullary substance, leaving scarcely enough space for the genital canals to pass.

The structure of the excretory canals varies according to the portion of the worm examined. A histological difference can also be observed between the dorsal and ventral canals in the strobila.

The longitudinal canals possess a fine membrane, which can occasionally be seen to be finely striated at right angles to the long axis of the canal. This membrane is the product of a layer of epithelial cells, which separate it from the parenchyma. These cells are best studied in the scolex; they are, as Bugge (1902) found in *Moniezia expansa* (Rud.), flat cells; their plasma is collected somewhat more above the nuclei than elsewhere, and their margins are not observable. Seen from the side, one observes that the layer is not equally thick everywhere, being thicker near the nuclei, thinner towards the margins. Exactly the same structure can be observed in *Stilesia hepatica*, Wolffhügel, where the

transverse canals present the same structure as the longitudinal canals.

I have not observed any special musculature of either the dorsal or the ventral canals in *Avitellina*.

In the strobila certain changes take place in the epithelial layer of the canals. The nuclei of the epithelial cells sink into the surrounding parenchyma, the membrane remaining connected to the epithelial cells by a fibrillar structure standing vertical to the surface of the canal (fig. 30). This arrangement is especially noticeable in the dorsal canal, but is also present in the ventral canal. Bugge (1898) figures the same radial arrangement of the fibres without comment.

Fig. 31 shows the dorsal canal at about 25 cm. from the scolex. The lumen has almost disappeared, being only 1 or 2μ wide. The membrane has consequently thickened considerably, and is seen to consist of two layers, a thin dark-staining inner and a thick light-staining outer layer. The outer layer is followed by a darker layer with radial structure, whose fibres appear to be continuous with the radial fibres belonging to the epithelial cells; the epithelial cells have sunk considerably deep into the surrounding parenchyma. Their plasma shows a fibrillar structure, arranged radially where in contact with the canal, and less definitely around the nucleus. The plasma stains very lightly with orange G, but takes eosin deeply. The nucleolus is large, its membrane stains distinctly; it contains a nucleolus and one or two chromatin bodies. At first the cells appear to lie close to the radial fibres; then they grow in length and their nuclei move further away from the canal; the portion of the cell surrounding the nucleus becomes rounded or retort-shaped, the plasma becoming less dense as it recedes from the canal. A connection by its fibrillæ with the canal is always to be made out. In appearance these cells suggest glands, which swell and increase in size as they come into function. Their connection with the dorsal canal is always evident; the radial fibres surrounding the canal certainly belong to them, not to the parenchyma. In cells which have reached their full

development the radial fibres are seen to enter the cell and to be continuous with the fibrillar plasma. I consider these modified epithelial cells of the dorsal canals to be nephrocytes; they do not appear to have attracted the attention of other observers as yet. As no flame-cells arise from the dorsal canals they must represent the terminal nephridial element of the dorsal canals.

In the ventral canals a similar change takes place. The epithelial cells also sink into the surrounding parenchyma (fig. 30), remaining connected with the membrane by radial fibrillæ. They do not, however, become directly modified into gland-cells, but indirectly give rise to the flame-cells and their capillaries. Figs. 32, 33, and 36 show the development of the flame-cells. The first stage is represented by fig. 32; here we see a single epithelial cell sinking into the parenchyma, away from the membrane of the ventral canal, but remaining attached to the canal. The next change apparently takes place very rapidly, but without doubt it consists in the multiplication of the cell, a row of five nuclei, as shown on fig. 33, being produced. These five nuclei belong to four developing flame-cells and their capillary cell. A group of young flame-cells is to be seen on the same figure. The development of the flame-cells in *Avitellina* thus goes very much on the lines described by Bugge (1902), only here we have four flame-cells in a group instead of only three, the capillary cell in each case originating in the same way. As Bugge, I have only found developing flame-cells quite close to the ventral canal, never at any distance from it. Examination of *Tænia serrata* gives the same results, only in this species the flame-cells are in groups of three, plus one capillary cell (see fig. 36).

I cannot agree with Young (1908) that the flame-cells develop from parenchyma cells, and that "the capillary cell is at least a parenchyma cell of separate origin from the flame-cells, and that the capillary is formed at first as a passage in parenchyma strands, to become modified later into a definite tube with a specially modified wall." On the other

hand, every stage of the development of the flame-cells observed by me in *Avitellina* has served to verify Bugge's statements.

The fully developed flame-cells do not present any remarkable differences from those figured and described by other authors, except that they almost regularly contain a small corpuscle in the plasma, whose nature I have not been able to identify (*x*). The cells always occur in groups of four, most frequently in the medullary substance, very rarely in the transverse muscle, hardly ever exterior to the muscle. They consist of a cell (figs. 34 and 37) with granular plasma, usually with irregular, star-shaped outline; the nucleus contains a nucleolus and a varying number of chromatin bodies. The flame usually stands vertically to the cell, more rarely tangentially; the flame is often quite close to the nucleus, but it is sometimes separated from the main portion of the cell and the nucleus by a longer or shorter "neck" of plasma. The flame arises from a basal plate, a meniscus-shaped body with its concave side turned towards the flame, its convex side embedded in the cell. The flame itself consists of cilia. The funnel is widest a little way from its base; near its middle it is suddenly thickened, a ring of the substance of the funnel projecting into and somewhat constricting the lumen. The capillary usually runs straight in the same direction as the funnel for about once or twice the length of the funnel, and then bends suddenly to one side; after a short distance it bends again to resume its original direction. The capillaries can often be followed for a considerable distance, and frequently present a very devious course. It is interesting to note that the flame-cells invariably lie at right angles to the longitudinal axis of the worm, so that they fall within the plane of a transverse section. They are fairly frequent in the scolex as well as in the strobila, but do not occur in the suckers.

The flame-cells have long been recognised to be unicellular glands (Pinter, Lang, Bugge, Blochmann), and the capillaries to be their ducts; both the flame-cells and the nephrocytes

of the dorsal canal have a common origin—the epithelial cells of the excretory system.

THE NERVOUS SYSTEM. Figs. 38-40.

The central nervous system of the strobila consists of a nerve lying lateral to the ventral canal on either side of the strobila. From this main nerve branches are given off in every segment, which are, however, very difficult to observe.

The nerves on entering the scolex first bend inwards, passing well into the central space enclosed by the suckers. At the level of the bottom of the hollow of the suckers four ganglia are found, two lying in the inner lateral angles formed by the suckers, two in the inner median angles. These four ganglia are connected by transverse commissures, so that the lateral ganglion of each side is connected to both the dorsal and the ventral median ganglia; the ganglia are further connected by a transverse commissure, connecting the two lateral ganglia, and a dorso-ventral commissure, connecting the two median ganglia. The transverse and the dorso-ventral commissures fuse in the middle of the scolex, where they cross each other and form a central ganglion. Each of the four peripheral ganglia gives off two nerves, which proceed at first anteriorly and then bend over and unite with the nerve of the neighbouring ganglion, the eight nerves thus forming four loops connecting the four ganglia. Anterior to these is a second central nerve-plate, poorer in ganglion cells, whose connection with the rest of the nervous system has not been made out.

The histology of the nervous system presents on the whole the usual structures. The lateral nerves in the strobila consists chiefly of neurofibrillæ, arranged parallel to each other on longitudinal sections, having a reticulated appearance on transverse sections. The neurofibrillæ are accompanied by scattered glia cells, which are chiefly found on the surface of the nerve. Ganglion cells were very rarely observed in the nerves of the strobila.

In the ganglia of the scolex nerve-cells are very prominent, their large size and deep staining (with Ehrlich's hæmatoxylin) making them most conspicuous. Multipolar ganglion cells were most frequently met with in the ganglia, bipolar in the nerves.

The multipolar ganglion cells (fig. 38) have a reticulated or spongy protoplasma, which stains dark blue with the hæmatoxylin. From the processes of the cells the neurofibrillæ can often be traced for some distance. The nuclei measure 8μ to 9μ long by 4.5μ to 8μ broad; they are vesicular, and contain a large nucleolus (up to 2μ in diameter) and one or two chromatin bodies. The nucleus itself is much paler than the surrounding plasma; the nucleolus stains rather lighter than the chromatin.

In the nerves arising from the scolex ganglia large bipolar ganglion cells are frequent; fig. 39 represents a portion of such a cell, one end not being in the plane of the section. The nucleus resembles that of a multipolar ganglion cell; the cell-plasma, however, contains tigroid bodies. From each end of the cell neurofibrillæ can be traced for a considerable distance. The long axis of the bipolar ganglion cells always lies in the direction of the nerve.

In the suckers one also finds large multipolar ganglion cells (fig. 40); they usually lie rather nearer to the delimiting membrane than to the cuticula; their processes usually unite with those of neighbouring nerve-cells, or they are connected with each other by their neurofibrillæ. A connection between the sucker ganglion cells and the "Sommer-Landois" cells of the subcuticular muscles of the suckers can frequently be made out.

The nuclei of the sucker ganglion cells measure from 6μ to 7μ in diameter; they are usually globular and contain a large nucleolus, measuring up to 2μ , and one or two chromatin bodies. Small glia cells are frequent along the surface of the cell processes or the neurofibrillæ.

I am not aware that anyone has yet pointed out the presence of ganglion cells in the suckers; I have already mentioned

them as occurring in *Stilesia centripunctata* (Rivolta) and *Anoplocephala magna* (Abilgaard), (Gough, 1909). I have recently also observed similar cells in *Tænia*, only in *Tænia* they are smaller than in *Anoplocephala* or *Avitellina*, agreeing in this respect with the other scolex ganglion cells. Recently, similar cells have been observed by Spätlich (1909), in the bothridia of *Tetrabothrium laccocephalus* and *T. macrocephalus*; he describes them as being large cells with granular plasma, which stains deeply with hæmatoxylin, and with a large nucleus in which several large chromatin masses are visible. These cells have branched processes and form a reticulation between the muscle-fibres by the anastomosing of the processes. These branched cells are more or less restricted to the middle of the bothridia, keeping distance from either surface. Spätlich thought that they might be glands, but states that his material was not sufficiently well preserved to allow definite conclusions.

It is strange that nobody has yet looked for ganglion cells in the suckers, as they could be expected to exist in order to control the working of those complicated muscular organs.

THE GENITAL ORGANS. Figs. 1-4 and 41-65; Text-fig. 2.

When the genital organs are in full activity, the receptaculum seminis is filled with spermatozoa and the oöcytes are passing from the ovarium into the uterus; the genital organs are disposed as shown on Text-fig. 2 or fig. 1. The position of the cirrus pouch to the vagina is extremely variable, as shown on fig. 43; it can lie anterior or posterior, dorsal or ventral to the vagina. The figure shows a sagittal section through about nine sections, passing through four cirri and vaginae; it will be seen that the utmost possible irregularity has been realised.

The vagina leads into the spermiduct, which shortly after having crossed the ventral canal widens and forms the pear-shaped receptaculum seminis (Text-fig. 2); from the wider

interior end of the receptaculum seminis arises the canalis seminalis (Befruchtungskanal—fertilisation duct), which, however, soon branches, sending one arm, the oviduct, to the ovarium, the other arm, the uterine duct, to the uterus. Fertilisation of the eggs probably takes place at the point of junction of the three ducts, as one cannot observe spermatozoa penetrating into either the oviduct or the uterine duct. The uterus originates as a simple, hollow, transverse tube, but fills with cells soon after the arrival of the ova, cells derived from the uterine walls completely surrounding and embedding the eggs.

At the same time the male sexual organs consist of a cirrus, which opens straight into the vagina; the vas deferens forms several twists and turns within the cirrus-pouch (fig. 4). Just before entering the cirrus pouch the vas deferens is considerably swollen with spermatozoa, forming a kind of vesicula seminalis. The vas deferens runs straight across the dorsal side of the segment, being dorsal to all the other genital organs, to the nerve and to the excretory canals. The testicles lie near the transverse axis, in four groups of from three to seven testicles, one group being lateral and one median to each ventral canal.

The changes that follow as the segments proceed from the anterior end of the strobila to the posterior end are, first the disappearance of the ovary and oviduct, then of the canalis seminalis, then the testicles disappear. The uterine duct, the receptaculum seminis and the cirrus pouch with the vagina remain long after their function is past. When the ovary is disappearing the paruterine organ begins to develop; finally all the eggs are enclosed in the paruterine organ (fig. 3).

In their first anlage the cirrus-pouch and the vagina appear to have a common origin in a clump of dark-staining nuclei which collect near one of the lateral margins. Later on this clump splits into two masses, which are surrounded by myoblasts (fig. 44). In the middle of each of these masses a central core of cells differentiates, those which are

to form the vagina advancing in their development perhaps somewhat more rapidly than those from which the cirrus arises. The central core is at first solid, at this stage it cuts itself off from the surrounding cells by the formation of a basal membrane. The cavity arises by the cells separating in the middle of the epithelial cord. At this stage we find the vagina lined with an epithelium; later on the cells of the epithelium atrophy and their nuclei disappear. The fully developed vagina (fig. 45) is lined with fine cilia, all pointing away from the pore; it is surrounded by a sheath of large cells, with round, dark-staining nuclei. The plasma of these cells does not stain; their membranes are, however, very distinct. In shape, the cells are prismatic, with all the sides delimited by planes, the ends of the cells bordering on the parenchym generally forming pyramids. These cells surround the vagina in a single layer; their function may be glandular (?), and they certainly help to give greater rigidity to the vagina.

The female sexual canals, spermiduct, oviduct, uterine duct, canalis seminalis and the receptaculum seminis all arise as solid cords of epithelial cells, which, after having produced a basal membrane, become hollow. As is the case with the vagina, the epithelium atrophies, and finally disappears. Oviduct, canalis seminalis and uterine duct are, when completely developed, lined with cilia. The receptaculum seminis is not a simple dilation of the spermiduct due to the action of the contents, but arises out of a clump of cells which already show the final shape of the organ.

The ovarium forms at a very early stage a clump of darker staining cells near the middle of the medullary layer. It is remarkable, when fully developed, as compared with the ovaries of other cestodes, on account of its compactness, and also by reason of the fewness of the oöcytes produced. The ovary is bean- or kidney-shaped, the oviduct inserting in a depression of its dorsal side; it consists of a number of lobes, separated from each other by septa (fig. 41); the lobes are all enclosed by a common outer membrane. The single lobes do

not contain very many eggs; an ovarium produces at the most fifty to one hundred eggs, a lobe probably not more than one dozen. Besides the eggs, each ovarium contains a number of smaller cells, probably abortive oöcytes, which lie scattered between the eggs and along the walls of the lobes. Fig. 42 represents a single such cell lying between three oöcytes, which have not been completely drawn. For these cells I propose the name ovarial nutritive cells, on account of their probable function. They are much smaller than the oöcytes, and are fairly rich in plasma. In shape they accommodate themselves to the space at their disposal between the eggs. They are very extensively in contact with the eggs, sending out plasmatic processes along and over the surface of the surrounding oöcytes. Their nuclei are oblong, rather pale, especially as compared to those of the oöcytes, and contain a varying number of small round chromatin bodies. The diameter of a nucleus averages $17\ \mu$ by $9\ \mu$.

There seems to me to be no possible doubt about the function of these cells. They act vicariously for the missing yolk-cells, and supply the oöcytes with nutriment by means of the processes touching and covering their surface. According to the accepted theory, the yolk-gland (vitelline gland) is only a modified ovarium, and the yolk-cells modified oöcytes. Should this theory be correct, it would render the mutation of oöcytes into nutritive cells within the ovarium easy to imagine, the chief difference from the state of things existing elsewhere being, that here both nutritive cell and oöcyte arise in the one ovary, elsewhere the oöcytes arise in the ovary and the nutritive cells (yolk-cells) in a modified ovary, the yolk-gland. A very important difference, however, still remains. The yolk-cell of other cestodes becomes attached to the oöcyte, and remains closely united to it for a long period after having handed over to the oöcyte its supply of yolk, being enclosed in the shell with the oöcyte. In *Avitellina* and *Stilesia* the nutritive cell is only temporarily connected with the oöcyte, it does not leave the ovarium when the oöcyte does, and finally one nutritive cell

has relations to several oöcytes at the same time. For all that, ovarial nutritive cell and yolk cell have in common, that both are modified oöcytes. The fact that in *Avitellina* and *Stilesia* the ovarium has in some degree the double function of ovary and vitelline gland, might be taken as proving the two genera as being primitive.

The oöcytes appear to mature in the ovarium, the reduction of the chromatin taking place before the eggs leave the ovarium. In this respect they differ from the oöcytes of *Tænia serrata*, studied by Janicki (1907), which mature in the uterus after the penetration of the spermatozoon into the egg-cell. The process of ripening is complicated by the passing of chromatin out of the nucleus into the plasma. Spätlich (1909) observed somewhat similar phenomena in *Tetrabothrium* before the mitoses take place. For the study of these changes it is necessary to use material that has been stained with iron-hæmatoxylin, and to compare with sections stained by other means, as very much depends on the technique of the specimen. Figs. 57-65 show in a series of eggs the most important changes that take place before maturation and immediately after fertilisation. Fig. 57 is the youngest stage illustrated; it represents an oöcyte that has already reached its full size. Its plasma shows no modifications whatever, being perfectly homogeneous. A nucleolus is not visible; the chromatin forms a large round mass. Fig. 58 shows a somewhat older oöcyte; a nucleolus has appeared, and in the plasma two centrosomes can be observed. The centrosomes in the cestode oöcyte are quite large objects, as already observed by other authors (e. g. Janicki, 1907). When they first appear they have a light centre surrounded by a dark ring. On the same cell one sees that the chromatin is lying quite on the nuclear membrane, causing it to press out somewhat. Fig. 59 is also of about the same stage; in the cell shown a portion of the nucleus appeared to be cutting itself off from the main portion. I cannot say whether this is a regular occurrence or no, as I have only observed it in a single cell. The next changes are

shown on fig. 60; here we find that the size of the chromatin body in the nucleus is reduced, and that now there have appeared two dark-staining bodies in the plasma, lying quite close to the nucleus and just opposite the chromatin body. In iron-hæmatoxylin these bodies stain to the same intense black as the chromatin; with Delafield's hæmatoxylin they also stain blue, but not quite as deep as the chromatin. I consider them to be portions of the chromatin which have been ejected from the nucleus; in the course of the further development of the egg they behave as the yolk-nucleus observed by Janicki does. They are certainly not the same as the yolk-nucleus observed by Spätlich in *Tetrabothrium*. I have not seen any structure to compare with Spätlich's yolk-nucleus. There are usually only two of these problematic bodies present; occasionally, however, as shown by fig. 62, a larger number can appear. Whilst they were at first situated close to the nucleus, in older ovaria we find that they have moved further away from it, until they have gone as far as possible away from the oöcyte nucleus. Fig. 61 shows the emigrant chromatin bodies moving away from the nucleus, and shows that they have also separated from each other at the same time. A further change in the nucleus can be noted, the linin threads are becoming distinct. Figs. 63-65 show that a mitosis is now becoming imminent; the chromatin mass has entirely disappeared, and the chromatin has rearranged itself on the linin threads; the nucleolus, at first large, is reducing its size. But it is also remarkable that not only has the chromatin in the nucleus changed its arrangement, but also the chromatin that had wandered into the plasma. In the place where the emigrant chromatin bodies had been one now finds a mass of fine granules, and finally the only remaining trace is a darker staining of the plasma in the vicinity of the place they had occupied. It is worth noting that a pair of centrosomes are often seen close to the emigrant chromatin before it dissolves.

The mitoses that follow have been observed, but in specimens stained with Ehrlich's hæmatoxylin considerable luck

is necessary to obtain material where mitoses are taking place, as they pass very quickly. Usually only two or three segments at the most show mitoses; those before and after not containing any; the difficulty of obtaining sections through the exact portion of the strobila is consequently easy to appreciate. There appear to be four chromosomes, the exact number being hard to count, as the karyokinetic figure is very small. I have not been able to make out the fate of the pole bodies, but when the eggs pass into the oviduct they are no longer to be found. The matured oöcytes are not enclosed in a membrane of any kind; they arrive naked in the uterus, not having a membrane of their own, and not receiving a shell, as there is no shell-gland. The fertilised oöcytes are at first not enclosed in shells or by membranes. Fig. 49 shows four oöcytes in the uterus in various stages of development; in oöcytes *a* and *b* one sees the spermatozoa as short rod-shaped bodies, stained black by the hæmatoxylin, lying in a dark area, stained bright red by eosin. Oöcyte *c* shows the sperma nucleus and the egg nucleus fusing in the same manner as described by v. Janicki (1907) for the eggs of *Tænia serrata*. The plasma of the eggs in these first stages after fertilisation is not homogeneous, but contains larger and smaller masses of differently staining substance.

The later fate of the eggs has not been followed up; at first, however, the multiplication of the nuclei is not followed by division of the plasma, so that up to four nuclei can be seen in an undivided mass of plasma. Later on one observes quite regularly that the cell division gives rise to a few macromeres, and to a greater number of micromeres, the macromeres probably giving rise to the egg envelopes, the micromeres to the embryo, as demonstrated by Janicki (1907) for *Tænia serrata*. The exact number of macromeres is difficult to ascertain accurately: one is always larger than the others; two can usually be recognised, but I am not certain whether there is a third or no.

Soon after the eggs arrive in the uterus they become

surrounded by smaller cells, derived from the walls of the uterus. These cells, which have already been observed by Fuhrmann (1909) in *Stilesia sjöstedti*, Fuhrmann, can be termed uterine nutritive cells; their function is without doubt nutritive, as already suggested by Fuhrmann. The uterine nutritive cells (fig. 49) soon fill all the space between the eggs. They are rich in plasma at first; later on their plasma decreases, and finally they atrophy and disappear entirely at or before the stage when the egg envelopes have developed.

Fuhrmann (1909) supposed the uterus of *Stilesia* to have no cavity originally. This is not correct for *Avitellina*; the uterus is a simple hollow tube at the time of the arrival of the eggs, the uterine nutritive cells appearing shortly afterwards.

The absence of a vitelline gland, as can be seen, has had such an influence on the cestode as to have caused cells of two separate and distinct origins to arise in two different organs to replace to some extent the nutritive function of the missing organ, that is, if the vitelline gland has been lost in the history of the genera. If, on the other hand, the lack of a vitelline gland is a primitive character, the acquisition of uterine nutritive cells must still be a recent adaptation, as one would otherwise expect to meet such cells in other cestodes.

The uterine wall cells, after having given rise to the uterine nutritive cells, next supply the origin of the egg-pouches or paruterine organ.¹ In the first stages of the development of this organ we find parallel layers of plasma containing nuclei splitting off the terminal wall of the uterus (fig. 51). The

¹ It is a question which I cannot attempt to decide, whether the paruterine organ of *Stilesia* and *Avitellina* is homologous to the paruterine organ of other cestodes, as where it has been observed previously it has generally been held to arise outside the uterus. I am retaining the name as being convenient and as referring to a more or less well-known structure, but without prejudice as to its origin in other species. The paruterine organs of various cestodes may quite possibly be of different origin, and may only be convergent structures, as Fuhrmann has shown that they can arise independently in various unrelated genera.

nuclei are oblong, with two or three chromatin bodies, and resemble to some extent those of the uterine nutritive cells. The presence of the developing paruterine organ causes the wall of the uterus to bulge outwards at a very early stage. The portion thus pressed outwards is at first hemispherical, opening directly into the main body of the uterus, this portion might be termed the paruterine pouch (fig. 53). The next changes take place very quickly, and we find the mouth of the pouch contracting, the contraction being effected by muscles running around this portion of the uterus. As the contraction proceeds the pouch becomes more nearly globular, until it is finally almost spherical, remaining connected with the remainder of the uterus by a very narrow passage. These changes of their receptacle are not without their effect on the lamellæ, whose shape has to accommodate itself to the changes in form of the pouch; as they remain arranged parallel to the walls of the pouch they, too, become spherical; but the lamellæ are also growing quickly, and some of them force their way into the uterus as concentric hemispheres, whilst others form concentric hemispheres within the pouch. The lamellæ projecting into the uterus appear to grow very quickly, pressing the uterus and its eggs further and further away from the pouch; the uterus, which was at first merely a transverse tube, becomes globular through the invasion of the lamellæ, which have become almost spherical. Finally, as the lamellæ are still growing and as they have no more room to spread outwards, the only outlet remaining for them is by doubling back. Depressions appear on the surface of the lamellæ, deepen, pass through the neck into the pouch, carrying the eggs, which are already enclosed in their two envelopes and contain embryos, with them into the paruterine pouch. Finally, the lamellæ appear to complete the process of retroversion and return entirely through the neck of the pouch: none are left outside the pouch at all events. The growing back always takes place at several points at the same time, thus giving rise to more than one pocket; six or seven are usually formed (figs. 53-56, *pp.*).

The lamellæ are at first composed of a mass of plasma belonging to several nuclei and are fairly thick; with further development the thickness of the lamellæ becomes less and less, and they finally resemble thin fibrillæ on section. There can, however, be no doubt that the structure is lamellar and not fibrillar, as in whatever direction a section may pass through a paruterine organ, one always sees a concentric arrangement of "fibres" running within the plane of the section, but never by any chance sections through fibrillæ. As the lamellæ grow older the nuclei atrophy and finally disappear.

Directly anterior to the uteri lie pads of fibrous tissue (fig. 52), whose probable function is to give support to the paruterine organ, and perhaps also to act as cushions between the uteri and paruterine organs of adjacent segments. These pads take their origin from myoblasts, which are very frequent close in front of the uteri when the eggs are beginning to enter that organ. The fibrous tissue, when fully developed, contains scattered nuclei, and its fibrillar structure is fairly apparent. It stains fairly vividly with eosin (fig. 48).

The cirrus and cirrus pouch arise out of a common anlage with the vagina, as already explained above. The dense mass of dark-staining nuclei, which gives rise to both, first splits into two masses; these become surrounded by myoblasts (fig. 44); then, in the middle of one of the two masses the vagina begins to develop, the cirrus and part of the vas deferens in the other. The epithelial cord arising in the middle of the cirrus pouch anlage is at a very early stage already convoluted, probably giving rise to the twisted portion of the vas deferens enclosed in the cirrus pouch.

The vas deferens is, like the female ducts, formed of a solid cord of epithelial cells surrounded by a basal membrane (fig. 46); the hollow arises later. As in the female ducts, the epithelium finally atrophies entirely. Its lumen is then clothed with ciliæ.

The cirrus is straight, $34\ \mu$ long by $3\ \mu$ in diameter; it is devoid of hooks, bristles or cilia of any kind; it opens

directly into the vagina. I have never observed an intromission of the cirrus into the vagina. The cirrus muscles consist almost entirely of a circular muscle; the longitudinal muscle usually observed between the circular muscle and the cirrus appears to be wanting or extremely weak. Cirrus and vas deferens within the cirrus pouch are suspended by loose parenchym cells, which stretch themselves from the walls of the pouch, passing around and gripping the vas deferens. They act as a kind of mesentery in function (fig. 50).

The muscles of the cirrus pouch are very feeble. The whole cirrus apparatus gives one the impression that the cestode has lost the habit of cross-fertilisation, and that self-fertilisation has become the rule.

CONCLUSIONS.

- (1) There is no subcuticula in the suckers.
- (2) The cuticula can arise independently of the subcuticula.
- (3) The subcuticular cells stand in direct connection with the dorsoventral and transverse muscles and form part of them.
- (4) The parenchyma muscles are produced by three kinds of myoblasts: (a) Bipolar myoblasts with terminal fibrillæ; (b) bipolar myoblasts with lateral fibrilla; (c) elongate bipolar myoblasts lying axially in the tubiform muscles of the longitudinal muscle.
- (5) The dorsal canals are surrounded by nephrocytes, which are homologous with the parent cells of the flame-cells.
- (6) There are ganglion cells in the suckers, connected by neurofibrillæ with the "Sommer-Landois" cells of the subcuticular muscles of the sucker.
- (7) In addition to oöcytes, nutritive cells are produced in the ovaries.
- (8) The oöcytes mature before leaving the ovarium.
- (9) The oöcytes arrive fertilised in the uterus.
- (10) The oöcytes in the uterus are surrounded by nutritive cells of uterine origin.
- (11) The paruterine organ is contained in a pouch of the uterus, and arises within the uterus; its structure is lamellar.

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EXPLANATION OF PLATES 12-14,

Illustrating Mr. Lewis Henry Gough’s “Monograph of the
Tapeworms of the Sub-family Avitellinæ, being a
Revision of the Genus *Stilesia*, and an Account of the
Histology of *Avitellina centripunctata* (Riv.).”

PLATE 12.

Avitellina centripunctata (Rivolta).

- Fig. 1.—Portion of three segments at male sexual ripeness. $\times 99$.
Fig. 2.—Oncosphere. $\times 588$.
Fig. 3.—Four segments with fully developed paruterine organs. $\times 99$.
Fig. 4.—Cirrus-pouch and vagina. $\times 380$.
Fig. 5.—Left half of the excretory canals of scolex, lateral view.
Fig. 6.—Right half of the excretory canals, lateral view.
Fig. 7.—Scolex.

Stilesia vittata, Railliet.

- Fig. 8.—Pore-side of a segment at male sexual ripeness. $\times 99$.
Fig. 9.—Oncosphere. $\times 588$.

Stilesia globipunctata (Rivolta).

- Fig. 10.—Pore-side of a segment at male sexual ripeness. $\times 99$.
Fig. 11.—Oncosphere. $\times 588$.
Fig. 12.—Scolex.

Stilesia hepatica, Wolffhügel.

- Fig. 13.—Pore-side of a segment at male sexual ripeness. $\times 99$.
Fig. 14.—Segment with developing paruterine organs. $\times 99$.
Fig. 15.—Oncosphere. $\times 588$.
Fig. 16.—Cirrus pouch and vagina. $\times 380$.

PLATE 13.

Avitellina centripunctata (Rivolta).

- Fig. 17.—Cuticula of the suckers. $\times 784$.
 Fig. 18.—Cuticula of the proglottids, silver nitrate fixation. $\times 784$.
 Fig. 19.—Subcuticula and cuticula. $\times 784$.
 Fig. 20.—Subcuticula near the lateral margin. $\times 784$.
 Fig. 21.—Cuticula and subcuticula in an old segment. $\times 392$.
 Figs. 22, 23.—Dorso-ventral muscles. $\times 784$.
 Figs. 24, 25.—Transverse muscles. $\times 784$.
 Figs. 26, 27.—Longitudinal muscles. $\times 784$.
 Fig. 28.—“Sommer-Landois” cell, from the scolex. $\times 784$.
 Fig. 29.—“Sommer-Landois” cells of the “subcuticular” muscles of the suckers. $\times 784$.
 Fig. 30.—Ventral canal, 25 cm. from the scolex, showing epithelial cells sinking into the parenchyma. $\times 784$.
 Fig. 31.—Dorsal canal, 50 cm. from the scolex, showing nephrocytes. $\times 784$.
 Fig. 32.—Epithelial cell of the ventral canal sinking into the parenchyma to become a parent of flame-cells. $\times 784$.
 Fig. 33.—Ventral canal, 1 cm. from the scolex, with developing flame-cells and a group of young flame-cells. $\times 784$.
 Fig. 34.—Flame-cell. $\times 784$.
 Fig. 35.—Group of flame-cells, reconstructed from three consecutive sections. $\times 392$.

Tania serrata. Rud.

- Fig. 36.—Developing flame-cells. $\times 784$.

Avitellina centripunctata (Rivolta).

- Fig. 37.—Flame-cell. $\times 2352$.
 Fig. 38.—Multipolar ganglion-cells from the central ganglion of the scolex. $\times 784$.
 Fig. 39.—Bipolar ganglion-cell from one of the nerves in the scolex, showing tigroid bodies. $\times 784$.
 Fig. 40.—Multipolar ganglion-cell from the suckers. $\times 784$.
 Fig. 41.—Young ovarium. $\times 588$.
 Fig. 42.—Nutritive cell lying between three oöcytes in a young ovarium. $\times 1146$.

PLATE 14.

Avitellina centripunctata (Rivolta).

Fig. 43.—Sagittal section through nine segments, showing cirrus pouches and vaginae of four segments. $\times 88$.

Fig. 44.—Anlage of vagina and cirrus. $\times 784$.

Fig. 45.—Transverse section of vagina. $\times 784$.

Fig. 46.—Anlage of vas deferens. $\times 1176$.

Fig. 47.—Developing oviduct. $\times 1176$.

Fig. 48.—Fibrous tissue. $\times 784$.

Fig. 49.—Portion of uterus, with oöcytes surrounded by nutritive cells. $\times 1176$.

Fig. 50.—Transverse section through a cirrus-pouch, showing the vas deferens suspended by "loose parenchym cells." $\times 784$.

Fig. 51.—Anlage of paruterine organ on the uterus-wall; three oöcytes are represented surrounded by nutritive cells. $\times 784$.

Fig. 52.—Developing paruterine organ. $\times 190$.

Fig. 53.—Developing paruterine organ; the "pockets" (*pp.*) are just forming. $\times 190$.

Fig. 54.—Paruterine organ: a somewhat older stage than fig. 53. $\times 190$.

Fig. 55.—Paruterine organ with eggs entering the pockets. $\times 190$.

Fig. 56.—Fully developed paruterine organ; all the eggs have entered the pockets of the pouch.

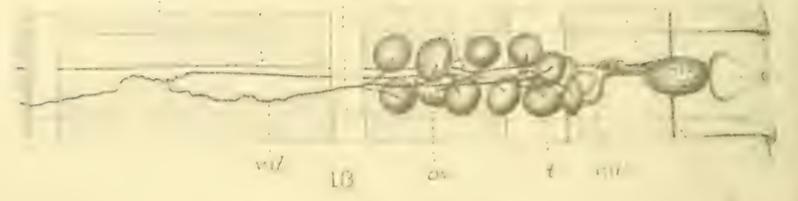
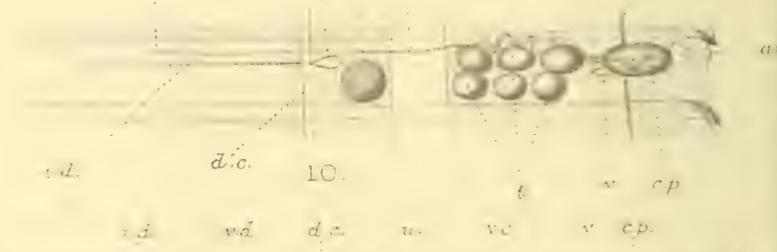
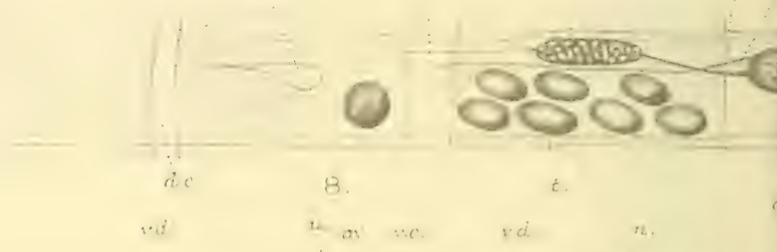
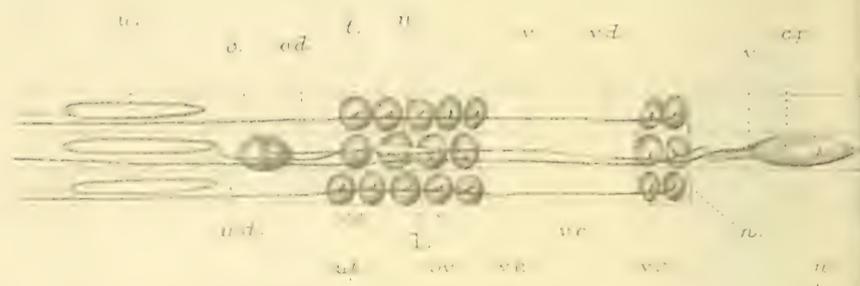
Figs. 57-65.—Oöcytes in various stages.

LIST OF ABBREVIATIONS USED.

[The abbreviations used are the same throughout the paper.]

a. Sucker. *at.* Cloaca. *b. m.* Basal membrane. *b. p.* Basal plate.
c. Cirrus. *c. c.* Connecting canal. *ch.* Chromatin. *c. l.* Comidial layer.
c. p. Cirrus pouch. *c. s.* Canalis seminalis. *cu.* Cuticula.
d. Dividing nucleus. *d. c.* Dorsal canal. *e.* Egg. *ep.* Epithelium.
ep. c. Epithelial cell. *f.* Fibrilla. *f. c.* Flame-cell. *f. c. d.* Developing flame-cell.
fl. Flame. *f. p.* Fibrous pad. *fu.* Funnel. *g.* Gland-cells surrounding the vagina.
gl. Glia-cells. *h. l.* Homogeneous layer. *i. d.* Interuterine duct. *k.* Capillary. *k. c.* Capillary cell. *l.* Lumen.
l. i. Inner layer. *l. o.* Outer layer. *m.* Muscle. *mb.* Myoblast. *m. f.* Muscle-fibrilla.
n. Nerve. *n. c.* Nutritive cell. *nf.* Neurofibrilla.

nl. Nucleolus. *nu.* Nucleus. *o.* Ovarium. *od.* Oviduct. *p.* Paruterine organ. *par.* "Parenchyma" cell, suspensory cell in the cirrus pouch. *pou.* Paruterine pouch. *r.* Nephrocyte. *r.f.* Radial fibres. *r.s.* Receptaculum seminis. *s.* Spermatozoon. *s.c.* Subcuticular cell. *s.l.* "Sommer-Landois" cell. *s.m.* Subcuticular muscle. *t.* Testicle. *t.b.* Tigroid bodies. *t.c.* Transverse canal. *u.* Uterus. *u.d.* Uterine duct. *v.* Vagina. *v.c.* Ventral canal. *v.d.* Vas deferens. *v.s.* Vesicula seminalis. *x.* Corpuscle. *y.* Centrosome. *z.* Fusing sperma and egg nucleus



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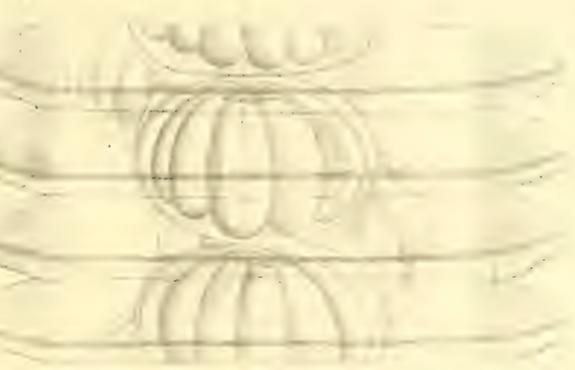
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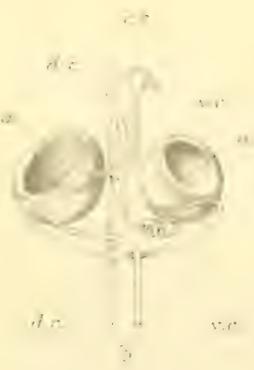
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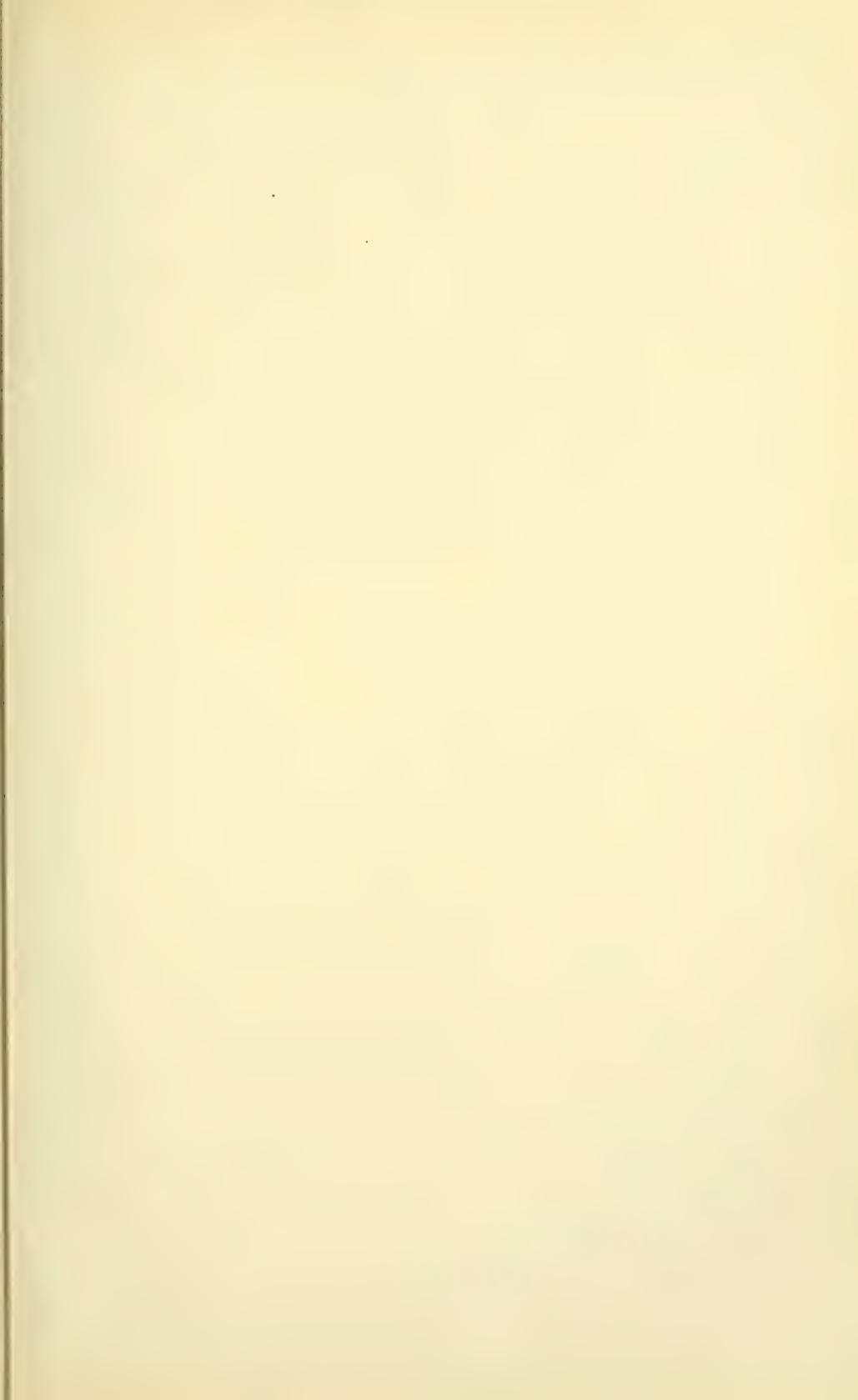
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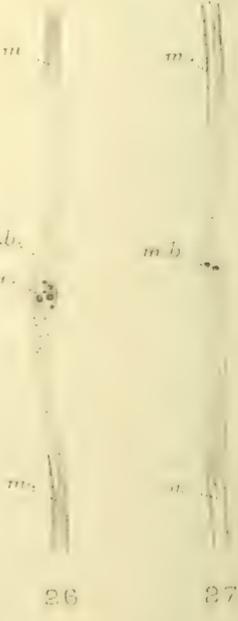
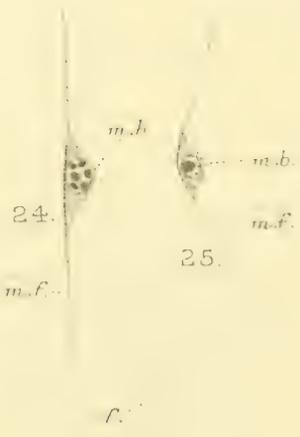
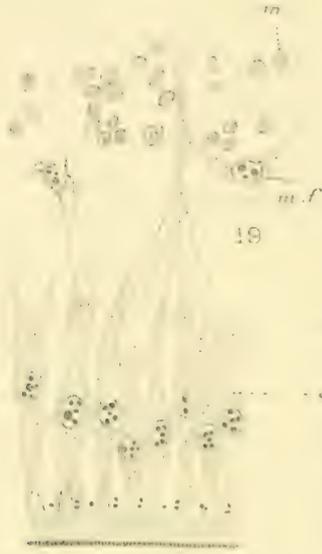
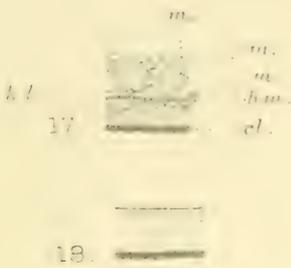


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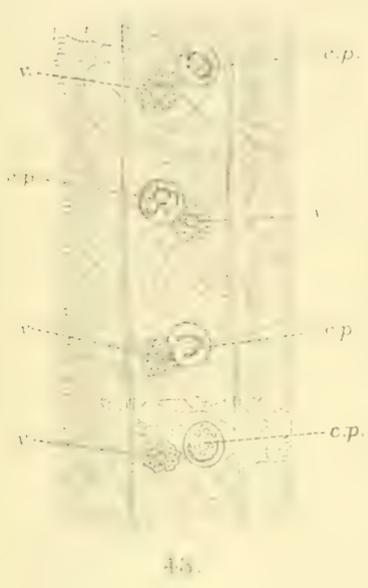


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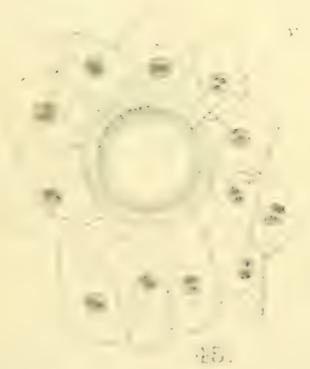
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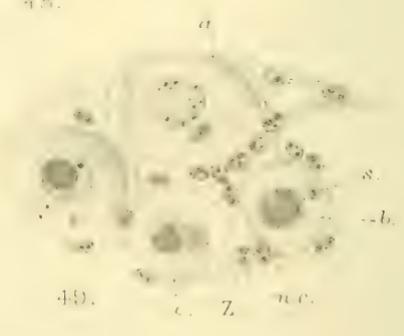
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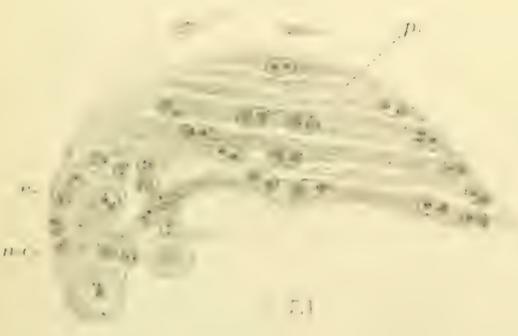
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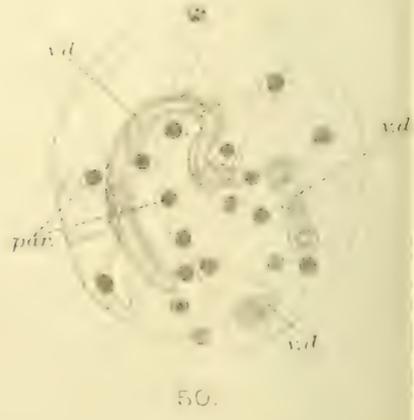
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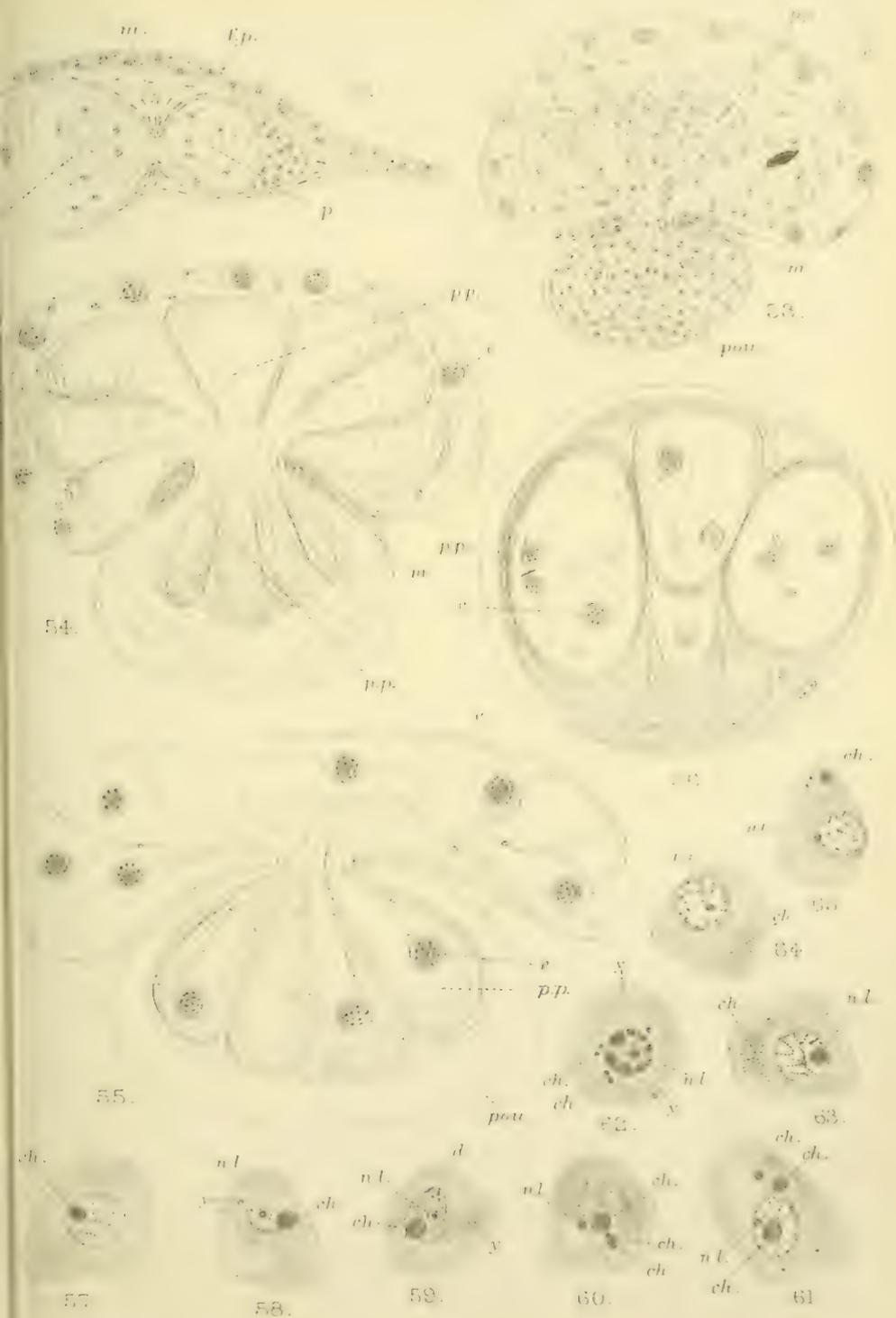
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Brief Notes on the Structure and Development of *Spirochæta anodontæ* Keysselitz.

By

W. Cecil Bosanquet, M.D.

With Plate 15.

MUCH uncertainty still prevails as to the structure and biological relationships of the spirochætes. Earlier observers—not altogether influenced perhaps by a desire to prove their affinities with the hæmoflagellates—equipped them with an undulating membrane and a complicated nuclear apparatus, the existence of which is very problematical; and much controversy has existed as to their mode of division, longitudinal or transverse. The very claim of the organisms found in Mollusca, as well as that of the smaller spirochætes, to be called by this name, depending as it must on their relationship to *Sp. plicatilis* Ehrenberg, the type-species, is not very firmly established, since the structure of the latter—a system of large waves, each broken by smaller undulations superposed on them, with a central axial rod and a series of dots, regarded as nuclei—is markedly different from that of the later additions to the genus. It is desirable, therefore, to record any observations which may tend to throw light on the morphology and life-history of these organisms.

My own observations have been carried out on *Sp. anodontæ*, a species easily obtained, and one which may be regarded as fairly typical of the spirochætes which are parasitic in Mollusca.

With regard to the so-called undulating membrane of

Sp. anodontæ I am inclined to agree with Schellack, who holds that the appearance which has been thus interpreted is an artifact, due to splitting of periplast, since I found that the better my preparations were fixed, the fewer were the examples of this condition. It was found almost invariably in films dried in the air (fig. 1), but very exceptionally in those fixed in osmic vapour. It seems possible that the periplast, if it is so to be called, adheres to the slide in the position in which death of the spirochæte occurs, and that subsequently the protoplasm of the organism shrinks and thus straightens its outline a little, leaving a line of periplast following a different line from that finally adopted. At other times the periplast seems to shrink most and to form a band uniting the cells of the spirochæte (fig. 2).

I have seen a considerable number of specimens which exhibit a dark line running along one side (figs. 3, 4). This probably corresponds with the "crest" described by Gross. Judging from the illustrations of spirochætes in section given by him and previously by Fantham, it would seem that the organism possesses a sheath which is loose enough to form a fold along one side in certain conditions. The sheath or periplast stains more darkly than the body-substance of the spirochæte, and hence the double layer appears as a dark line. Splitting of the sheath into fibrillæ seems sometimes to occur, as in fig. 6.

On the other hand, I find it difficult to agree with Schellack that two separate species of spirochæte are present together in *Anodonta*, one having blunt (figs. 1, 3, 4) and the other sharp extremities (figs. 7, 8, 9). The amount of difference seen is scarcely sufficient to suggest a specific differentiation. Further, the great variation in the length and thickness of the individuals in each class thus formed is such as to negative the possibility of distinguishing species by length, as he further suggests. It is noteworthy that Schellack distinguishes two species of spirochæte in more than one kind of mollusc, and that Gross also describes two, very similarly differentiated, in *Pecten jacobæus*. This rather strongly suggests that

the same spirochæte may present different shapes and sizes. I have never seen specimens of *Sp. anodontæ* with transverse lines near the middle of the length of the organism, pointing to transverse division by means of a septum here formed, as is depicted by Schellack.

As to the actual method of division, I had the opportunity of observing an instance of what appeared to be longitudinal fission. This was in a preparation containing living spirochætes in a drop of fluid from the mantle-cavity of the mussel. My attention was attracted to an individual which remained fixed at one point, near to the edge of the preparation, and wriggled feebly, instead of dashing about like the others. I then noticed that this spirochæte seemed to possess two "tails," if this expression is allowable (fig. 11*a*); and on watching it I noticed that a line, dark at one moment, refringent at another, as the creature moved, was gradually extending along the body of the spirochæte from the point of bifurcation towards the opposite extremity. Fission proceeded along this line, but not quite regularly as in tearing a strip of calico, since at one moment there was visible a sort of loop in the "anterior" part of the organism—using this term for the portion which remained single, longest—before the process of division had extended quite to this point from "behind" (figs. 11*b*, *c*). When the longitudinal fissure had extended nearly to the "anterior" end, the organism suddenly jerked itself away out of the field of the microscope and I was unable to see the final separation of the daughter, individuals; but as I was unable, on searching through the preparation, to find the dividing spirochæte again, I could only conclude that the separation took place almost immediately, the two resulting spirochætes being then indistinguishable from the other individuals present.

A possible source of error in this observation lies in the slenderness of the organisms and in the difficulty of accurately observing them during movement. It is scarcely possible to exclude the explanation that an organism may become folded upon itself as a preliminary to undergoing transverse fission

(figs. 12, 13). This alternative possibility was strongly brought to my mind by a preparation in which many living spirochætes appeared to have loops at one extremity, while they revolved rapidly on a longitudinal axis. As their movements slackened, these organisms appeared to have a shape which might be compared with that of a hair-pin having its "legs" twisted together. On fixing and staining this preparation, specimens were found both of the condition just described (fig. 14), and also of what seemed to be a succeeding stage, in which division had taken place at the bend of the loop (fig. 15). I could find no instance of a spirochæte simply looped at one extremity, as might have been expected to occur if longitudinal division took place, starting at a point a little distant from the extremity of the spirochæte. I was at first tempted to regard the pairs of closely apposed organisms here seen as being instances of conjugation, but the explanation just given seems more probable in view of the looped forms seen alive in the preparation. Division by "incurvation" is stated by Gross to occur in the spirochæte present in *Pecten jacobæus* (called by him *Cristispira pectinis*), and he believes it to be the characteristic of all spirochætes. Nuttall, Fautham and Porter state that in the small spirochætes both transverse and longitudinal division occurs. If this be so, it would be interesting to ascertain whether any difference in environment is responsible for their adoption of each method respectively, or whether the two processes occur at different points in the life-cycle.

With regard to the internal structure of *Sp. anodontæ*, I have always found that specimens taken from the crystalline style of the mussel stain homogeneously like *Bacteria* (fig. 7). Those, however, which are present in the stomach of the mollusc, especially after the style has dissolved, show beading of the substance of the organism, as is depicted by Keysselitiz in his original memoir (figs. 16, 17, 18). Since the style is apparently the most favourable medium for the life of the spirochæte, I am inclined to look upon the beaded forms as either involutory or developmental. The fol-

lowing observation supports the view that the change is, in some cases at least, one of development.

A piece of crystalline style was dissolved in tap-water and sealed up with vaseline for forty-eight hours under a coverslip on a slide. At the end of this period the cover was removed, and the preparation was fixed in osmic vapour and stained with thionine. The spirochætes, which had originally been very numerous, were found to have almost entirely disappeared, while their place was taken by elongated, variously curved bodies, closely corresponding with them in length and thickness, containing irregular masses of staining substance (figs. 19 and 20). Some definite spirochætes present showed beading of protoplasm and separation into segments (segmentation, plasmolysis?), and it was possible to trace a fairly definite series of gradations between such spirochætes, as seen in this and other preparations, and strings of bead-like or coccoid bodies, which seemed to constitute the final stage of development (figs. 16, 18, 21, 22). It seems then that *Sp. anodontæ* goes through a stage in development in which it breaks up into coccoid bodies, just as, according to Leishman, *Sp. duttoni* breaks up into similar bodies inside the body of the tick. If this be so we have, on the one hand interesting evidence of the generic unity of the small and large spirochætes, and on the other, of the affinity of the whole group with the Bacteria rather than with the Protozoa.

The portion of style used for the above observation was taken from a mussel which had been kept at a temperature just above freezing-point. The spirochætes seemed specially numerous and lively at this temperature, and dividing forms were numerous. I also found some organisms in the water in which the mussels were kept. Most of these were stiff and motionless, but one or two individuals were actively moving when first seen. The addition of water to a piece of the style seems to have the effect of rendering a large number of the organisms motionless and rigid (fig. 23), and it is tempting to speculate as to whether the difference between

what has been called a *Treponema*, in which the curls are more or less rigid, and a *Spirochæte*, in which there is more flexibility, may not be dependent to some extent on the medium in which the organism is examined. Accompanying the spirochætes in the water were a considerable number of spirillar organisms, exactly resembling them in microscopical structure (figs. 23, 24 *a, b*). It seems possible that the segmenting spirochætes (fig. 19) may divide first into short spirilla in some instances before undergoing development into the coccoid stage. At a low temperature the style of the mussel is more slowly dissolved than at the temperature of the room. I have found that, as a rule, this body entirely disappears in about a week or ten days after the mussel is taken up from its native place. One mussel, which was kept for three weeks in an aquarium, where presumably food was plentiful, formed a new style, so far as could be judged, since all the other mussels of the same batch had lost theirs long before. This re-formed style was quite free of spirochætes.

My best thanks are due to Prof. Minchin for allowing me to use his laboratory, and for his advice and encouragement; also to Miss Rhodes, who has drawn the illustrations to this paper.

LISTER INSTITUTE,
November 4th, 1910.

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EXPLANATION OF PLATE 15,

Illustrating Dr. W. Cecil Bosanquet's paper on "Brief Notes on the Structure and Development of Spirochæta anodontæ Keysselitz."

[All the figures, except Figs. 1 and 11, are from specimens fixed in osmic vapour and stained with thionin, and all are drawn at a uniform magnification of 2000.]

Fig. 1.—Spirochæta anodontæ, showing so-called undulating membrane. From a film dried in the air and stained with iron-hæmatoxylin.

Fig. 2.—Sp. anodontæ—long thin specimen from crystalline style.

Fig. 3.—Same. Long thick specimen.

Fig. 4.—Same. Short thick specimen.

Fig. 5.—Same. Short thin specimen. †

Fig. 6.—Same, showing splitting of (?) sheath.

Fig. 7.—Same. Slender specimen showing uniformity of structure and absence of "undulating membrane," like a bacterium.

Fig. 8.—Same. Short specimen with pointed ends.

Fig. 9.—Same. Longer specimen with pointed ends and somewhat stiff curls (not well shown in the figure).

Fig. 10.—Same. Short thin specimen with apparent undulating membrane.

Fig. 11.—*a, b, c*, stages in apparent longitudinal division of Sp. anodontæ (diagrammatic).

Figs. 12, 13.—Stages in "incurvation" (?) of Sp. anodontæ.

Figs. 14, 15.—Complete incurvation (?) and subsequent transverse division.

Figs. 16, 17, 18.—Sp. anodontæ, from the alimentary canal of the mussel, showing plasmolysis (?).

Figs. 19, 20, 21, 22.—Apparent development of spirochætes into coccoid bodies. From a preparation of crystalline style dissolved in water and kept for forty-eight hours.

Fig. 23.—Spirochæte from water in which mussels had been kept, for comparison of structure with figs. 24 *a, b*, showing spirilla from same water.

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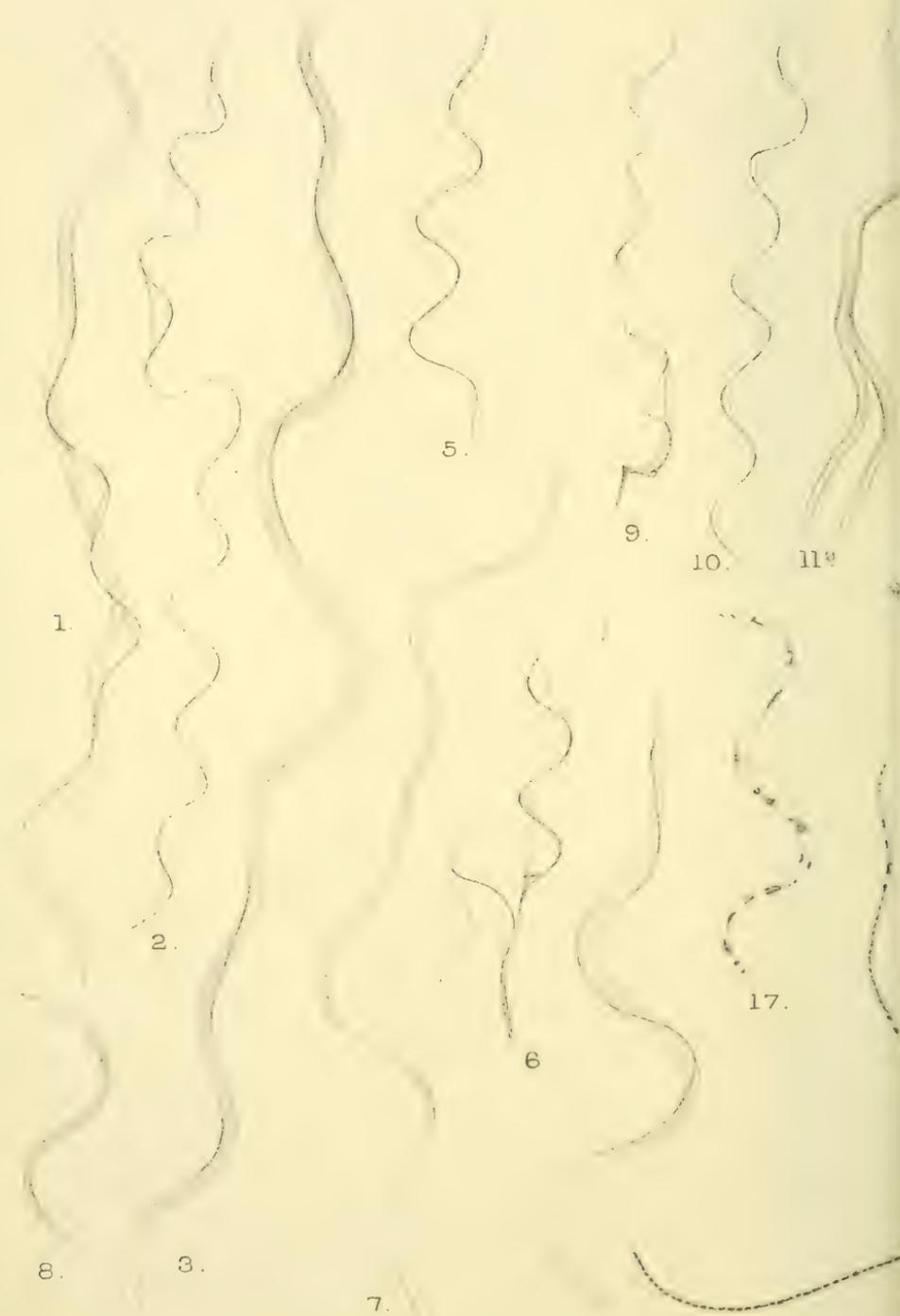
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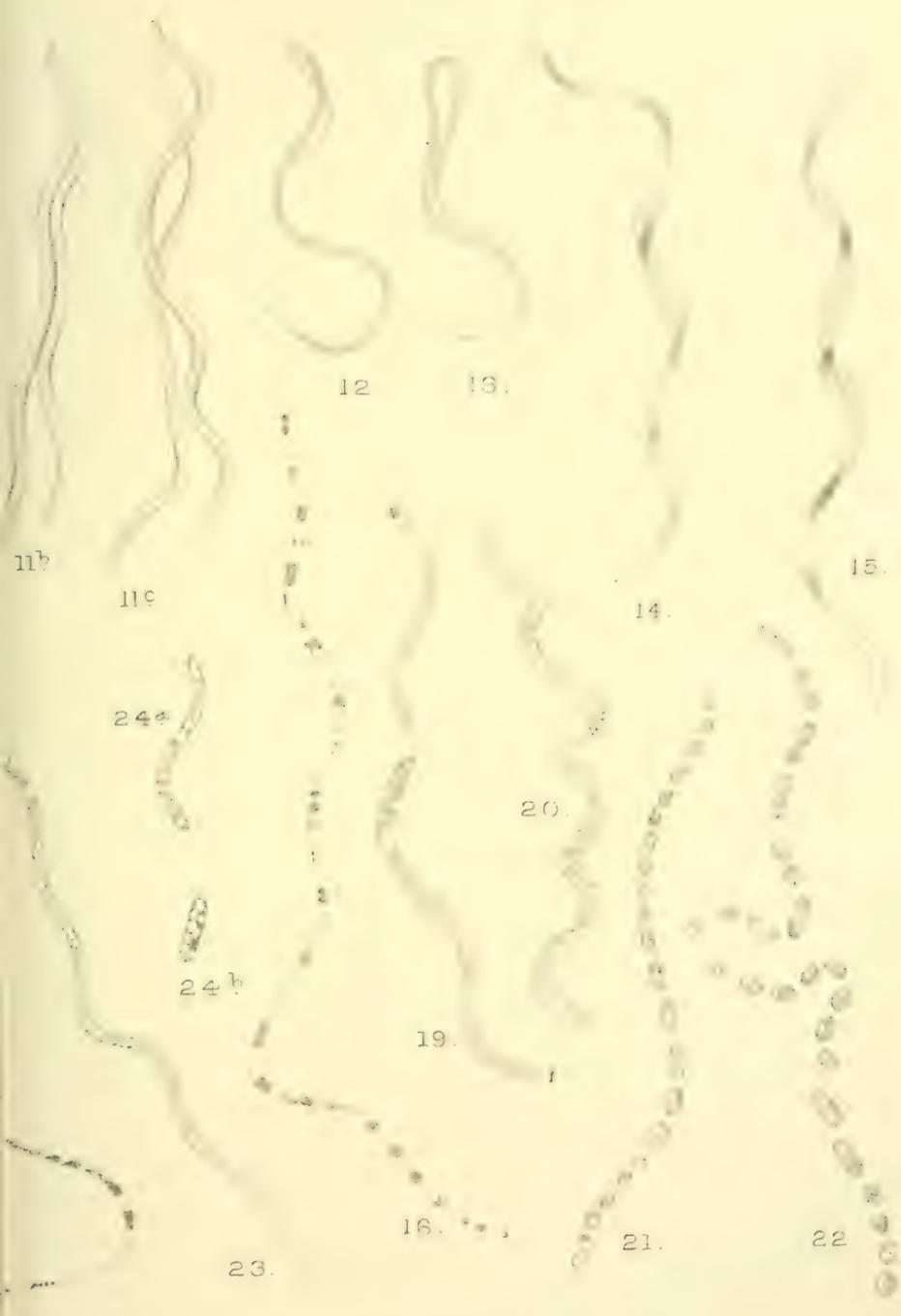
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Contributions to the Cytology of the Bacteria.

By

C. Clifford Dobell.

Fellow of Trinity College, Cambridge; Lecturer at the Imperial
College of Science and Technology, London.

With Plates 16-19, and 1 Text-figure.

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“Ich hoffe zuversichtlich, dass wir nicht mehr allzu weit von dem Augenblicke entfernt sind, wann es klar werden wird, dass die verschiedenartigsten Angaben, insofern dieselben einer ernsten und gewissenhaften wissenschaftlichen Arbeit entspringen, alle in reinen Einklang gebracht werden, so dass ein neues schönes Gebäude, das der Bacterien-cytologie, in der allerfeinsten der Wissenschaften hoch emporragen wird.”—Mencl (1910).

INTRODUCTION.

It is a remarkable fact that modern cytology, which has recently made such rapid strides as the result of the enthusiastic investigations of a vast army of workers, has almost lost sight of the Bacteria. Cytologists and protistologists alike have been content, for the most part, with assuming that the Bacteria are a group of simple organisms, possessing but little structural differentiation, and have then left them alone. Yet no biologist would deny, I think, that it is of the utmost importance that we should possess exact detailed knowledge of the structure and life-history of this immense group of living beings. More than one of the current conceptions in biology must undergo profound modification when we have precise information regarding the Bacteria.

If anyone endeavours, at the present moment, to ascertain from the vast bacteriological literature, which has been pouring out for many years past, the present state of knowledge regarding the structure of Bacteria, he will find that the whole matter is in a state of utter chaos. He will find that

the most divergent views are held regarding the various structures present in the bacterial cell. He will find, for example, regarding that most important of all cell-structures—the nucleus, that all views regarding its existence are held—from that which tells him that there is no nucleus of any sort, to that which tells him that the whole cell is to be regarded as a free nucleus.

Now the reason for this divergence of opinion is not far to seek. For many years the Bacteria have been entrusted to the bacteriologists, and only an occasional botanist or zoologist has ventured to poach on their preserves. Yet to the bacteriologists, the Bacteria are but a means to an end—they study them in order to cure a cold or make a cheese. Modern bacteriological methods are excellent and adequate when applied to medical diagnosis or industrial needs, but they are inadequate when applied to a study of the Bacteria themselves.¹ It is for this reason that professed bacteriologists possess such remarkably diverse opinions regarding the normal structure of Bacteria, and it is for this reason also that what little is definitely known of their cytology is due largely to the labours of a few zoologists and botanists. The bacteriologists are, of course, not to blame for this. Their aims are wholly different from those of the protistologist or cytologist. It is from these that our knowledge of the structure of Bacteria must come.

The great majority of Bacteria which have been described have taught us nothing concerning the internal structure of the bacterial cell. Nearly all the pathogenic forms are of exceedingly small size; and in addition to this great disadvantage they have mainly been studied after fixing and staining in the usual bacteriological manner, which renders them worthless for cytological purposes. It is desirable, in the first place, to study the largest and most easily investigated

¹ The truth of this can easily be seen by anyone who will consult the vast number of text-books on bacteriology which are in current use. In the majority of these, the cytology of Bacteria is not noticed at all, or else dismissed with a few inaccurate remarks made at random.

forms, and to examine them after treatment by suitable cytological methods.

The foregoing considerations have led me to a study of the cytology of the Bacteria. During the last four years I have devoted a considerable amount of time and labour in an endeavour to arrive at positive conclusions regarding the structure of the bacterial cell. It has been my object to discover large Bacteria which can be investigated cytologically with comparative ease—both whilst living and after suitable fixation and staining. The present paper represents the greater part of the results of my work, which—though still in progress—has led me to conclusions which are sufficiently definite to appear to me worth publication. I do not claim that the problem of the cytology of the Bacteria has been solved. My results are here given merely as a contribution towards a solution of the problem: I know only too well how incomplete and imperfect they are.

My main object has been to discover whether the Bacteria are nucleate or enucleate cells. It is useless to speculate upon the "simplicity," "primitiveness," "lack of differentiation," etc., which this important group is supposed to display, when such a simple point as this remains in doubt. I have endeavoured to find out whether a nucleus is present, and—if present—what form or forms it may assume. As staining reactions and micro-chemical tests appear to me to have been a signal failure in this direction, I have attacked the problem from another point of view—the morphological. I hoped—and I confess I am not altogether disappointed—that a study of the morphological elements present in the cell, and their behaviour during the various phases of the life-cycle, would throw considerable light upon the matter. Such results as I have obtained are, at least, very definite. They are, moreover, supported by the less important—as I believe—results derived from staining reactions.

As there is already a very extensive and confusing literature dealing with the structure of Bacteria, I have thought it advisable to give a brief historic review of the more important

work which has been done previously on the subject. I shall then give my own observations—recording them quite independently of the work of others—and reserve a full discussion of the whole matter to the final section of the paper.

My work was begun in the Zoological Laboratory in Cambridge. Afterwards I continued it whilst working in the Zoological Institute in Munich, and at the Zoological Station in Naples.¹ Subsequently I was able to add to my results whilst visiting Ceylon in 1909, during my tenure of the Balfour Studentship of Cambridge University. I have completed my work up to its present state at the Imperial College of Science and Technology, London. I desire here to record my indebtedness to all those who have—in one way or another—assisted in the furtherance of my work in the various places mentioned.

HISTORIC.

In the pages which now follow, I have attempted to give a brief historic account of the most important work which has been contributed towards a solution of the problem of the nucleus in Bacteria. It is obviously impossible—in a paper of the present scope—to enter encyclopædically into all the work which has been done in this connection.

In dealing with the cytology of Bacteria, it is of the very greatest importance to consider the technique by means of which the various workers have reached their results. I shall therefore make a special point of noting in each case—wherever possible—the methods of fixation, staining, etc., which have been used. When this is done, it becomes apparent that a large part of what has been written upon the bacterial nucleus is practically worthless—owing to the inadequacy of the technique employed.

The older observers were mostly content to regard the Bacteria as enucleate—Monera, as Haeckel termed such

¹ Whilst occupying the British Association Table in 1908, under a grant from the Goldsmiths' Company.

supposed forms.¹ Early workers (e.g. Cohn) noticed, indeed, granular bodies in many Bacteria, but they were unable to reach any definite conclusions regarding their significance.

If we turn to older books on bacteriology, we find it usually stated that no nucleus is to be found in these organisms. De Bary (1884) says: "Nuclei have not yet been observed in Bacteria" (p. 492). Similarly, Zopf (1885) states: "Until now, nuclei have been looked for in vain in bacterial cells" (p. 14). Hüppe (1886), whilst pointing out that no nucleus had ever been shown to exist in Bacteria, suggested that the whole bacterial cell might be the homologue of the nucleus of other forms. This view has found many subsequent adherents.

One of the very first to investigate the structure of Bacteria was Kunstler (1887). He described in *Spirillum tenue*—after fixation with osmic acid, and staining with "noir Collin" or hæmatoxylin—an alveolar structure of the protoplasm, with numerous granules. In the later publications of Kunstler and his colleagues, descriptions which seem essentially similar are given of a number of different Bacteria. The descriptions are usually so incomplete, however, the figures usually so diagrammatic, and the technique employed usually so imperfectly indicated, that I find great difficulty in interpreting his results. (See Kunstler et Busquet [1897, 1898], Kunstler [1900], Kunstler et Gineste [1906, 1906A], etc.) As a rule, Kunstler appears to think that there is, in most Bacteria, no structure comparable with a nucleus.

Schottelius (1888) claimed to have found nuclei in various Bacteria (*B. anthracis*, cocci, etc.). These nuclei are said to be in the form of a short rod (bacilli) or spherule (cocci), and to divide in the process of cell-division. They are said to be visible in the living cells, but more distinct in dry films stained with gentian violet. The method of fixation is not given.

¹ It is perhaps worthy of note that, so late as 1894, it was still dogmatically stated by Haeckel that Bacteria contain no nucleus ('Systematische Phylogenie der Protisten und Pflanzen').

Babes (1889) found stainable granules—whose presence he had recorded at an earlier date—in various bacterial cells. Later (Babes, 1895), he named them “metachromatic granules,” but he was unable to determine their precise significance.

Ernst (1888) found similar granules in the cells of *Bacillus xerosis*. They were observed in dry, flame-fixed cells, stained with methylene blue and Bismarck brown. He believed that they took part in spore-formation. Subsequently (Ernst, 1889) he found similar granules—using similar methods—in a number of other Bacteria. He proposed the name “sporogenic granules” for them, and regarded them as probably of a nuclear nature. Still later (Ernst, 1902), he described “chromatophil” granules—of uncertain significance—in many Bacteria (*B. megatherium*, water Bacteria, etc.). These granules were coloured by intra-vitam staining with methylene blue and neutral red.

The carefully conducted and classic work of Bütschli (1890, 1892, 1896, 1902) can here be considered in its main outlines only. After studying the Cyanophyceæ, Bütschli turned his attention to the large sulphur Bacteria.¹ In these he believes that the protoplasm, which has an alveolar or honeycomb structure, is differentiated into a peripheral layer and a denser “central body.” In the meshes of the latter, granules which stain red with hæmatoxylin (“red granules”) are present. He regards the “central body” with its “red granules” as the homologue of the nucleus of other cells, and the peripheral layer as the homologue of the cytoplasm. In the smaller Bacteria which he investigated, he found that the peripheral layer was relatively greatly reduced in size, or altogether absent—the greater part, or the whole of the cell being therefore constituted by the “central body.” He was therefore led to regard the whole cell as homologous with a nucleus. The observations were made not only upon living cells, but also upon cells fixed, stained and variously treated by a number of different reagents.

¹ The earlier work of Winogradsky (1888) and others, upon this group, did little to elucidate the structure of the cells.

Wahrlich (1890, 1891) studying a number of different forms (*B. subtilis*, *B. megatherium*, etc.), arrived at conclusions essentially the same as those of Bütschli. He believed, from their chemical and staining reactions, that Bacteria contain chromatin. Young cells are homogeneous, chromatic; older cells show a reticulum of linin in which granules of chromatin are suspended. The chromatin granules fuse to form spores. He concludes that Bacteria are therefore really nuclei. All his work appears to be based upon a study of dried cover-slip preparations.

Zettnow (1891), using Löffler's flagellar stain—which has little value from a cytological point of view—agreed with Bütschli's conclusions regarding small Bacteria. Later (Zettnow, 1897) he extended his observations to large *Spirilla*, using chiefly *intra-vitam* staining with methylene blue, and drawing the same conclusions as before. Still later (Zettnow, 1899), he examined a number of Bacteria stained by Romanowski's method, but after flame-fixation. His conclusions regarding structure were essentially the same once more—that Bacteria consist entirely, or in some cases chiefly, of nuclear substance.

Protopopoff (1891) found granules which stain with fuchsin in a *Bacillus* from a cow's tongue, and in *Actinomyces*. He interpreted them as being of a nuclear nature, though on very slender evidence. The method of fixation is not stated.

Wager (1891) described a nucleus, containing two deeply staining rods and surrounded by a very thin membrane, in a *Bacillus* from the scum on water containing decaying *Spirogyra*. The division of the nucleus is briefly described. The method of fixation is not given, but it is stated that cover-glass preparations were stained with fuchsin. Wager (1895) again described structures which he believed to be nuclei in various other Bacteria, but gave only a very fragmentary account both of the structures themselves and of the technique employed.

Frenzel (1891, 1892) gives a description of several species of Bacteria—chiefly from a study of living cells—and draws analogy between spores and nuclei.

In 1892 Sjöbring described large vesicular nuclei, which divide by mitosis, in *B. anthracis*, hay Bacteria, the *Vibrio* of fowl-cholera and several micrococci. Fixation is stated to have been effected with nitric acid (alone, or with alcohol) without previous drying. The stains used were carbol methylene blue or carbol magenta.

Trambusti and Galeotti (1892) investigated a large *Bacillus* from water. The preparations were either dried, or fixed with HNO_3 , and stained with safranin. The organisms stain at first uniformly, but later show a differentiation into darkly staining longitudinally placed rods, and granules. Subsequently young cells appear to be formed endogenously. The authors compare the structural changes with mitosis, though the reason for this is far from obvious.

Mitrophanow (1893) studied the structure of various sulphur Bacteria (*Beggiatoa*, *Chromatium*, *Ophidomonas*, etc.), also of *Cladotrix*, *Spirilla*, *Bacilli*, etc. He employed *intra-vitam* staining with methylene blue, and also examined organisms after fixation with various reagents and treatment with various stains. He believed that a nucleus was present in all the forms examined. Various modifications were described and figured. He did not agree with Bütschli's interpretation of the structures present in the bacterial cell. He believed "que toutes les bactéries que nous étudions ne peuvent être aucunement considérées comme des organismes sans noyau; de même on ne peut pas leur attribuer exclusivement une nature de noyau. Elles apparaissent comme des cellules dans divers stades de complication, laquelle est exprimée par la séparation plus ou moins complète du noyau."

Podwysozki (1893) gives an account of the structure of the cholera *Vibrio*, as seen in dried preparations stained with Ziehl-Neelsen and in cells treated simply with fuchsin. He finds a nucleus-like oval mass of "chromatin" in the cell, and other bodies of different (undetermined) nature. In place of the oval mass of chromatin, two or more masses may sometimes be seen—appearances which he regards as due to degeneration.

Schewiakoff (1893) finds a structure like that described by

Bütschli in sulphur Bacteria, in a large freshwater organism which he names *Achromatium oxaliferum*. This organism resembles the sulphur Bacteria in general form, but contains calcium oxalate—probably in combination with a carbohydrate—instead of sulphur. There is a “central body” present, containing colourable granules which undergo division.

Ilkewicz (1894), studying *B. anthracis* after flame-fixation and a complicated staining process, found darkly staining bodies present, which he believed to be spore-rudiments. He suggests that it is these structures which Sjöbring mistook for nuclei.

A. Fischer (1894) explains the protoplasmic differentiation described by Bütschli as due to plasmolysis. In this, as in subsequent memoirs (Fischer, 1897, 1899, 1903), he maintains that a “central body” does not exist: that the granules are probably reserve material, and neither nuclei nor chromatin: and that no nucleus has been demonstrated in Bacteria. The cell is not the equivalent of a nucleus. His conclusions are based upon elaborate studies of fixation and staining methods. It is hardly necessary to enter here into the polemics which have taken place between Fischer and Bütschli.

Migula (1894), after a study of *Bacillus oxalaticus*, reaches the conclusion that no “central body” is present in this form. Colourable granules—insoluble in pepsin—are present, but no definite interpretation of them is given. In a subsequent work (Migula, 1897), after reviewing the literature he concludes: “Ueber die Bedeutung der Körnchen in der Bakterienzelle lassen sich nur sehr subjektive Vermutungen hegen; ich möchte sie als die ersten Anfänge einer Zellkernbildung betrachten.” More recently (Migula 1904), he expresses the opinion that the existence of a nucleus is still an open question.

That nuclear structures occur in many Bacteria is believed by Löwit (1896). His conclusions are based, however, upon dried preparations stained with Löffler’s flagellar stain.

A. Meyer (1897), using various methods, found granules which he interpreted as nuclei in *B. asterosporus* and *B.*

tumescens. In a later paper (Meyer, 1899) he extended these observations to a number of other Bacteria. He employed various methods—chiefly fixation with formol and staining with fuchsin. The granules, which are nuclei, may be from one to six in number in each cell. In 1904 he gave a detailed account of the chemical and staining reactions of “volutin” granules in Bacteria and other organisms. More recently (Meyer, 1908) he affirms that his “nuclei” are not volutin, but condemns the nuclear structures described by the majority of other workers.

Wagner (1898) discovered a nucleus in the form of a granule, dividing with a dumbbell figure—one in every cell—in *B. coli* and *B. typhosus*. His preparations were “dried in the usual way” and stained by a very elaborate method.

“Chromatin” bodies were found in various forms of Bacteria by Ziemann (1898). He made dry films, fixed in the flame or in alcohol, and stained by Romanowski’s method.

Macallum (1899) investigated three species of *Beggiatoa*, after various methods of treatment. He finds no such differentiation as described by Bütschli. Compounds of masked iron and organic phosphorus are uniformly diffused through all the protoplasm, and these compounds also occur in certain granules which stain with hæmatoxylin. “There is no specialised chromatin-holding structure in the shape of a nucleus of any kind.”

Rowland (1899) records the results of staining various Bacteria—chiefly with roseine, without fixation. Deeply stainable granules were found, though no very definite interpretation was given to them. He appears to think that they may be partly nuclear and partly excretory.

Under the name *Bacterium gammari*, a large nucleate organism—inhabiting the body cavity and hæmolymph of *Gammarus zschokkei* (from Garschina Lake, Switzerland)—was described by Vejdovský (1900). The organisms were treated by various cytological methods. Each cell has a

distinct nucleus lying towards the centre. Later (Vejdovský, 1904), he describes stages in the mitotic division of this nucleus, and records similar nuclei in certain filamentous Bacteria inhabiting the gut of *Bryodrilus ehlersi*.

Marpmann (1900) suggests—amongst other things—that enucleate Bacteria may exist. His observations are very fragmentary, and all made upon flame-fixed organisms.

Feinberg (1900) describes “nuclei” of various forms in various species of Bacteria (*B. coli*, *B. anthracis*, *Micrococci*, etc.). The observations were made upon organisms stained by Romanowski's method. The method of fixation is not given; presumably the preparations were dried and flame-fixed. (See here also Zettnow, 1900.)

Marx and Woithe (1900) arrive at the conclusion that the Babes-Ernst granules afford an index of virulence—greater numbers indicating a greater degree of pathogenicity. They further state that the organisms containing these granules are the “Träger und Erhalter der Art.” They also make the statement that “the Babes-Ernst granules are products of maximal condensation and typical localisation of the euchromatic substance of the bacterial cell.” The illuminating nature of such a statement is obvious. Regarding the relation between the granules and virulence, the statement of Marx and Woithe has been controverted by Ascoli (1901), Gauss (1902), Schumburg (1902), Krompecher (1901), Ficker (1903), Guilliermond (1906), and others.

Krompecher (1901), working on various organisms, draws a distinction between “metachromatic granules” and “Babes-Ernst granules,” on the grounds of staining reactions. He leaves the significance of the granules in doubt. (See here also Mühlischlegel [1900], Marx [1902], etc.)

Hinze (1901) found scattered granules, which he believed to be chromatin, in the cells of *Beggiatoa*. Later (Hinze, 1903), he described similar bodies in another large sulphur bacterium—*Thiophysa volutans*. Various methods of fixation (Flemming, etc.) and staining (Heidenhain, etc.) were employed. The granules are said to divide by a process

of simple constriction. An ordinary form of nucleus was not found.

Nakanishi (1901) describes nuclei in a large number of Bacteria (Cocci, Bacilli, Spirilla) stained with methylene blue, either *intra-vitam*, or after fixation with formol vapour. He finds minute spherical nuclei in Cocci; nuclei in the form of a granule, rodlet, or filament in Bacilli; and granular or filamentar nuclei in Spirilla. He also finds nuclei in spores. He gives an excellent account of his technique, good figures, and strong evidence for the conclusion that the structures he observed are really nuclei. His interpretations have been unfavourably criticised by Ascoli (1901 A), Ficker (1903), Preisz (1904), Meyer (1908), and others.

Schaudinn (1902) inaugurated a new era in bacteriology by studying cytologically the whole life-cycle of the gigantic *Bacillus bütschlii* in the gut of the common cockroach. He described a nucleus in the form of scattered granules of a chromatic substance (chromidia) throughout the greater part of the life-cycle. During spore-formation the granules arrange themselves in a spiral and finally become aggregated into dense masses in the fully formed spore. A process interpreted as a modified sexual act (autogamy) was discovered. In the following year (Schaudinn, 1903) he described analogous conditions in *Bacillus sporonema*, a small marine organism.

Meyer's pupil Grimme (1902) has given a lengthy and elaborate account of the chemical and staining reactions of many different kinds of granules which occur in many different Bacteria. After a discussion of the various kinds of granules which he studied—especially the “metachromatic granules” (“*Volutanskugeln*”)—he finally decides in favour of the nuclear views of Meyer. The “nuclear” granules of most other observers are probably not nuclei. (In connection with these granules see also Guilliermond [1906, 1910, etc.], Meyer [1904], Eisenberg [1910], etc.).

Under the name *Spirillum colossus*, Errera (1902) describes an enormous spirillar form. Darkly staining masses

of variable form are seen in dried and stained preparations. Their interpretation is not indicated. (This organism is certainly worthy of a careful cytological study.)

Federowitsch (1902) studied *B. megatherium*, *B. pyocyaneus*, and other Bacteria. He found stainable granules, which play a part in spore-formation, in the cells. But he believes that "no nucleus like that of higher cells" is present. The method of fixation is not given; Weigert's stain was employed.

Růžička (1903) finds granules present in many Bacteria after fixation with HgCl_2 and staining with methylene blue. A definite interpretation is not given to the granules. In later papers (Růžička, 1908, 1909, etc.) he advocates the view that the bacterial cell represents a naked nucleus.

Ficker (1903) discusses the problem of the nucleus in Bacteria. He expresses the opinion that it is premature to draw any conclusions with regard to either granules or nuclei.

Mencl (1904), using careful cytological methods, finds typical nuclei in Bacilli inhabiting the gut of the cockroach. He also finds nuclei in *B. megatherium*. In 1905 he describes nuclei of many different forms in filamentous water Bacteria (*Cladotrix*, etc.), after staining *intra-vitam* with polychrome methylene blue. Later (Mencl, 1907) he gives a minute description of *Bact. gammari*, describing the various appearances seen in resting and mitotically dividing nuclei. He also published in the same year (Mencl, 1907A) a more detailed account of the symbiotic Bacteria of the cockroach. Quite recently he has given a description of the nuclei in *Sarcina* and *Micrococci* as revealed by staining with polychrome methylene blue *intra-vitam* and subsequently clearing in glycerine. Mencl's results have been adversely criticised by Guilliermond (1907, 1908, 1910) and Meyer (1908). The latter states that Mencl's nuclei are really volutin granules; the former believes they are the septa formed in the cells during cell-division. Mencl (1909) has replied to Guilliermond's criticisms and maintains the correctness of his own interpretations.

Dietrich (1904), after reviewing the literature on the subject, says: "Wir wollen nur noch als Hauptergebnis betonen, dass alle Versuche, Kerne in Bakterien zu finden, als gescheitert zu betrachten sind."

Preisz (1904) gives an elaborate account of the structure of the anthrax *Bacillus*. He studied the organisms after mixing them with alcoholic fuchsin, formol-fuchsin, or methylene blue. He maintains that the nuclei described by Schottelius, Nakanishi, etc., are really more deeply coloured portions of the cytoplasm. The real nuclei are in the form of minute spherical corpuscles, one or more in each cell. They undergo division. They are distinct from the metachromatic granules of Babes and Ernst, and from the acid-fast granules of Bunge. A nucleus enters into each spore. He finds similar nuclei in *B. cohærens*, *B. tetani*, and *B. asterosporus*. His conclusions are therefore essentially the same as those of Meyer. (Cf. here also Georgevitch [1910].)

Rayman and Kruis (1904) describe typical nuclei—similar to those found by Vejdovský and Mencl—in a variety of Bacteria (*B. mycoides*, *B. tumescens*, etc.). They are found in young cells only. The method of treatment is peculiar—fixation by desiccation (in a desiccator) and staining with iron-hæmatoxylin and purpurin. Excellent photo-micrographs are given. The conclusions of these investigators are challenged by Guilliermond (1908).

Swellengrebel (1906) records the results of a minute cytological and micro-chemical investigation of *Bacillus maximum buccalis*. He finds a nucleus present in the form of a more or less complete spiral or zig-zag filament. In the following year (Swellengrebel, 1907), he describes two large spherical nuclei in *Bacterium binucleatum*—an organism from the human mouth. He also describes spiral or zig-zag nuclear filaments or rodlets in *Spirillum giganteum* (Swellengrebel, 1907A), and subsequently (1909A) in certain filamentous Bacteria (*Sphærotilus*, *Thiothrix*). His results have been questioned by Hölling (1907), Zettnow

(1908), and Guilliermond (1908). The various objections raised against his work have been answered by Swellengrebel (1908, 1909), who maintains the correctness of his conclusions.

Guilliermond (1907) gives an excellent brief review of previous results upon the cytology of Bacteria. In the following year (Guilliermond, 1908) he describes the structure of a number of Bacilli (*B. radicosus*, *B. mycoides*, *B. megatherium*, etc.). He believes that in all these the nucleus is in the form of granules of chromatin (chromidia)—distinct from the metachromatic granules—scattered through the cytoplasm. These granules become massed together to form the spores. He criticises the results obtained by many other investigators. Various cytological fixing and staining methods were employed in his researches. In a more recent paper, Guilliermond (1909) describes nuclei in the form of spiral filaments—like those found by myself—in two species of *Bacillus* (from the gut of *Echinocardium*) and a large *Spirillum*.

In 1908 I gave the results of cytological researches which I had undertaken upon the structure and life-history of several Bacteria. I described a new large disporic *Bacillus*—*B. flexilis*, from the gut of frogs and toads—whose life-history is essentially the same as that of *B. bütschlii* described by Schaudinn (1902). I also described another organism—which I named *Bacillus spirogyra*—from the same hosts, in which the nucleus is in the form of a spiral or zig-zag filament. I described further in *Spirillum monospora*—from the frog and toad also—a nucleus of the chromidial form. The chromidia mass themselves together in forming the spores. In 1909 I gave a more detailed description of *B. spirogyra*. I discussed the nature of the nuclear filament, and described the part it played in spore-formation—a process which I described in detail. I described in addition the structure and method of spore-formation in *B. lunula*, which resembles in these respects *B. spirogyra*. As a result of this work, I reached the conclusion that the

“autogamy” of *B. bütschlii* (Schaudinn, 1902) and *B. flexilis* was probably not a sexual process at all.

Amato (1908) describes results obtained by staining several Bacteria (*B. mycoides*, *Sp. volutans*, etc.) *intra-vitam* with Brillanteresyblau. He believes that in the spore, and at the beginning of development, a relatively large spherical nucleus is present, which breaks up subsequently into chromidia. The divergent views of different observers may have an explanation in the fact that they observed similar organisms, but at different stages in their development.

Dangeard (1909) records the results of a re-investigation of *Chromatium*. By fixing with Flemming or Perenyi, and staining with various stains (especially Flemming's triple), he confirms the description of this organism given by Bütschli. A “central body” corresponding to a nucleus is present. Additional evidence for regarding the “central body” as a nucleus is afforded by the fact that a rhizoplast can sometimes be seen connecting the flagellum with this body.

Ambrož (1909) gives a lengthy description of *Bacillus nitri*. As a result, he reaches the same conclusion as Růžicka—that Bacteria are nuclei. Fixation is said to have been effected with a concentrated solution of “ HCl_2 ,”¹ and staining chiefly with Giemsa.

Under the name “*Hillhousia*” *mirabilis*, West and Griffiths (1909) describe a very large sulphur bacterium. There is said to be a protoplasmic network present, containing granules believed not to be chromatin. “Nothing of the nature of a definite nucleus is present.” Details—especially as regards the method of using formol as a fixative—are too scanty for this conclusion to be accepted without further evidence. No reference is made to the work of Bütschli, Schewiakoff, Hinze and others, on similar forms.

Recently, an account of the structure of the long forms of *B. coli*, *B. typhosus*, etc.—produced by growing these organisms on culture media containing aniline dyes—has been

¹ I presume this means HgCl_2 .— and not HCl , as given by Guilliermond in a review of this paper in ‘*Bull. Inst. Pasteur*.’

given by Vay (1910). He finds large irregular masses of darkly stained substance—which he calls chromatin—in these organisms. He does not appear to be aware that the production of these forms on coloured media had already been described by Walker and Murray (1904).

Such, then, is a very condensed account of the chief work which has hitherto been published concerning the problem of the nucleus in Bacteria.

In all work in which inadequate technique has been employed—for example, in all studies in which only dried and flame-fixed organisms have been examined—the conclusions attained can have little value from a cytological point of view. In many publications, moreover, the descriptions both of results and of methods are so meagre as to render discussion of them either unprofitable or impossible. Therefore I shall—on either or both of these grounds—eliminate the following works from any further discussion :

Kunstler and Busquet (1897, 1898), Kunstler (1900), Kunstler and Gineste (1906, 1906A), Schottelius (1888), Zettnow (1891, 1899), Protopopoff (1891), Wager (1891, 1895), Sjöbring (1892), Trambusti and Galeotti (1892), Ilkewicz (1894), Löwit (1896), Wagner (1898), Ziemann (1898), Marpmann (1900), Feinberg (1900), Errera (1902), Federowitsch (1902), West and Griffiths (1909), Vay (1910).

I think most cytologists will agree with me that no profitable discussion of these papers is possible.

MATERIAL AND METHODS.

As I have already indicated above, I have made a special point of working upon the largest forms of Bacteria which I have been able to find; but I have studied in addition a number of small forms, when they have been suitable.

Small Bacteria are not only very difficult to investigate on account of the limitations imposed by the microscope, but they are also in many cases unsuitable in other ways for cytological study. They occur frequently in media which render

the making of good microscopical preparations exceedingly difficult and laborious, and they contain granules (reserve material, etc.), which are relatively of such a size as to obscure much of the structure of the living substance itself. For the latter reason, the sulphur Bacteria, in spite of their large size in many cases, appear to me to be unfavourable objects for study—as a starting-point, that is to say, on our way to a comprehension of the organisation of Bacteria.

Another point that has seemed to me of some importance is this. Much of the work which has been done upon the structure of Bacteria has been based on a study of organisms which have been kept in cultures for a greater or less period of time. It seems to me highly probable that the discordant results of different workers may in many cases be due to cultural differences in the organisms studied. Different culture media may be used in the cultivation of Bacteria: but although "pure" cultures may be obtained in half a dozen of these, it does not follow that all or any of the colonies so obtained consist of normal individuals. Bacteria are not found in nature as a rule in pure cultures, and this is a point which should not be overlooked when considering their normal structure. Culture methods are of the greatest service in the separation of various microbes from one another, but it does not at all follow that all pure cultures of a given organism are identical, or that they contain individuals which are in every way the same as those living in their natural environment. I have therefore not studied Bacteria grown in artificial culture media, but have confined my attention for the present to organisms in their natural habitat. The fact that the Bacteria which I have investigated are not—for the most part—previously described and named "species" from pure cultures, is therefore not an objection which can be urged against my results, but a necessary consequence of the point of view from which I have attacked the problem.

As a source of material, I have found the intestinal contents of various animals most useful. The contents of the large intestine in many animals is swarming with Bacteria, frequently

of large size. The consistency of the intestinal contents, moreover, is usually such as to render the making of microscopic preparations (smears, etc.) comparatively easy. I have found the contents of the large intestine of Amphibia and Reptilia especially suitable; but insects, mammals and other animals also contain a rich supply of suitable material which is as yet almost untouched. Most of the organisms which I am about to describe have been obtained from frogs, toads and lizards.

I have found in all the animals which I have studied that the Bacteria in the large intestine vary enormously—in different individuals—both as regards the number of different forms, and the number of microbes as a whole. In the frog, for example, some individuals may contain very few Bacteria—mostly of the same form—whilst others may contain countless numbers of Bacteria of the most diverse forms. This is, of course, only what one would expect.

As the source of the material will be found under the description of each organism, I shall here say nothing more detailed regarding this, but will now devote a few words to a description of the technique which I have employed in my researches. I have already (Dobell, 1908) given a brief account of some of the methods which I have used.

I have tried most of the methods of fixation and staining which are usually employed in cytological work. It is usually necessary to modify the ordinary procedure in one way or another when dealing with Bacteria. In my experience, the usual methods of fixation (e. g. corrosive sublimate, Flemming's solution, Hermann's solution, osmic acid, formalin, various picric acid and bichromate solutions, etc.) may all—under suitable conditions, and with careful procedure—be made to give excellent results. Fixation is most easily and effectively accomplished by making a wet film of the intestinal contents—or other medium in which the Bacteria occur—on a coverslip, and then dropping it film-side downwards upon the fixing solution. Drying previous to fixation is, of course, to be avoided. The usual bacteriological method

of making dry films and fixing them by passing them through a flame is quite worthless from a cytological point of view, owing to the plasmolysis and distortion which it brings about.

When the medium containing the Bacteria is too watery to allow of fixable films being made, gelatine or albumen may be added until a film of suitable consistency is obtained. If the medium be too thick, one must of course be careful to use isotonic salt solutions for its dilution.

Most of the ordinary cytological stains (e.g. Delafield's hæmatoxylin, carmine, safranin, etc.) I have found unsuitable for Bacteria. They—like most of the ordinary aniline derivatives—are liable to stain the whole cell uniformly, without differentiating the internal structures. This is largely due to the marked affinity which the cell wall has for many stains, causing it completely to obscure the finer structures present in the protoplasm.

After trying a large number of combinations of fixatives and stains, I have latterly confined myself almost entirely to two methods. Both of these have proved of the greatest value. They are (1) fixation with osmic acid or formalin, followed by staining with one of the modifications of Romanowski's method, and (2) fixation with Schaudinn's sublimate-alcohol (2 : 1) followed by staining with Heidenhain's iron-alum hæmatoxylin. The latter method is now so well known (see, for instance, Schaudinn, 1902) that I will not re-describe it. It is of course a wet film method, and its only disadvantage is that it is exceedingly difficult to use, owing to the difficulty of obtaining exactly the right degree of differentiation. Indeed with different degrees of differentiation quite different appearances may be produced in the same Bacteria, and it is therefore necessary to be very cautious in interpreting the results. Nevertheless, I believe this method to be one of the most valuable for the study of the structure of Bacteria.

With regard to the first method, I have found it so simple and easy to use that I can strongly recommend it to others. My method of procedure is as follows. I take a drop of the medium containing the Bacteria and place it in the centre of

a carefully cleaned glass slide (or coverslip) by means of a platinum loop. I then place a drop of 1 per cent. osmic acid or strong formol (40 per cent. formaldehyde, Schering) beside the first drop, and then mix both together and spread the fluid in a thin and even film on the slide. I then allow the film to dry, which usually takes a few minutes. No heating should be used to accelerate the process. The slide or coverslip with the dried film is then placed in absolute alcohol for about ten to fifteen minutes. It is then removed, and the film allowed to dry once more. I then stain the film with Giemsa's or Leishman's stain in the usual way. After staining I differentiate in 30 per cent. alcohol—wash in distilled water—dry by blotting with a cigarette paper—and mount in cedar wood oil or neutral Canada balsam. Chromatin structures are coloured a bright red; the cytoplasm being blue, lilac or pink, according to the degree of differentiation. The structure of many Bacteria is revealed with remarkable distinctness by this method—its chief disadvantage being that the preparations sooner or later fade, and cannot as a rule be satisfactorily re-stained.

The above method of fixation—which I term the drop method—calls for some further comments. In the first place, it might be urged that the drying which takes place would be liable to injure the organisms, and give rise to misleading appearances. This is not so, however. If the Bacteria are fixed with osmic acid or formol before drying is allowed to take place they are not plasmolysed or injured in any way. It is only when drying takes place before fixation that such disastrous results ensue.

I have made many preparations by other methods as controls. I have made wet films and fixed them by immersion in 1 per cent. osmic acid or formol: I have also made wet films and fixed them by exposure to osmic vapour: and I have then stained these films by modifications of Romanowski's method and mounted them in balsam without allowing any drying to take place at any stage in the proceedings (cf. Dobell, 1908). The final results obtained in all these cases are almost in-

distinguishable from one another. The only real difference observable is that the organisms which have been dried appear slightly broader than those which have not—owing to the slight flattening which drying brings about. The internal structures appear exactly alike. Controls with wet films fixed with sublimate-alcohol and stained with Heidenhain's iron-haematoxylin give confirmatory results. I therefore think that the drop method of fixation, when employed in the manner described, gives reliable cytological results in the case of Bacteria.¹ On account of the ease with which osmic acid or formol may be employed in this manner, I have used them more frequently than any other fixatives.

Another point which calls for comment concerns the use of alcohol after fixation. When "osmic acid" (more correctly, osmium tetroxide, OsO_4) is used—either in solution or in the form of vapour—it is, of course, unnecessary to treat the preparations subsequently with alcohol—so far as complete fixation is concerned. In practice, however, I find that films fixed by osmic vapour or by the drop method adhere to the slide or coverslip better if they are hardened in absolute alcohol for a short time after fixation. When formol is employed, however, it is absolutely necessary to employ alcohol subsequently. As is well known, formaldehyde fixes protoplasmic structures without precipitating them in an insoluble form. It is therefore necessary to place the fixed structures in strong alcohol before proceeding further—otherwise fixation may be completely undone in subsequent treatment.²

¹ I may add that beautiful preparations of small flagellates and other Protista may also be obtained in this way.

² Cf. Gustav Mann (1902). This point seems worthy of attention. I note that Swellengrebel (1906) fixes Bacteria by the drop method, using formalin. But he does not appear to use alcohol subsequently, so that many of the appearances which he describes may be due to imperfect fixation. If it is desired to use formalin alone—without using alcohol at all—and to use stains in watery solution, the fixation may be preserved by adding a small percentage of formalin to all the stains, etc., employed after the original fixation. If it is desired to dilute the formalin used in fixation, this should be done with isotonic salt solution—not with water.

Sometimes excellent results may be obtained by making ordinary dry films, fixing with absolute alcohol, and staining in the usual way with Giemsa or Leishman. This method is not to be relied upon, however, and should never be employed alone. Giemsa's new wet method (vide Giemsa, 1909, 1910) appears to give excellent results, but I have not used it myself for Bacteria.

I have employed intra-vitam stains in many cases, but with little success—so far as nuclear structures are concerned. I have used neutral red, Brillantcresylblau, and methylene blue. Many other workers appear to have been more successful with these stains (e. g. Mencl, who has obtained most striking results with polychrome methylene blue). In my experience, only non-living structures in the cells (metachromatic granules, etc.) can be stained during life. But doubtless much depends upon the stain itself. Different samples of methylene blue—for example—may give quite different cytological results.

DESCRIPTIONS OF THE FORMS INVESTIGATED.

Having already given as briefly as possible the most important results which have been reached by previous work on the cytology of Bacteria (see p. 399), I shall pass on to a detailed description of my own observations. In this description I shall make no attempt to compare or to correlate my own results with those of others—my object being to give only the facts which my own work has disclosed. A discussion of all the results—obtained by other workers and by myself—will be reserved for a subsequent section of the paper (see p. 462, et seq.).

In describing the various forms which I have investigated, I have—for convenience—divided the organisms into five main groups. These are the Cocci, Bacilli, Spirilla, "fusiform Bacteria," and a group of other organisms which resemble—but are not—non-motile rod Bacteria. I shall deal with each of these groups separately, and in this order.

I. COCCUS FORMS.

Cocci of various sizes are very common in the large intestines of many different animals. Unfortunately, however, they are usually of very small size, and hence exceedingly difficult to study accurately. I have examined many cocci from the large intestines of frogs and toads (*Rana temporaria*, *R. esculenta*, *Bufo vulgaris*), of newts (*Triton vulgaris*), of cockroaches (*Periplaneta americana* and *Stylopyga orientalis*) and of several different snakes. All these have proved to be of little value, because the organisms were usually so small that I could not be certain of their structure as seen under the microscope. The living organisms were usually very refractile, and showed no internal structure which could be definitely separated from appearances due to optical phenomena. For instance in a small *Micrococcus*—examined under a high power—a dark spot of varying size can often be distinctly seen in the centre of the organism.¹ This is not, I believe, a definite body—such as a nucleus—lying in the cell, but is merely an appearance caused by optical phenomena connected with the microscope.

(A) *Micrococci*.

Only two *Micrococci* of suitable size for investigation have come under my notice, but they have both revealed a structure which is quite unmistakable. Both forms were found in the large intestines of lizards—*Lacerta muralis* and *Mabuia carinata*.

Micrococci from *Lacerta muralis*.

The lizards were obtained in the neighbourhood of Naples. I found that nearly all of them harboured a large *Micrococcus* in greater or less numbers.

¹ These appearances probably led Schottelius (1888) to believe that he could see a nucleus in living Bacteria.

The living Micrococci, examined in the contents of the large intestine immediately after removal from the lizard, showed no very definite structure. I have been unable to convince myself of the presence of any internal structures from an examination of living organisms alone.

With stained preparations, however, the case is very different. I have obtained the best results after fixation with 1 per cent. osmic acid or formalin, and after staining with Giemsa's or Leishman's stain in the manner already described (see p. 415). The following descriptions apply to organisms treated in this manner.

The Micrococci occur singly, in pairs, or in chains. They are usually perfectly spherical, and have a diameter (in fixed and stained specimens) varying from rather less than 1μ , up to 2μ . All intermediate sizes may be found. It is possible, of course, that the different sized forms are really different species. They all occur together, and in company with many other forms. But it is quite immaterial, for my purposes, whether they are one species or one hundred, for they all show a structure which is the same in each individual, and it is with their structure that I am concerned.

Every individual, after fixing and staining (cf. Pl. 17, fig. 45), shows a uniformly coloured cytoplasm, a well-marked cell wall, and a centrally situated, darkly staining body. This central body is always present. It is roughly in the form of a spherical granule, but may appear more or less square or triangular in optical section. It always takes up the chromatin stain strongly.

Among the ordinary "resting" forms just described, a number of dividing organisms can usually be found. The details of the process of division can be followed in stained specimens with great clearness, and present features of considerable interest.

Division—which results in the formation of two equal daughter-cells—takes place as follows (see figs. 46–49). In the first place, the central body becomes elongated—the cell itself also exhibiting a slightly rod-like form—and assumes a

characteristic dumb-bell shape (fig. 46). The long axis of the dumb-bell coincides frequently with the long axis of the cell, but it is also often seen to be slightly displaced from this axis—occupying a somewhat oblique position in the cell. The ends of the dumb-bell separate from one another, but remain attached by the slender intermediate strand for some time. When the central body has reached this stage, a constriction appears in the middle of the cell in a plane at right angles to the long axis of the dumb-bell figure. The cell now presents the appearance shown in fig. 47 (Pl. 17). A little later the ends of the dumb-bell lose their connection with one another, through the disappearance of the connecting strand. The constriction of the cell wall is now more marked (fig. 48). After the two new central bodies have been formed in this way from the original body, the cytoplasmic constriction becomes complete, and two daughter-cells are formed which lie at first in close contact with one another (fig. 49). In this Diplococcus-condition the daughter-cells may remain; or they may separate forming two free Micrococci; or they may divide again, and so give rise to a chain of coccus forms. Division always takes place in the manner just described—the central body dividing with the formation of a characteristic dumb-bell figure, and being followed by the fission of the cytoplasm.

Now I think there can be little cause for complaint if I call the central deeply staining body a nucleus. This body is a constant morphological feature of every cell: it divides with the formation of figures which are closely comparable with those of a very simple amitosis—on a very small scale: and it takes up the nuclear stain strongly. I shall discuss this more fully in a later part of the paper, and will henceforward call the central body the nucleus.

As I have pointed out above, the dividing nucleus not uncommonly occupies a slightly oblique position in the cell. It also shows occasionally another modification, which is of the greatest interest—a modification which is characterised by the dividing nucleus assuming the form of a zig-zag

filament. This condition may be more or less strongly marked: it may take the form of a simple bend, or it may take the form of a spiral filament consisting of one or more turns (see fig. 52).

It might be urged that the bilobed cells which contain a zig-zag, bent, or spiral filament are really different organisms from those under consideration. The proof that this is not the case lies in the fact that all stages can be found together in the same chain of organisms (fig. 52). There can be very little doubt that these chains are formed from the successive divisions of an originally single *Micrococcus*. In the short chain depicted in fig. 52, a pair of such forms is seen at the lower end of the chain. Above these, four dividing cocci are seen which show various modifications of the dividing nucleus, from a slightly distorted dumb-bell figure to a zig-zag or spiral filament.

I regard this configuration of the nucleus as of considerable significance. The matter will be discussed at greater length in a subsequent section of the paper (see p. 471).

Cocco-bacillar Forms from *Lacerta muralis*.

Now in addition to the coccus forms which I have just described, there are many organisms which cannot be very definitely classified with either coccus forms or bacillar forms, but which occupy an intermediate position. These forms (fig. 50) present the appearance of a slightly elongated sphere, or of a very short rod with rounded ends. The shortest, most spherical forms (fig. 50, upper right-hand individual) have a nucleus which is in the form of a short and usually bent rodlet. The longest forms (fig. 50, lower and upper left-hand individuals) show a nucleus which is in the form of a filament arranged in a more or less zig-zag or spiral manner. That such organisms are in a "resting" (i. e. not dividing) state, appears certain from the fact that no cytoplasmic constriction can be seen (compare figs. 45-50 and 52).

As I have already noted, the ordinary *Micrococcus*

forms show slight irregularities in the contour of the nucleus; and it is, in fact, frequently impossible to decide whether an individual should be described as a Micrococcus or a cocco-bacillar form. All intermediate gradations occur, so that—although an absolute proof is lacking—I believe that all these forms, from typical Micrococcus to typical Bacillus, are really stages in the life-cycle of one and the same organism.¹ For the present, however, I will confine myself to describing the morphological features of these forms—merely pointing out that, side by side in the same host, all forms occur from typical, spherical cocci with a spherical nucleus, to typical rod-shaped bacilli with a zig-zag or spiral nuclear filament.

Micrococci from *Mabuia carinata*.

These Micrococci were obtained from the large intestine of the Brahminy lizard (*Mabuia carinata*), caught in Ceylon (Colombo). They are of smaller size than those just described, and I have examined, relatively, only a small amount of material.

The organisms (Pl. 16, figs. 42–44) have an average diameter of about 1.5μ , or rather less. They are spherical, and show a centrally placed nucleus just as in the case of the Micrococci from *Lacerta muralis* (cf. fig. 44). The method of division appears to be exactly the same, and I have therefore not figured it in detail. Allowing for the difference in size, figures 46–49 would be equally good representations of the dividing individuals of this form.

Coccus or cocco-bacillar forms in the gut of *M. carinata* also show the zig-zag form of nucleus (fig. 43). I have not, however, a complete set of stages between cocci and bacilli, as in the case of the Bacteria from *Lacerta muralis*.

These Micrococci do not present any other features of special interest. I have described them because they are the only other cocci which have furnished me with unequivocal evidence regarding their cytology.

¹ For further consideration of this see p. 484.

(B) *Sarcina*.

After investigating the structure of the ordinary *Micrococcus* forms, I naturally became curious to see what sort of structures could be found in the *Sarcinæ*. For some time I endeavoured to ascertain the exact structure of a *Sarcina* which is very common in the English frog and toad, but I was unable to reach any definite conclusions owing to the very small size of the individual cells. Other *Sarcinæ* from other animals proved equally difficult, but at last I discovered a large and suitable form in the large intestine of a Ceylon toad. This organism I will now describe.

Sarcinæ from *Bufo melanostictus*.

These *Sarcinæ* were obtained from a single toad which was captured near Colombo. All the preparations were made by fixing in 1 per cent. osmic acid and staining with Giemsa's stain. The following description therefore applies to organisms treated in this manner.

Sarcina is, of course, simply a colony of cocci, arranged typically in groups of eight individuals in three dimensions of space. The groups originate by the successive "cleavages"—like a developing egg—of a single coccus cell.

The individual cocci which compose the cell-groups of the *Sarcina* under consideration are of very large size. They measure on the average a little over $2\ \mu$ in diameter—some cells attaining a diameter of $2.5\ \mu$.

In the living organism, it can be seen that nearly every cell contains one large refractile granule. This is probably reserve material of some sort. Sometimes this granule may be absent and occasionally two such structures are to be seen. No other internal structures can be made out with certainty in the fresh state.

Upon staining the organisms, however, the structure of the cell can be readily demonstrated (see figs. 24–29, Pl. 16). The cytoplasm appears a uniform blue,¹ and sometimes shows a

¹ Or pink, if the blue is extracted with alcohol after staining.

faint granular or alveolar structure. The refractile granules remain unstained, or after prolonged staining may take on a faint yellowish-pink tinge. In each cell a dark red granule—corresponding with the nucleus described above in *Micrococci*—can always be found. The position of this nucleus in the cell varies. It does not always lie in the centre, but is usually near this point, and very often in contact with the refractile granule (cf. figs. 24–29).

In resting cells, the nucleus has always this form of a simple granule. This is seen in fig. 24, which shows a two-cell stage. Division of the nucleus precedes the division of the cytoplasm, and is effected in the same way as the nuclear division of the *Micrococci* described above. The granule elongates slightly, assumes a dumb-bell figure, and then separates into two daughter-granules. Fig. 27 shows a three-cell stage, in which the two daughter-cells on the left have completed division, whilst the nucleus of the cell on the right is dividing. Fig. 26 shows a later stage. The two cells on the left contain dividing nuclei, whilst the single cell on the right contains two daughter-nuclei—cytoplasmic fission having not yet occurred. Fig. 28 shows a four-cell stage, each cell containing a resting nucleus. In fig. 25, one of the nuclei (upper left-hand cell) has divided into two, and in fig. 29 three out of the four cells show dividing nuclei. The eight-cell stage which results from the division of these four cells shows exactly the same sort of nuclei.

Judging from the large number of cells which showed dividing nuclei, I should think that cell division takes place very slowly in this organism, but I made no observations on this point on the living organisms.

It will be apparent, I think, to anyone who will compare the figures of the *Sarcina* with those of the *Micrococci*, that the structure of the cell and its nucleus—both during rest and during division—is essentially the same in both forms.

I will now pass on to a description of the bacillar forms which I have been able to investigate.

2. BACILLAR FORMS.

In two previous papers (Dobell, 1908, 1909), I have given a description of the structure and method of spore-formation in two large Bacteria which I obtained from the large intestines of frogs and toads. These two forms I named *Bacillus flexilis* and *Bacillus spirogyra*. The former is characterised by having a nucleus in the form of chromidia scattered through the cytoplasm: the latter by having a nucleus in the form of a spiral or zig-zag filament. *B. flexilis*, moreover, is a very large, flexible organism and forms two spores: whereas *B. spirogyra* is considerably smaller, rigid, and forms a single spore. As most of the Bacilli which I am now about to describe are organised in a manner similar to that of these forms, I shall—for convenience—refer to them frequently as Bacilli of the *flexilis* type or *spirogyra* type; meaning thereby that the organisms under discussion are structurally similar to one of these forms, though implying nothing as regards difference or identity of species.

(A) Bacilli of the *flexilis* Type.

(1) *Bacillus flexilis*.—Although I have already given a detailed account of this organism (Dobell, 1908), I shall here add a few further observations on its structure, as it seems to me of considerable importance that its cytology should be made absolutely certain.

My original figures were drawn from preparations stained by Giemsa's method. The various modifications of this method which I employed I have already given—as also several other methods which gave me satisfactory results. I would here emphasise the fact that all methods which give reliable cytological results reveal exactly the same structure in this organism. They show a number of deeply staining granules scattered through the cytoplasm—an appearance which I have interpreted as a nucleus in the form of chromidia. Subsequent work on this and allied forms has convinced me of the correctness of this interpretation.

On account of its very large size, *B. flexilis* is particularly well suited for observations upon its structure. I will now describe the appearances which it presents when fixed by a good wet method and stained by a good cytological stain.

I have given two figures of organisms so treated (Pl. 18, figs. 119, 120). Fixation, sublimate-alcohol (Schaudinn); stain, Heidenhain's iron-haematoxylin. It may be noted here that although this method gives good results on the whole, it is very difficult to obtain uniformly sharp differentiation. Different individuals behave differently towards the stain, so that in the same preparation well-stained, over-stained and under-stained organisms are often found side by side.

When examined under the highest magnification which I have been able to use (Zeiss 2 mm. apochromatic oil-immersion, compensating ocular 18) the following internal structure can be made out. The cytoplasm appears homogeneous and very finely granular (as it does in life) or else shows a rather indistinct alveolar arrangement (cf. fig. 120). The very well-marked cytoplasmic alveoli described by Schaudinn (1902) in *B. bütschlii* are very much more distinct than anything I have ever seen in *B. flexilis*. In the latter the cytoplasm is, at most, slightly alveolar. A number of round black granules can be seen scattered through each cell (figs. 119, 120). They are usually more numerous towards the periphery than in the centre. These granules constitute—I believe—the nucleus, and are probably composed largely of chromatin. Their behaviour during spore-formation I have already described (Dobell, 1908). No other structures are to be seen.

I believe that the figures (figs. 119, 120) give a faithful picture of the structure of *B. flexilis*. I am convinced that were other structures present—e. g. a vesicular nucleus, or a nuclear filament—they would have been visible in some of my preparations. I conclude, therefore, that the internal structure of *B. flexilis* consists simply of a faintly alveolar cytoplasm in which small granules of chromatin are imbedded.

Having said so much about *B. flexilis* itself, I will now

describe some very similar forms which I have been able to examine.

(2) Bacilli of the flexilis type from *Lacerta muralis*.—I found these organisms in the large intestine of *Lacerta muralis* captured in the neighbourhood of Naples. Many lizards which I examined contained these organisms, but I have no record of the exact percentage of animals infected. Possibly it is the same organism which Prowazek¹ observed in lizards in Rovigno. But from his very brief mention of it as “ein grosser mit zwei Polkörpern ausgestatteter Bazillus,” it is impossible to be certain.

These bacilli are of very large size, but they are usually smaller than *B. flexilis*. The largest individuals which I have measured are about 30μ in length, with a breadth of rather less than 2μ . The ends are blunter than in *B. flexilis*, but the organisms are flexible in the same way. Figs. 83 and 84 (Pl. 17) show two of these forms after fixation with osmic vapour and staining with Giemsa. Figs. 133 and 134 (Pl. 18) show two other individuals—one of them (fig. 134) undergoing division—after fixation with sublimate-alcohol and staining with Heidenhain. It will be seen that all of them show essentially the same structure—a structure, moreover, identical with that of *B. flexilis*. There is a uniform or slightly alveolar cytoplasm containing chromatin granules scattered through it.

Division takes place in this organism by constriction—not by septation—as in *B. flexilis*. I have not observed its method of forming spores, but I believe that it is probably a disporic form.

In addition to this organism of a typical flexilis form, I found some smaller Bacteria, which show a similar kind of organisation, in the large intestine of *L. muralis*. They were of different sizes and forms, and may be different species or stages in the life of the same species. It is impossible to be certain from a comparison of the fixed and stained organ-

¹ “Untersuchungen über einige parasitische Flagellaten,” ‘*Arb. kaiserl. Gesundheitsamte*,’ xxi, 1904, p. 1.

isms only. Some of these organisms are of large size, and it is easy to determine their structure. Two individuals of long, slender form are shown in figs. 64 and 65 (Pl. 17). They exhibit a pale, uniformly stained cytoplasm, with relatively large chromatin granules distributed through it. The cytoplasm is generally free from chromatin granules at the extreme ends of the organism.

This form divides like *B. flexilis*. It is slightly flexible, and motile. I have not observed spore-formation. The average length is about $11\ \mu$, the breadth a little less than $1\ \mu$.

I have seen a good many forms which are intermediate in size between these forms and the large *flexilis* forms described above in the same host. I think it possible that there may be some genetic connection between them, though it does not appear very probable.

(3) Bacilli of the *flexilis* type from *Mabuia carinata*.—In the large intestine of a Ceylon lizard, *Mabuia carinata*, I found an organism which is very similar to the *flexilis* forms from European frogs, toads, and lizards. The infected *Mabuia* were taken in Colombo.

This organism is shown in figs. 21–23, Pl. 16. It is motile, flexible, disporic (see fig. 23), and divides by constriction. It shows a nuclear apparatus exactly like that of *B. flexilis*. I have not been able to obtain all the stages in spore-formation, so that I do not know whether it displays the same remarkable phenomena during this process as are seen in *B. bütschlii* and *B. flexilis*. But such stages as I have found are very like those of *B. flexilis*. The average length of the organism is about $17\ \mu$ —a good deal less than that of *B. flexilis* or the *flexilis* forms from *Lacerta muralis*.

As far as my observations go, the ordinary forms of these organisms are therefore closely similar. There are differences which distinguish these three forms (i. e. those from the frog and toad [*B. flexilis*], from *Lacerta*, and from *Mabuia*) from one another, but cytologically they are all three essentially similar.

In the preparations of the contents of the large intestine of *Mabuia*, I also found some small Bacteria which show a similar structure. These forms (fig. 41, Pl. 16; fig. 76, Pl. 17) do not exceed about 10μ in length, and are very slender. They all show a nucleus consisting of scattered granules of chromatin during the vegetative existence of the organism.

The Bacilli of the *flexilis* type from frogs, toads, *Lacerta*, and *Mabuia* all appear to have but one form of nucleus—that of a diffuse system of chromidia. But in addition to these forms, I have encountered two other organisms which—whilst apparently belonging to the same group—present certain features which separate them from the others. These two organisms were found in the common English newt and the Naples lizard, but to my great regret my observations—recorded in the ensuing pages—are very incomplete on both of them.

(4) Bacilli of a modified *flexilis* form from *Triton vulgaris* and *Lacerta muralis*.

(A) The form from *Triton vulgaris*.—I recorded in a previous paper (Dobell, 1908, p. 122) the fact that an organism similar to *B. flexilis* occurs in the newt. Unfortunately it is—as far as my observations go—exceedingly rare. I have found it only once, inhabiting the large intestine of a *T. vulgaris* captured in the vicinity of Cambridge. Subsequent search for the organism in other newts has up to the present been fruitless.

The organism is very long and slender. It is also the most flexible of all the *flexilis* forms which I have encountered. Many individuals attain a length of 30μ and more, though the breadth is only about 1μ .

The living organisms were actively motile. They showed a number of granules of various sizes in the cytoplasm, but no other clearly visible structures.

After fixation with formalin, and staining with Giemsa (see p. 415), the organisms could be seen to have the following structure (see figs. 79–82, Pl. 17). Most of the cells presented a nuclear apparatus like that of *B. flexilis* (fig. 79);

that is to say, they showed a number of red granules scattered irregularly through the cytoplasm. The latter showed no distinct structure as a rule.

Now, in addition to these forms of the characteristic flexilis type, there were other forms which possessed quite a different sort of nuclear arrangement (figs. 80, 82). They showed a variable number of large, nucleus-like bodies arranged in a single row along the whole length of the organism. The cytoplasm of these forms was finely granular (fig. 80), or sometimes distinctly alveolar (fig. 82). I observed a number of forms which appeared to be intermediate in structure between these forms and the ordinary chromidial forms; but I cannot state with absolute certainty that these different forms are not really different species. Though I believe that all the forms belong to one and the same species—representing different phases in the life-history—the fact that they are possibly different species living side by side cannot be ignored.

This organism is—like the other flexilis forms—disporic. It forms two terminal spores in each cell, but unfortunately I did not succeed in observing all the stages of spore formation. I have seen the living, actively motile, spore-bearing individuals, and also some isolated stages in the formation of the spores. Fig. 81 shows one of these—with two large chromatin spore-rudiments (as in *B. flexilis*) at the ends of the cell. I have not found stages in which the chromatin is arranged in a spiral filament—as in *B. bütschlii* and *B. flexilis* during spore-formation. It is possible, therefore, that spore-formation does not take place in the same manner as in *B. flexilis*, and that the forms which show large nuclei arranged in a row are really forms which are about to sporulate. This seems to me improbable, however, because both long and short individuals (figs. 80, 82) may show this arrangement of the chromatin, whilst it is usually in long individuals only that spores are found.

As far as my observations go, therefore, it appears probable that this particular organism, whilst presenting the flexible

form, disporic habit, and chromidial nucleus characteristic of other *flexilis* forms, has also stages in its life-cycle during which the chromatin is arranged in a series of large nucleus-like masses. The significance of this arrangement has not been determined.

(B) The form from *Lacerta muralis*.—I obtained this organism in a single lizard which I caught near Naples (Pozzuoli). As in the case of the preceding organism, I have been able to make only very fragmentary observations upon it. Unfortunately, I examined it only casually in the living state—believing it to be the ordinary *flexilis* form described above (p. 428). When alive it showed active movements and was flexible—like the ordinary *flexilis* forms from the lizard.

To my great regret I made only a single preparation of this organism—a dry smear on a coverslip, fixed in absolute alcohol and stained by Giemsa's method. All my observations, therefore, are based on this scanty and unsatisfactorily treated material.

It might be urged that the appearances presented by the organisms in this preparation are due to imperfect fixation. This may perhaps be true to some extent, but I do not think it is altogether justified. In the first place, other organisms in the same preparation (e.g. some *spirogyra* forms and other bacilli) appear quite normal. In the second place, drying and fixing in this manner never produces similar appearances in the ordinary *flexilis* forms.

Some of the organisms in this slide are shown in figs. 85–90 (Pl. 17). Many of them are of the form seen in fig. 89—that is to say, they present the appearance of long, slender Bacteria of the *flexilis* type. They possess a nucleus of the chromidial type—many of the chromatin granules being, however, relatively very large. Large individuals reach rather over 40μ in length, though most of them are narrower than the ordinary *flexilis* forms (cf. figs. 89 and 83).

I have found in addition to these characteristic *flexilis* forms a number of individuals which display quite a different kind of nuclear arrangement. The chromatin is in large,

more or less spherical, nucleus-like masses (figs. 85, 88, 90). Some individuals possess three or four of these structures (fig. 85), whilst others—usually much shorter—possess but one (fig. 88). These “nuclei” bear a strong resemblance to the nucleus-like spore-rudiments of *B. flexilis* and similar forms, and it is possible that they are of a similar nature. Spores, however, are not present in any of the organisms.

A third modification of the chromatin which I found is that shown in figs. 86 and 87. It will be seen that the chromatic elements are neither in the form of diffuse chromidia, nor in the form of aggregated “nuclei,” but in the form of a broken spiral filament. In no single case did I observe a complete filament like that of *B. spirogyra*. In the organism depicted in fig. 86 the chromatin is disposed as follows: a dumb-bell-shaped figure, two nucleus-like masses, and a short spiral filament with enlarged ends. Fig. 87 shows a single, nucleus-like mass, a short spiral filament, a small chromatin rodlet, a small dumb-bell figure, and a second more drawn out spiral filament.

After examining all these different forms of nuclear apparatus, my impression is that they are different stages in the life-history which are connected with one another in the following way. The chromidial form (fig. 89) becomes converted into the “nucleated” form (fig. 85) by the aggregation of chromatin at various centres; this nucleated form then gives rise to the form with the spiral filaments (fig. 87) owing to the drawing out of the nuclei by a process similar to that seen occasionally in the division of the nuclei in *Micrococci* (compare fig. 52). This is very strongly suggested in the organisms shown in figs. 86 and 87.

That this interpretation of the appearances is correct I cannot, of course, be certain. But the appearances are so suggestive that I cannot refrain from making the suggestion that in this organism we have a clue to much that is obscure in the morphology of the bacterial nucleus. It seems to me highly probable that in this organism three different arrangements of the chromatin—chromidia, spherical nucleus, and

spiral filament—succeed one another at different times in the life-cycle. But I will reserve a discussion of this for another part of the paper (see p. 481).

(B) Bacilli of the spirogyra Type.

Under this heading I will describe those Bacilli which possess a structure like that of *Bacillus spirogyra*. I have encountered many Bacteria of this form, and believe the type of organisation which they display to be a very common one.

(1) *Bacillus spirogyra*.—I have already given a full account (Dobell, 1909) of the structure and life-history of this *Bacillus*, which occurs in the large intestine of *Rana temporaria* and *Bufo vulgaris*. My published figures are all of organisms fixed with formalin and stained with Giemsa. As this method involves drying before mounting (see p. 415), I have thought it advisable to publish a few more figures of the organism after treatment by a more approved method, though—as I have pointed out on p. 416—drying after fixation with osmic acid or formalin does not appreciably affect the appearance of the cells as a rule.

Figs. 117 and 118 (Pl. 18) are accurate drawings of *Bacillus spirogyra* fixed with corrosive sublimate and acetic acid and stained with Heidenhain's iron-hæmatoxylin. No drying was allowed to take place at any stage during the making of the preparation—a moist film cover-glass preparation of the undiluted contents of the large intestine of a toad (*B. vulgaris*). It is very difficult to obtain good preparations of this organism with iron-hæmatoxylin, on account of the strong affinity of the pellicle for the stain. During differentiation the nuclear filament and the pellicle give up the stain at almost the same rate, so that one is apt to over-differentiate. But when differentiation has been stopped at exactly the right moment, very distinct figures of the cell-structure can be obtained.

The appearances presented by the cells after this method of treatment are essentially the same as those obtained by

osmic or formalin fixation and Giemsa staining. This can easily be seen by anyone who will compare figs. 117 and 118 with the figures which I have already published (Dobell, 1909). The cytoplasm has a uniform appearance—no granular or alveolar structure being apparent. In each cell a darkly staining filament (cf. fig. 117) can be seen. This filament usually has a more or less strongly marked spiral or zig-zag disposition, and extends throughout nearly the whole length of the cell. Its behaviour during cell-division and spore-formation I have already described in detail (Dobell, 1909).

In my previous papers I described this filament as a nucleus. As I now believe that this interpretation is completely justified from my subsequent observations,¹ I shall continue to refer to this structure by this name. I will say no more about the nucleus of *B. spirogyra* here, as I have already discussed it at some length in my previous papers, but I will now pass to a description of some other Bacteria which possess the same kind of structure.

(2) Bacilli of the *spirogyra* type from *Lacerta muralis*.—In my description of the cocco-bacillar forms which I found in the gut of *Lacerta muralis*, I pointed out (p. 422) that many cocci and cocco-bacilli showed nuclei of a more or less spiral form (cf. fig. 50, Pl. 17). Larger Bacteria of a more definite bacillar form show an even greater structural resemblance to *B. spirogyra*.

In the first place, there is a large Bacillus, which I found very frequently in the lizards whose rectal contents I examined, which is almost identical—as regards structure—with the *B. spirogyra* which I found in English frogs and toads. It is a large motile organism (figs. 55–60, Pl. 17), rod-shaped, with rounded ends, and reaches a length of 9 μ or slightly over. Dividing forms frequently exceed 10 μ in length.

The nucleus—as in *B. spirogyra*—is in the form of a filament of chromatin, arranged in a more or less spiral or zig-zag manner. It may be almost linear (fig. 55), thrown into a few coils (fig. 57), or much contorted (fig. 60). Sometimes

¹ See discussion on p. 466, et seq.

it has a much shortened and thickened form (fig. 56). It displays, in fact, the same range of variations which I have already described in *B. spirogyra*.

Division of the cell is preceded by division of the nucleus into two portions (cf. figs. 59, 60), exactly as in *B. spirogyra*. The organism is, moreover, monosporic—forming a single terminal spore in each cell (fig. 58). I have not followed out the whole of the stages in spore formation, but it appears closely similar to what I have already described in detail in the form from the frog and toad. (See Dobell, 1909.)

These figures (figs. 55–60) are all from preparations treated by the osmic-Giemsa method (p. 415). I have, however, obtained confirmatory results by other methods. In figs. 91–95 (Pl. 18) organisms are shown which have been fixed with sublimate-alcohol and stained with Heidenhain's iron hæmatoxylin. It will be seen that the structure of the cells is essentially the same as in the osmic-Giemsa preparations. This organism presents the same difficulties in staining by iron-hæmatoxylin as *B. spirogyra* (see p. 434). The individuals depicted are therefore selected specimens in which differentiation is particularly good.

As in the case of *B. spirogyra*, there can be no possible doubt of the existence of the spiral filament which I regard as the nucleus. Giemsa preparations reveal it always with the greatest distinctness, and Heidenhain staining—when successful—does the same. Though my interpretation may be wrong, the structures which I have described and figured exist beyond all shadow of doubt.

I found a large number of organisms of the same type which are intermediate in size between the forms just described and the cocco-bacillar forms described on p. 422. In fact I found such a gradually graded series of forms that it is difficult to me to avoid the conclusion that the smallest cocci and the largest bacilli are genetically connected. Direct proof of this most important point is wanting. Nevertheless, whether the different forms are different species or one and the same

does not in any way affect the fact that all the different forms exist. I have represented some of the small bacillar forms in fig. 51 (Pl. 17). They are so closely similar to the ordinary spirogyra forms, that it will be unnecessary to enter into a minute description of them. I would emphasise the fact, however, that many organisms exist which are intermediate in size and structure between these forms and the cocco-bacillar forms (fig. 50) on the one hand, and the long spirogyra forms (figs. 55-60) on the other. The importance of this fact will be discussed later (see p. 484, et seq.).

Amongst the smaller bacilli inhabiting the large intestine of *L. muralis* is a form which—though not of the characteristic spirogyra type—may conveniently be described here. The organism (figs. 53, 54, Pl. 17) is a short rod, about $3\ \mu$ in length. Each cell contains a nucleus in the form of a short, straight chromatin rod (fig. 53). This rod divides in the process of cell division (fig. 54), behaving like the spiral filament of ordinary spirogyra forms. This form is of interest because it is but a little removed from many of the cocco-bacillar forms. It may, indeed, be derived from a coccus-form by simple elongation. It is—structurally—a drawn-out coccus such as those in fig. 45, the nucleus assuming a straight rod-like shape instead of the spiral or zig-zag of many cocco-bacilli (cf. fig. 50). Some similar organisms from another lizard are shown in fig. 109 (Pl. 18). The drawings were made from a preparation fixed with sublimate alcohol and stained with Heidenhain. Inside each cell a short, darkly stained rod can be seen. I interpret this as the nucleus, though it may possibly be a spore-rudiment. In the case of the previously described individuals (figs. 53 and 54), however, such an explanation can hardly be advanced. For the deeply staining rods are present in forms which are undergoing division (fig. 54) and divide into two transversely during this process.

Another form of *Bacillus* which I found is one of large size (up to $8\ \mu$ in length) which has its nucleus constantly in the short, thick varicose condition which can sometimes be

seen in ordinary spirogyra forms (fig. 56). Some of these organisms are shown in fig. 63, Pl. 17. The nucleus is considerably shorter than the cell, and always has the thickened, knotted appearance shown in the figures; otherwise it resembles the nucleus of typical spirogyra forms.

(3) Bacilli of the spirogyra type from *Mabuia carinata*.—In these lizards, captured in Colombo, I found a large number of spirogyra forms in addition to the cocci and flexilis forms already described. All the preparations which I made of the contents of the large intestine were fixed with osmic acid and stained with Giemsa's or Leishman's stain. The following descriptions are therefore based upon material so treated.

The largest forms which I found (figs. 30–35, Pl. 16) are of considerable size. They reach a length of 16μ . They show the organisation characteristic of spirogyra type organisms with great distinctness. Forms with a nearly straight nuclear filament (fig. 35), forms with a simple spiral or zig-zag (fig. 34) and forms with a very much twisted filament (fig. 31) were found. In fact all the modifications which are seen in *B. spirogyra* itself were encountered. A modification which I have observed comparatively rarely in *B. spirogyra* was fairly common in this form. In this modification the nuclear filament appears to be split at certain points, thus giving rise to open loops in these places (cf. fig. 32).

Division takes place as in *B. spirogyra* (fig. 33). It will be unnecessary therefore to describe it in detail. A number of short forms (fig. 36), which measured about 7μ in length, were seen. They are apparently young forms resulting from the transverse divisions of the long individuals, but some of them may possibly be another species.

This organism is—like *B. spirogyra* and the spirogyra forms from *Lacerta*—monosporic. It forms a single terminal spore in each cell (see fig. 39). Details of spore-formation were not observed, but it appears to take place in a manner similar to that of *B. spirogyra*.

I found a few forms which appeared to belong to this organism, but which presented unusual features. I have figured two of these (figs. 37, 38, Pl. 16). The organism represented in fig. 37 has finely granular, darkly coloured cytoplasm, containing several large deeply stained chromatin masses. The organism shown in fig. 38 contains what appears to be a broken-up spiral filament together with some small chromatic granules. Whether these forms are normal stages in the life-cycle or degenerate forms, I am unable to decide. At first I took them for degeneration forms, but in view of the nuclear conditions which I have observed in *B. saccobranchi* (see p. 441), it seems possible that they represent normal events in the life-history. Their presence in my preparations seems worthy of record.

Another form, which I believe to be certainly a degenerate form, is that shown in fig. 40. I have seen and described (Dobell, 1909, Pl. 13, fig. 19) similar degenerate forms in *B. spirogyra*.

Many small Bacteria from the large intestine of *Mabuia carinata* showed nuclei of the *spirogyra* type. Drawings of some of these organisms will be found on Pl. 17. Fig. 69 shows a long slender form—11 μ long, and less than 1 μ broad. Through almost the entire length of the organism, a delicate chromatin filament—irregularly coiled and zig-zagged—can be seen to extend. In spite of its small size, the filament can be seen with the greatest clearness. Figs. 70, 71 and 73 show similar, but smaller organisms. In each of these the characteristic nuclear filament is plainly visible. Figs. 72 and 74 are smaller forms (length of fig. 72 = 2 μ) with relatively short, thick and straight nuclear filaments. The nucleus appears to be like that of the organism shown in fig. 56, but on a very small scale. Fig. 75 shows a dividing form of the same kind of organism as fig. 72. With the exception of certain forms from the blood of *Saccobranchus* (see p. 441), these are the smallest Bacilli in which I have succeeded in differentiating a nuclear filament with absolute precision. All the figures are faithful copies of the organisms

which were in my preparations. Their structure was as distinct as that of much larger forms.

Two other organisms are shown in figs. 77 and 78. In the form shown in fig. 77, there is apparently a large nuclear mass occupying most of the middle region of the cell. I found several organisms like this one. They were all equally distinct, and appeared normal. But it is possible they are really plasmolysed forms of one of the other varieties. I cannot be absolutely certain that they are not, as I have not sufficient material for comparison. Fig. 78 is drawn from an organism which has its nucleus in the form of a slightly bent rod. I found a fair number of individuals of this form. It appears to be a large form similar to the *Lacerta* organisms shown in fig. 53. It is also somewhat similar to the forms shown in fig. 63.

I believe that a number of very small Bacteria which occur in frogs, toads, and lizards have the same structure as these small Bacteria which I have just described. But I have, up to the present, been unable to convince myself that this is so. With very small Bacilli it is usually very difficult to be absolutely certain of the exact structure. Only in very favourable cases, when staining has been exactly right—as in these minute forms from *Mabuia*—can satisfactory results be attained.

(4) Bacilli of the spirogyra type from *Bufo melanostictus*.—In the large intestine of this toad—taken near Colombo—I found several Bacilli which showed the spirogyra type of nuclear structure. I have given figures of two different forms which I encountered (see Pl. 17, figs. 61, 62).

The larger forms (fig. 62) were usually of the shape of a bent rod. They attained a length of 10μ . In these organisms the nuclear filament was usually linear rather than twisted—like the straight form of filament in *B. spirogyra*. It may be described as a slender thread with here and there large knot-like swellings on it (fig. 62). I did not see any individuals with definite spiral or zig-zag filaments.

The smaller forms measured about 2μ – 2.5μ in length. They were of short, oblong form (fig. 61), and each contained a very distinct spiral or S-shaped nuclear filament. I give pictures of three individuals of this form in fig. 61, and will not describe them in greater detail.

(c) *Bacillus saccobranchi* n. sp. and Associated Forms.

I shall now describe a new *Bacillus* which shows a form of nuclear apparatus differing in many ways from typical *flexilis* or *spirogyra* organisms. As it is convenient to have a name for this organism, I propose to call it *Bacillus saccobranchi*.

(1) *B. saccobranchi* n. sp.—I obtained this *Bacillus* in the following manner. Whilst I was working at the Colombo Museum, I examined the blood of a number of fish for trypanosomes. Among these I examined a number of individuals of *Saccobranchus fossilis* caught in the Colombo Lake. Some of them contained *T. saccobranchi* Cast. et Will. in their blood, but the majority showed no blood-parasites of any sort.

One day two *Saccobranchi* were brought to me for examination. As I was unable at the moment to examine their blood microscopically, I placed them in water in a large earthenware chatty. On the following day, when I was about to make an examination of the blood of these two fish, I found that one of them had died during the night. As it appeared quite fresh, however, I decided to examine its blood for Protozoa. The fresh blood, taken from the heart, showed no Protozoa, but contained some very large and actively motile bacilli, together with a number of smaller Bacteria.¹ As the bacilli seemed to me particularly suitable for study—both on account of their size and the medium in

¹ I never found Bacteria in the blood of other fish which I killed and examined immediately after killing. It is possible that these Bacilli caused the death of this particular fish, though there is no conclusive evidence that this is so.

which they were living—I made some moist films in the following way. The blood was spread in a thin film upon a slide and immediately—without drying—exposed to osmic vapour for about 30 seconds. The slide was then transferred immediately to absolute alcohol, where it was left to harden for 15 minutes. After this treatment the film was stained in the usual way in Giemsa's stain—finally being allowed to dry. I made several preparations in this way, and also by the ordinary drying method with fixation in absolute alcohol. In the preparations fixed with osmic vapour, both the blood-corpuscles and Bacteria were beautifully preserved, and the description of the organisms which follows is taken from these preparations.

The largest individuals of this form are of considerable size (figs. 4–6, Pl. 16). They attain a length of as much as $16\ \mu$, and a breadth of $2\ \mu$. The cytoplasm contains no inclusions and appears usually homogeneous, though occasionally it has a slightly alveolar structure. In all the Giemsa preparations the cytoplasm is coloured a bright blue (see figs. 1–20)—as I did not extract the colour with alcohol after staining.

In all the organisms the chromatin is stained red—in sharp contrast with the cytoplasm (see figs. 1–20). It is distributed through the cell in various ways, which can be classified conveniently into three main types of structure, with intermediate conditions.

The first type of nuclear structure is a typical spirogyra form (figs. 1, 2, 4, 12). The nucleus is in the form of a spiral or zig-zag filament, extending through the greater part of the length of the cell. As in *B. spirogyra*, the filament may be comparatively straight (fig. 1) or much contorted (fig. 2). All intermediate conditions occur. During division the filament behaves in the same way as in *B. spirogyra*: that is to say, it divides transversely into two, a half of the original filament remaining in each daughter-cell (see fig. 2).

The second type of nuclear structure is that shown in figs. 3, 5–9. The organisms of this type show nuclei of very irregular form. They possess chromatin structures, in the

form of short, irregular, broken, bent, branched, and twisted filaments (cf. figs. 3, 6, etc.). These filaments are sometimes connected with one another to form irregular networks (fig. 9). In addition to the filamentar chromatin bodies, granules and irregular masses of various shapes and sizes are frequently present (figs. 3, 5, etc.). This irregular form of nucleus appears to be derived from the spirogyra form through the breaking up and rearrangement of the elements composing the spiral filament. Figs. 6 and 8 show organisms which suggest this very clearly. Indeed, I have seen so many forms intermediate between these forms and the typical spirogyra forms that I have no doubt at all that they are different nuclear stages which succeed one another at different periods in the life-history.

The third type of nuclear structure is the chromidial type, characteristic of the flexilis group of organisms (fig. 10). In the organisms of this type—which were present in comparatively small numbers in my preparations—all the chromatin is in the form of minute, diffusely arranged granules (fig. 10). It can generally be seen that the chromatin structures (filaments, networks, etc.) of the other forms are built up of aggregated granules of chromatin. The organisms with a chromidial type of nucleus appear to be derived from the forms with irregular nuclei by the breaking apart of these chromatin granules. In a form such as that shown in fig. 5, the irregular chromatin filaments and masses appear to be breaking up. Other organisms show later stages in this process—intermediate between figs. 5 and 10. I believe, therefore, that the chromidial type is derived from the irregular type through the dispersal of the chromatin granules.

As a result of examining a considerable number of individuals, therefore, I have come to the following conclusions: *B. saccobranchi* may possess a nucleus of the characteristic spirogyra type, or of the characteristic flexilis type. It shows in addition a large number of forms intermediate between these two types. The nucleus in the intermediate

forms may have the form of a broken filament, of an irregular arrangement of branched filaments or chromatin masses, or of an irregular network. So many different forms exist that it is difficult to classify them accurately.

On account of the large number of intermediate forms, it seems to me certain that all these different organisms are not really different species. They grade into one another almost imperceptibly, so that I regard the interpretation of them as different species as excluded. Whether the spirogyra type gives rise to the irregular type, and this in turn to the flexilis type, or whether the process is in the reverse order, cannot, of course, be stated with absolute certainty from an examination of fixed and stained material alone. The former interpretation, however, appears to me the more probable. It is really immaterial which of these interpretations is correct. The point of interest is that all these different nuclear modifications occur in the same organisms. I shall discuss the matter more fully later (see p. 471).

This organism is, like typical spirogyra forms, monosporic. The spore is formed towards—but not absolutely at—one end of the cell (see fig. 14). I have not found all the stages in spore-formation in my preparations, but such stages as I have seen are similar to those of *B. spirogyra*. As in this form also, the spore-forming individuals are short—measuring on an average about 8μ in length. A certain amount of chromatin, also, is left over in the formation of the spore (fig. 14).

(2) Forms associated with *B. saccobranchi*.—I have already noted above (p. 441) that I found a number of smaller Bacteria in my preparations of the blood of *Saccobranchus*. These forms I will now describe.

First of all, I should point out that although I found many very small individuals, which were easily distinguished from the large forms which I have called *B. saccobranchi*, yet many of the smaller forms were really but little smaller than this form. Indeed, I found so many organisms of all sizes intermediate between the largest *B. saccobranchi* and the

most minute Bacilli, that I cannot avoid the impression that all these different forms are really connected with one another. This would be a point of great interest if it could be established, but unfortunately no definite conclusion can be reached by simply examining dead organisms.¹

I found a number of individuals—of smaller size than the large bacilli—which showed a very distinct nucleus of the spirogyra type. Some of these are shown in figs. 11 and 15. They measure about $6\ \mu$ in length, and are considerably narrower than the large organisms which I call *B. saccobranchi*. Each individual will be seen to contain a characteristic nuclear filament of the spirogyra type.

Some smaller forms, with a similar structure, are shown in fig. 20. They measure about $3\ \mu$ in length, and are much narrower than the preceding forms: otherwise they are closely similar. Nuclear filaments of various forms are seen in each cell.

Some still smaller organisms— $2\ \mu$ and less in length—are depicted in fig. 19. In spite of their very minute size, they show a nuclear filament with great distinctness. They appear to possess a nucleus exactly like that of the spirogyra forms of *B. saccobranchi* (fig. 2, etc.), but on a very small scale. These are the smallest bacilli which I found in the blood of *Saccobranchus*.

I would again emphasise the fact that the various individuals depicted in figs. 2, 11, 20, and 19 are organisms selected on account of the difference in their sizes. Actually, a number of intermediate-sized organisms of precisely similar form were seen.

I have added some figures of other organisms which were of interest. The Bacillus shown in fig. 13 shows a nucleus of a form which I observed in a few individuals. It is in the

¹ If the organisms are all of one species, it follows that the name *B. saccobranchi* should be given to all. As the matter is so doubtful, however, I have restricted this name to the large forms only, though I do not wish to imply thereby that the large forms are specifically distinct.

form of a short, irregular mass of chromatin, which appears sometimes to be really a spiral filament contracted upon itself. Fig. 17 shows a pair of Bacilli with rod-like nuclei. Fig. 18 appears to be a similar kind of organism undergoing division. Fig. 16 is an individual which is like a *B. saccobranchus*, with a nucleus of the irregular type, on a smaller scale. None of the smaller forms showed nuclei of a distinct chromidial type.

3. SPIRILLAR FORMS.

I have endeavoured to elucidate the structure of a number of spirillar forms, but have succeeded in reaching definite results in only four different forms. Yet these four different forms have shown me three different types of nuclear apparatus, and these I shall now describe.

(a) *Spirilla* with Nuclei of a Chromidial Type.

In a previous paper (Dobell, 1908) I described briefly the structure and method of spore-formation which is found in the *Spirillum*—which I named *S. monospora*—which occurs very commonly in the large intestine of frogs and toads. I described this organism as containing a number of scattered granules, stainable with chromatin stains. These granules I regarded as constituting a nucleus of the same sort as that of *B. flexilis* and similar forms. They enter into the formation of the spore, forming a nucleus-like spore rudiment—as in *B. flexilis*, *B. spirogyra*, *B. lunula*, and many other Bacteria.

I have made many attempts to obtain a more detailed knowledge of *S. monospora*. My efforts, however, have been unavailing. The facts—as far as they go—which I have already recorded are the only ones which I have so far been able to discover. I therefore abandoned this form and looked for other and more favourable objects for investigation.

One of the first organisms to which I then turned my attention was the large *Spirillum* which inhabits the hind gut of the common cockroach, *Stylopyga orientalis*. I hoped—from the large size of this organism, and from the descriptions which have been given of it by others¹—that I should find some new and illuminating structures in this *Spirillum*. But my hopes have not been altogether fulfilled.

The only structures which I have been able to make out in this form with absolute precision are essentially the same as in the form from frogs and toads. The *Spirillum* of the cockroach possesses an exceedingly thick cell wall, which has a strong affinity for many stains, and which renders it very difficult to study cytologically. I have obtained the most satisfactory results with wet films fixed in sublimate-alcohol and stained with iron-hæmatoxylin. But differentiation of the contents of the cell is very difficult to obtain, and succeeds only occasionally. It has usually been my experience that

¹ According to Guilliermond (1907) and others, an organism containing a typical nucleus has been described by Kunstler and Gineste ('C. R. Assoc. Anatomistes,' 1904), under the name *Spirillum periplaneticum*, from the intestine of *Periplaneta orientalis*. Unfortunately I have not been able to see this paper. In two subsequent notes, however (Kunstler and Gineste, 1906, 1906A) these authors describe and figure this organism, but do not mention or depict a nucleus, and give the host as *P. americana*. In the first note (1906) a reticular structure of the cytoplasm is figured and described. In the second note (1906A) "spherular elements" are described in the cytoplasm, and the average dimensions are given as $8\mu \times 3\mu$. It is possible that this is the same organism which I call the "large *Spirillum* from *St. orientalis*," but I cannot be certain from the incomplete notes of Kunstler and Gineste which are at my disposal. Fantham ('Quart. Journ. Micros. Sci.,' 1908) casually describes "spirillar forms occurring in the hind gut of the cockroach." He observed "a diffuse nucleus . . . consisting of a number of chromatin masses seen to be connected by a lightly staining spiral in successfully stained specimens. At other times the achromatic thread was not evident." Diagrammatic figures are given. They are not like anything I have seen in *Spirilla* from *S. orientalis*. I do not attach any importance to these fragmentary observations.

when the colour has been sufficiently extracted from the cell wall to enable one to see the internal structures, then the cell contents have also been so much decolorised as to be almost invisible. Now and then, however, clear pictures of the protoplasmic structures can be obtained.

In well-stained individuals I have invariably found the same structure present. There is no vesicular nucleus visible and no nuclear filament. The only structures which can be seen are shown in figs. 110–112 (Pl. 18). The cytoplasm is alveolar, the alveoli sometimes extending across the whole width of the cell, sometimes being of smaller size. At various points in the walls of the alveoli lie deeply staining granules. They are usually few in number, very irregularly arranged, but generally in close proximity to the cell wall. Occasionally two or more granules appear at first sight to be connected with one another by fine intervening filaments; but it seems to me that in all cases these filaments are really nothing more than the alveolar walls. I have never found any structure at all comparable with the nuclear filament of *B. spirogyra* and similar forms.

My interpretation of the appearances observed in the large *Spirillum* of the cockroach is therefore this: The structure is essentially the same as that of *B. flexilis* and allied organisms. The cytoplasm is alveolar and the nucleus is in the form of chromidia—consisting of a few small, scattered granules of chromatin lying in the walls of the alveoli. Neither vesicular nucleus nor nuclear filament exists in the forms which I have examined. The structure is therefore closely similar to that of *S. monospora*.

(b) *Spirilla* with Nuclei of Filamentar Form.

There is a large *Spirillum*¹ which lives in the gut of *Lacerta muralis*. I have found it much easier to study than the large forms from the frog and the cockroach, and

¹ These are the organisms to which I referred in a previous paper (Dobell, 1909, p. 583).

have found that it possesses a different kind of organisation. It is—like the other forms—difficult to stain satisfactorily, but I have obtained some beautiful results with iron-hæmatoxylin. My figures (figs. 96–108, Pl. 18) are all drawn from wet film preparations, fixed with sublimate alcohol and stained with Heidenhain's iron-hæmatoxylin. My best preparations are from the contents of the large intestine of lizards which I obtained from a dealer in Munich; but similar—I believe the same—*Spirilla* also occur in Naples lizards, and have furnished me with additional material.

The structure of these *Spirilla* is shown in figs. 96–108. Long individuals attain a length of as much as $13\ \mu$ (fig. 102) and more, whilst the shortest individuals measure about $4\ \mu$ (figs. 103–105). All forms display a structure which is essentially the same, and which differs markedly from that of other forms which I have investigated.

In fixed and stained organisms¹ the cytoplasm has, as a rule, a very distinct alveolar structure (cf. figs. 96, 100, 102, etc.). The alveoli may extend right across the cell, being arranged as a single row of large chambers (fig. 96), or they may be of variable smaller sizes (e. g. fig. 107). The only other structure which is normally present in well-stained individuals is a darkly staining filament, like that of *B. spirogyra*, which I interpret in this case also as the nucleus (see figs. 96–108). Both the form and position of this filament may vary. It is frequently in the form of a short and nearly straight rod (figs. 96, 104, etc.), occupying a central (figs. 104, 107) or terminal (fig. 96) position, or any intermediate site in the cell. It may be in the form of a more or less twisted and varicose spiral or zig-zag (figs. 101, 102, etc.), sometimes showing knob-like thickenings at the ends (figs. 100, 105), and sometimes running almost from one extremity of the cell to the other (figs. 100, 101, etc.). Inspec-

¹ As in the case of other *Spirilla* I have been unable to reach any definite conclusions regarding structure from a study of the living organisms. The living organisms, however, have been studied in all cases.

tion of the figures on Pl. 18 will give a better idea of the various modifications which occur in this filament than pages of description.

The filament is a morphological element present in each cell. It is a body which is independent of the walls of the cytoplasmic alveoli: that is to say, it is not merely an accidental appearance due to portions of the alveolar walls taking up the stain more strongly than others. It can be seen in many cases that the filament crosses the alveoli, and is a quite independent structure (cf. figs. 96, 102, etc.). I believe there can be no doubt whatsoever that it is a structure of precisely the same sort as occurs in *B. spirogyra* and so many other similar organisms.

Additional evidence in support of this view is derived from a study of the behaviour of this filament during cell division. Just as in the Bacilli of the *spirogyra* type, division of the filament precedes division of the cell as a whole (see fig. 108). After the filament has divided into two approximately equal parts, the cell divides across the middle (figs. 97, 98). It will be noted that during division the filament must lie in a central position in the cell, and that immediately after division it must occupy a more terminal position in the daughter-cells. This no doubt to a large extent explains the variable position of the filament, in different cells, alluded to above. On purely morphological grounds, therefore, it seems to me justifiable to regard the filament in these *Spirilla* as a nucleus of the same sort as occurs in Bacilli of the *spirogyra* type. I shall discuss these filaments and their interpretation later (see p. 466): for the present I wish simply to establish the fact that a filament of this sort is present in these *Spirilla* and in many Bacilli.

Occasionally darkly staining granules are present in the cytoplasm of the *Spirilla* from the lizard. They are few in number, and not a constant morphological feature of the cell (cf. figs. 101, 102). I regard them as probably composed of reserve material of some sort. As a rule these organisms are free from granular inclusions.

(c) Spirilla with Spherical Nuclei.

The fourth and last spirillar form concerning whose structure I have reached definite conclusions is exceedingly minute. It is an organism which occurs commonly in the hind gut of the common cockroach (*S. orientalis*) in company with the larger form already described.

The organism in question has the characteristic form of a small Spirillum or Vibrio (figs. 121-132, Pl. 18). The smallest Vibrio forms have a length of rather less than 2μ (fig. 132) and upwards. In the largest Spirillum forms the length rarely exceeds 4.5μ (fig. 130). In all cases the organisms are very narrow—the largest having a breadth of approximately 0.5μ , the smallest, considerably less. Yet, in spite of their very small size, these organisms possess an internal structure which may be demonstrated with the greatest clearness.

I have obtained very good results with wet films, fixed with sublimate-alcohol and stained with Heidenhain. All the figures (figs. 121-132) on Pl. 18 are from individuals treated in this manner. Differentiation may be obtained with comparative ease.

Every individual—when properly stained—shows a single, darkly staining, spherical body situated somewhere within it (cf. figs. 121-124, 130-132). I regard this body as a nucleus. This body—or, as I shall call it, this nucleus, may lie in the centre of the cell (fig. 123, etc.) or at one extremity (fig. 121), or in an intermediate position. It always has the form of a minute round or oval granule, which appears—under the highest magnification which has been available—quite homogeneous. It is a constant morphological feature of every cell. No cytoplasmic structure can be made out with absolute certainty, on account of the very small size of most of the organisms. Faint indications of an alveolar structure seem to me, however, to be sometimes present. In addition, it may be noted that the ends of the cells frequently stain deeply, so that in optical section an individual often appears

to have its ends covered with little dark caps—an appearance seen in many stained Bacteria.

During the division of an organism into two, the nucleus behaves in a characteristic manner. It lies, before division, towards the centre of the cell (fig. 130). Whilst in this position, it divides into two daughter-nuclei, with the formation of a minute dumb-bell figure (figs. 125, 126). These daughter-nuclei then separate to a variable distance from one another (fig. 127) and cytoplasmic fission follows (figs. 128, 129). According to the degree of separation which has occurred between the daughter-nuclei, the nuclei of the daughter-cells may lie in a terminal (fig. 129) or central (fig. 128) position.

The general appearance of the nucleus in this Spirillum—both during rest, and during division—is therefore closely similar to that of the nucleus which I have already described in Micrococci and Sarcina.

4. "FUSIFORM BACTERIA."

At various times, I have encountered a number of organisms belonging to the group of so-called "fusiform Bacteria." These organisms are very commonly found in the intestines of animals. I have studied their structure in quite a number of forms, and all appear to be organised in essentially the same manner.

My own impression—after observing many different organisms of this class—is that they are really not Bacteria at all, but more probably belong to the Fungi. Perhaps they are related to the yeasts. But as I have no conclusive evidence—from their life-histories—of their real affinities, and as they are usually regarded as Bacteria, I will describe here the forms which I have investigated.

(A) "Fusiform Bacteria" from *Lacerta muralis*.

I found fusiform organisms fairly common in the large intestines of the lizard which I captured near Naples. They were

generally to be found in my preparations, but usually in small numbers.

Good preparations can be obtained by the osmic acid or formalin method with Giemsa staining (see p. 415), and Heidenhain's iron-hæmatoxylin—when sharp differentiation has been achieved—gives very good pictures of the structure of these organisms.

In Pl. 18, figs. 113 and 114, will be seen some fusiform organisms from a moist film preparation of the contents of the large intestine of *L. muralis*, fixed with sublimate-alcohol and stained with Heidenhain. It will be seen that each cell contains a single, darkly staining, centrally situated, spherical mass, which I believe to be the nucleus. I have not obtained any satisfactory preparations which show a division of this body in this particular form, but from analogy with similar organisms, I have little doubt that it divides in the course of cell division.

Small single individuals (fig. 114) have the characteristic spindle shape, and measure $4\mu - 4.5\mu$ in length. The ends are sharply pointed. No other structures besides the nucleus were visible in the cells.

In this form—as in all the other fusiform organisms which I have investigated—double individuals are very common (fig. 113). They arise from dividing single individuals through an incomplete separation of the daughter-cells—in the same way that *Diplococcus* forms are produced by *Micrococci*.

Smaller forms than those just described also occur in the lizard. They all show a similar structure. Fig. 68, Pl. 17 is of a small double form, stained with Giemsa's stain. Each individual shows a distinct nucleus, as in the Heidenhain-stained individuals. The length of this double organism was approximately 5μ .

(B) "Fusiform Bacteria" from Frogs and Toads.

The common English frog and toad (*R. temporaria* and *B. vulgaris*) occasionally contain fusiform organisms in

their large intestines. They appear to be the same in both hosts, but I have found them more often in the toad than in the frog. Many of them, however, are of very small size, so that they are easily overlooked unless one is specially on the look-out for them.

As a rule these organisms occur in the double form (fig. 115)—single spindles being comparatively rare in any preparations. The ends of the double spindles are more rounded than in the forms from the lizard. The nuclei are of the same type. Fig. 115 is an individual (double) from the large intestine of *Bufo vulgaris* (moist film, sublimate-alcohol and Heidenhain). Heidenhain and Giemsa reveal the same structure. The length of the double individual depicted in this figure was ca. 10 μ .

(c) "Fusiform Bacteria" from *Triton vulgaris*.

The common newt contains several different forms of fusiform organism. They are similar to those of the frog and lizard, and usually occur in the double form. I have not made a very careful study of these organisms, but they show certain features of interest.

Each individual (figs. 66, 67, Pl. 17), contains a single nucleus. This may take the form of a simple, apparently solid granule of chromatin (cf. fig. 67, upper nucleus), or occasionally it appears to be a vesicular structure with one large karyosome (cf. fig. 67, lower nucleus). From the small size of these nuclei it is often very difficult to be quite certain of their exact structure. Another type of nucleus, which I observed in some of the larger forms from the newt, is that shown in fig. 66. This figure shows an ordinary double form in which the upper individual is dividing into two. It will be seen that all three nuclei which are present are in the form of double granules, arranged transversely across the cells. All these forms were seen in Giemsa preparations, which were fixed with formalin, and dried before mounting. It is therefore not impossible that

these double nuclei are artifacts produced by the breaking into two of a single granule through drying. This seems to me improbable, however.

(D) "Fusiform Bacteria" from *Stylopyga orientalis*.

The common cockroach harbours a fusiform organism very like those which I have already described. The forms which I have encountered are usually of small size, and are generally of the double spindle form.

Every cell contains a nucleus (see fig. 116). It is frequently of a rather square shape, and sometimes I have found nuclei which appear to be dividing (cf. lower nucleus in fig. 116). All the dividing nuclei which I have seen are of this rather curious form—that of two chromatin masses separated by a variable distance. I have not been able to make out any other details of the process of nuclear division, but I have not made a very careful study of these organisms. My only knowledge of them is derived from occasional individuals which I have found in preparations made for other organisms.

5. ON SOME NUCLEATED BACTERIUM-LIKE ORGANISMS.

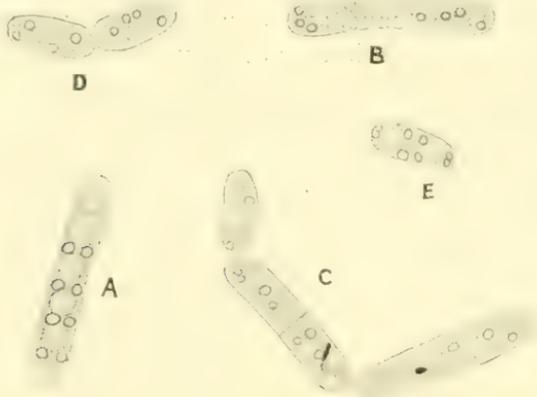
In this section I shall describe some curious organisms which present—at certain stages in their lives—a strong resemblance to Bacteria, but which are really to be placed among the Fungi. For a considerable time I believed these organisms to be Bacteria which possessed a typical vesicular nucleus—like that of *Bacterium gammari*—and I believe that other observers may have fallen into a similar error. I think, therefore, that no excuse is needed for publishing my results in some detail in the present paper.

The organisms under consideration have all been found in the large intestines of animals. I have found them in several insects, snakes, lizards, and Amphibia, and I have also encountered forms which I believe to be of a similar nature in the rectal contents of mammals. However, I have made no careful

investigation of these. The forms which I have studied most carefully are those occurring in frogs and toads (*Rana temporaria* and *Bufo vulgaris*), in the common cockroach (*Stylopyga orientalis*), and in *Boa constrictor*. It is upon the organisms from the large intestine of this snake that I have made the most complete series of observations, and I will therefore record them in some detail at this point.

The living organisms, when removed from the host, and examined under an immersion, appear as large, bacterium-like;

TEXT-FIG.



Bacterium-like organisms from large intestine of *Boa constrictor*. Living organisms. (Zeiss, 2.5 mm. apochromatic water immersion \times compens.-oc. 18.)

non-motile rods (see Text-fig.). They may occur singly, in pairs, or in chains. The average length of the largest individuals is about 14μ . Smaller individuals are very common, and many do not exceed 4μ . All intermediate sizes are to be found.

The rods all have rounded ends, and many of the longer individuals show a slight curvature (cf. Text-fig. B). The internal structure in the living cells is very easily seen, though the nucleus can be satisfactorily demonstrated in stained preparations only. The cytoplasm is finely granular, and contains as a rule a number of refractive bodies—probably reserve

material. In addition to these, pale vacuoles are usually to be seen. They may be irregularly scattered through the cytoplasm (B, C) or arranged in a single line down the middle of the organism (A). In the latter case the refractive bodies frequently occur in pairs between the vacuoles—as shown in fig. A. Intra-vitam staining¹ brings out the refractive bodies very sharply, but does not reveal the nucleus.

Multiplication can be easily observed under the microscope. It is accomplished by the rods undergoing a transverse fission in a manner which closely resembles that of many Bacteria. If long cells be carefully scrutinised, some of them can be found which show faint indications of a septum towards the middle of the organism. The septum makes its first appearance as two faint transverse lines, extending towards one another from opposite sides, in the centre of the cell. A little later the lines appear to meet, so that a delicate septum extends right across the middle of the cell (see Text-fig. c—middle individual). The septum becomes thicker, and cuts the parent cell into two equal daughter-cells. After separation—which now takes place—the contiguous ends of the daughter-cells are square, but they rapidly assume a rounded appearance (cf. text-fig. D). The whole process of division—as seen under the microscope—takes several hours, proceeding very slowly. I have not been able to follow the division of the nucleus satisfactorily in the living organisms.

All the material upon which these observations are based came from the large intestine of a single *B. constrictor* which had been in captivity for some time. I have, therefore, no data to indicate the frequency with which the parasite occurs in this snake.

On examining the contents of the large intestine of the snake, soon after death, I found a large number of organisms present in the stages which I have just described. My first conclusion—not unnaturally—was that I was dealing with a large species of Bacterium. Had I not made further observations upon the subsequent development of the organ-

¹ With neutral red, methylene blue or Brillanteresylblau.

isms, this conclusion might have appeared to some extent justified. After fixing and staining some of the cells, a large nucleus was seen to be present. It therefore appeared to me at the time that I had discovered a new Bacterium which possessed a well-marked nucleus, and hence belonged to the group of organisms of which *B. gammari* is the type.

Stained examples of this organism from the boa are shown in figs. 137, 138, 140, 141 and 144 (Pl. 19) and in fig. 135 (Pl. 18). Owing to the watery nature of the rectal contents, and to the large amount of grit present, it was found very difficult to obtain good wet-film preparations. Most of my stained preparations were therefore made by allowing some of the fluid containing the organisms to dry upon a slide;¹ then fixing the dried film in absolute alcohol; and finally staining with Giemsa's stain. As a cytological method this is of course unsatisfactory; but the results obtained were, in the main, good enough for arriving at conclusions regarding the general structure of the cells. In most cases the nucleus had undergone a certain amount of fragmentation—owing to drying—but it frequently showed its vesicular structure quite clearly.

In fig. 137 a number of small individuals are depicted—each showing a distinct nucleus. Fig. 138 shows two larger individuals, of the characteristic Bacterium form, with rounded ends. Fig. 141 shows a similar organism, but with ends of a squarer form. The nucleus is in all cases unmistakable. In fig. 140 a chain consisting of four organisms of a more or less bent form is seen. The nuclei are all somewhat broken up through drying. Fig. 144 shows another chain of four individuals, of smaller size, and each containing a vesicular nucleus. Forms intermediate in size between these small forms and the larger forms occur, so that there is no reason for regarding them as different species. I propose to call all the individuals which have the rod-form characteristic

¹ At the time when these observations were made (1906) I had not discovered the osmic acid drop method of fixation which has since proved so useful.

of Bacteria, the bacterioid forms—to distinguish them from other forms.

The other forms which this organism is able to assume appeared in the course of a few days in the contents of the snake's large intestine, which had been kept as a culture in a glass vessel. They were not found inhabiting the snake. Multiplication of the bacterioid forms continued for several days, after which the other forms made their appearance. The ordinary bacterioid individuals (such as fig. 138, etc.) were seen to become more rounded (fig. 142), finally assuming the oval form characteristic of a yeast. In this yeast-like condition the organisms continued to multiply—but by budding, and not by transverse fission (see fig. 145). I propose to call these yeast-like forms the zymoid forms—to distinguish them from the rod-like bacterioid forms.

The zymoid forms are exactly like any other ordinary yeast. They possess an oval form, a vesicular nucleus, and reproduce by budding (cf. figs. 136 [Pl. 18] and 145 [Pl. 19]). They are, indeed, exactly like other yeasts with which I am familiar in the rectal contents of frogs, toads, lizards and many other animals.

That the zymoid forms are directly derived from the bacterioid forms—and are not really independent organisms—I can assert with absolute certainty. I have observed the transformation in living organisms kept under observation for several days. All intermediate forms, moreover, were found in my fixed and stained preparations, and it was no uncommon thing to observe bacterioid and zymoid individuals composing one and the same chain (fig. 151). Both bacterioid and zymoid forms existed side by side in my cultures for many days, but finally the bacterioid forms were almost completely supplanted by the zymoid forms.

Curious further changes were also observed. Many of the bacterioid forms developed outgrowths, which sometimes grew to a considerable length (see fig. 148). Many of the zymoid forms also gave rise to outgrowths—in some cases of very large size. These outgrowths began as short finger-like

processes (fig. 146), into which the nucleus sometimes entered (fig. 149). In some cases, division of both nucleus and cytoplasm occurred—the finger-like outgrowth being separated off as a more or less bacterioid cell (fig. 150). At other times, the outgrowths continued to grow in length without cell division taking place. They often attained a considerable length, and underwent branching (fig. 147)—looking like the beginnings of a mycelium. Although I kept the organisms under observation for many weeks, I never found any other stages in development. Apparently, the conditions under which the organisms were kept were such as to inhibit further growth.

Now after observing the changes which my original Bacterium-like organisms underwent, I came to the conclusion that I was really dealing with a fungus closely allied to the yeasts. It seems to me more than probable that the organisms are really fungi, a part only of whose life-cycle has come under my notice. I believe the resemblance of the original bacterioid forms to Bacteria is purely accidental, and the organisms have nothing whatever to do with this group.

As I have already noted, forms similar to these from *Boa constrictor* occur in the intestines of a variety of animals. It is therefore necessary to be on one's guard when investigating Bacteria derived from such sources. Unless observations be made upon the development of the living organisms, one may easily be led into error.

I must point out that the finer details of nuclear division—of both bacterioid and zymoid forms—have not been thoroughly investigated. This is due to the fact that perfect fixation was usually impossible. Division is, I believe, amitotic: and this is certainly true of the form which occurs in the frog—a form upon which I have made a number of careful observations. As these, however, are still incomplete, and indicate that this form is very closely similar to that from the boa, I do not wish to enter into a fuller description at present.

In conclusion, I would emphasise the fact that the foregoing

observations in no way invalidate the contentions of Vejdovský and Mencl regarding *Bacterium gammari*. I see no reason at present for doubting that this organism belongs to the Bacteria. My own investigations have shown merely that certain organisms, which appear to resemble *B. gammari* at one stage in their lives, are really not Bacteria at all, but belong to the Fungi.

SUMMARY OF RESULTS.

I will now summarise the results which I have recorded in some detail in the foregoing pages. In this section I shall consider my own work only, without reference to the work of others. A full discussion will be found in the next section of the paper (p. 462 et seq.).

(1) All the Bacteria which I have been able to investigate with precision contain a structure (or structures) which I believe to be a nucleus. The reason for regarding these structures as nuclei is two-fold—first, from purely morphological considerations; secondly, from their staining reactions (see discussion, p. 462).

(2) The Bacteria studied belong to four different groups—namely, Cocci, Bacilli, Spirilla, and so-called “fusiform Bacteria.”

(3) The Coccus forms studied possess a single, centrally placed, spherical nucleus in each cell. It divides by a simple amitosis. This type of nuclear organisation has been found in forms belonging to the genera *Micrococcus* and *Sarcina*.

(4) Cocco-bacillar forms which have been investigated show a nucleus in the form of a straight or bent rodlet, or of a more or less spiral or zig-zag filament.

(5) Bacillar forms show several different types of nuclear differentiation. The nucleus may be in the form of chromidia scattered through the cell (flexilis type, etc.); in the form of a more or less straight, spiral or zig-zag filament (spirogyra type, etc.); or in the form of irregular strands

and networks (*B. saccobranchi*). There is evidence to show that a nucleus in all these three forms may occur at different times in the same organism (*B. saccobranchi*). There is also evidence that spherical nuclei, filamentar nuclei, and chromidial nuclei may occur in the same organism at different stages in its life-history (Bacilli of modified *flexilis* form from Triton and *Lacerta*).

(6) Spirillar forms which I have studied show three different types of nucleus: the chromidial (*Sp. monospora*, etc.); the filamentar (*Spirillum* from *Lacerta*); and the spherical type (small *Spirillum* from *Stylopyga*), which divides by amitosis, and resembles the nucleus of *Coccus* forms.

(7) "Fusiform Bacteria" possess a single, usually spherical, nucleus in each cell.

(8) A number of large, parasitic, non-motile, rod-like organisms, possessing a vesicular nucleus, which appear at first sight to be Bacteria, are really Fungi allied to the yeasts.

GENERAL DISCUSSION.

Now that I have briefly reviewed the more important literature bearing upon the cytology of the Bacteria, and have given my own observations in some detail, I am in a position to discuss my results. My main object, as I have already pointed out, has been to decide the question, whether or not the Bacteria are nucleate cells. The chief part of this discussion will therefore be directed towards answering this question.

As I have already indicated, many of the observations which have been made by others upon the cytology of the Bacteria, are based upon material which has been so imperfectly fixed and stained that it is useless to consider them. Of the researches reviewed in the "Historic" section (p. 399), therefore, only a part can be profitably considered here. Furthermore, it is impossible to enter into a minute discussion of many excellent contributions to the subject—extending,

as they do in the aggregate, over many hundreds of pages. Consequently, I crave forgiveness for the many sins of omission which must be apparent to anyone who reads the ensuing remarks.

Metachromatic Granules.—Considerable confusion exists in bacteriological literature regarding a number of granular cell-inclusions which I shall call metachromatic granules. Recent work has, however, done much to clear up this confusion, and I believe that the interpretation of these granules is now perfectly plain, and there is no cause for any further misunderstanding regarding their nature and significance. For an excellent summary of our present knowledge of these bodies, I would refer the reader to a recent paper by Guilliermond (1910).

The first to observe these granules in Bacteria appears to have been Babes. It was he, also, who subsequently named them "metachromatische Körperchen." There seems to be little doubt that the majority of colourable granules which have been described in bacterial cells really belong to this class of bodies. Different observers have given different names to the granules, and this has been largely the cause of the confusion which at present exists regarding them. It appears to me certain that the "metachromatic bodies" of Babes, the "sporogenic granules" of Ernst, the "red granules" (in part only) of Bütschli, the "chromatin granules" (in part) of Wahrlich and many others and of Meyer's earlier papers, the "granules" of Fischer, the "Volutanskugeln" of Grimme, the "volutine" granules of Meyer, the "toxigen granules" of von Behring, the "Babes-Ernst bodies" of many bacteriologists, and many other kinds of granule described by many other workers—all these are in reality the same, namely the bodies which I shall call metachromatic granules. This name aptly designates these bodies, and has been used throughout by Guilliermond¹ in his important researches into their nature; and I hope—with him—that it will find universal acceptance

¹ Guilliermond's actual name is "corpuseules métachromatiques."

and so help to clear away the confusion which now surrounds these bodies.

Metachromatic granules are found not only in many Bacteria, but also in Fungi, Algæ, Cyanophyceæ, Protozoa, and probably in many of the "higher" groups of animals and plants. Their presence in Bacteria can therefore not be used as evidence of the affinities of this group.

Regarding the chemical and staining properties of these granules, we now have a considerable mass of information—chiefly from the work of Guilliermond, Grimme and A. Meyer.¹ Their most characteristic property is that they stain red with many blue or violet stains (e. g. methylene blue, hæmatoxylin, etc.). After fixation they have a strong affinity for so-called "nuclear" stains—which has given rise to their confusion with chromatin.

Chemically considered, the metachromatic granules are probably to be regarded as composed of nucleic acid combined with an organic base (cf. Meyer, Guilliermond).

The biological significance of the metachromatic granules appears to be definitely decided. They are non-living (metaplasmic) reserve material. They are not living morphological derivatives of either nucleus or cytoplasm, but merely stored up food substance. The evidence for this appears to me overwhelming. The most important fact has been established, I believe, that they are not a constituent of the living protoplasm: they are transient, non-living elements of the cell. That they are in any way an index of the virulence of the organisms containing them, as maintained by Marx and Woihte (1900), is negatived by the work of Ascoli (1901), Krompecher (1901), Gauss (1902), Schumburg (1902), Ficker (1903), Guilliermond (1906) and others. The biological distribution of the granules throughout other organisms also speaks strongly against such a view.

It might be urged, with some justification, that the "chromidial nucleus" described in Bacteria by Schaudinn, Guilliermond and myself is really nothing more than a diffuse

¹ See also Eisenberg (1910).

system of metachromatic granules. Such a supposition has already been considered and rejected by both Schaudinn and Guilliermond. I have also had occasion already to speak against this view, and I shall now enter into it more fully.

The Bacilli of the spirogyra type which I have described, also the Micrococci, and Spirilla with a filamentar or spherical nucleus, are in the majority of cases entirely free from granular inclusions in the cytoplasm. The nuclear structures which I have described are the only constant internal structures present. It is therefore useless to argue about metachromatic granules in these forms, unless it be assumed that the nuclear filaments, etc., are metachromatic bodies—an assumption for which there is not a shred of evidence, and which is entirely opposed to the facts. It remains therefore to consider the Bacilli and Spirilla (chiefly the organisms of the flexilis type, and the Spirilla from the frog and cockroach) in which I have described a chromidial nucleus.

In the first place, I must point out that the two methods of staining—namely the Heidenhain and Romanowski methods—which I have chiefly used are not sufficient to distinguish between chromatic and metachromatic substances by means of differential staining. Both chromatic and metachromatic granules are stained black with Heidenhain and red with Romanowski. Neither method, therefore, can be used as an index of the chemical nature of the granules. In the second place, I think it highly probable that metachromatic granules do exist, side by side with the nuclear granules, in many Bacteria with chromidial nuclei (cf. also Schaudinn [1903], and Guilliermond [1908]). *Bacillus flexilis* itself, and also other Bacilli of the same type, contain granular inclusions which may easily be stained intra-vitam with neutral red, methylene blue and Brillanteresylblau. All these granules have a faintly reddish tinge when so treated. The same is true of *Spirillum monospora*. These colourable granules are few in number, however, as compared with the number visible after Heidenhain or Romanowski staining. I believe therefore that they are metachromatic

granules (reserve material) which are present in addition to the granules constituting the nuclear apparatus.

That some of the "red granules" described by Bütschli, and the "chromatin granules" of Wahrlich, A. Meyer and others are also really metachromatic granules, I think extremely probable. Yet I believe that many of these granules seen by these observers are of a nuclear nature—as in the case of my own Bacteria. Guilliermond (1908), moreover, found granules of both chromatic and metachromatic material in a number of forms which he investigated.

Now the evidence for regarding the greater part of the granules in my Bacteria as of a nuclear nature is not derived chiefly from their staining reactions—which I regard as of secondary importance—but is morphological. I shall consider this in detail in the ensuing section.

Morphological Evidence that Bacteria are Nucleate Cells.—Before proceeding any further, it is necessary to consider for a moment what is meant by the term nucleus. Various more or less unsatisfactory definitions have been given, and I do not propose to add to their number. To define any well-known thing—such as a nucleus—is merely to confine one's idea of the thing to certain arbitrarily chosen properties which it possesses, and to lay oneself open to the attacks of the verbal quibbler. It is absurd to define a nucleus in terms of certain of its chemical characteristics alone. Still more absurd is it to base a definition upon its staining reactions; for—apart from the fact that it cannot, in most cases, be definitely proved whether staining is a chemical or physical phenomenon—it is well known to every cytologist that different nuclei may display a very wide range of difference in their staining capacities. And yet I think every biologist knows what he means when he speaks about a nucleus. He means a morphological element of the living cell—a structure which could have been discovered even if chemistry were completely unknown, and staining had never been invented. The concept "nucleus" is fundamentally one of form—the idea, that is to say, belongs primarily

to the province of morphology, not of chemistry or physics. It is necessary to bear this in mind when discussing it. Hence whether a given body is a nucleus or not can only be decided by studying its morphology and then comparing it with other structures which we agree to call nuclei. Chemical properties and staining reactions may aid us materially in reaching a conclusion, but they cannot alone be used as criteria at present.¹ If they could, then a pound of nuclear substance—if it could be obtained—would be a nucleus.

One more point must be mentioned here. It has been many times asserted that Bacteria consist entirely of nucleus, or entirely of cytoplasm—because no cellular differentiation like that of other organisms has been discovered. That Bacteria are composed of cytoplasm is not frequently stated in so many words, but it is often tacitly assumed when speaking of these organisms as enucleate. But that Bacteria are nuclei has been definitely stated by many workers—especially in recent years by Ružička. Now, apart from any work which may have led to such an interpretation, I should like to point out that such statements are, a priori, nonsense. By “nucleus” and “cytoplasm” are meant definite morphological elements into which most—probably all—cells are differentiated. There is good experimental evidence that neither nucleus nor cytoplasm—specialised parts both of the living protoplasm—is capable of living independently of the other for any length of time. To call a *Bacillus* a naked nucleus is, therefore, a misapplication of a word in common use. An organism may have a structure similar to that of many nuclei, it may have similar chemical and staining characters,² but to call it a nucleus in consequence is—far

¹ In connection with the nucleus in Bacteria somewhat similar views have already been expressed by Schaudinn (1903). It is curious to note how many other writers are so profoundly impressed with the importance of chromatin that they frequently use “chromatin” and “nucleus” as though they were synonymous.

² It should also be emphasised that the “special affinity for chromatin stains,” which is often attributed to Bacteria, is—as Fischer has pointed out—a myth.

from giving a satisfactory interpretation—simply to misuse words. That Bacteria are composed of a substance similar to cytoplasm may readily be granted; but to say that they consist of cytoplasm is merely to use the word “cytoplasm” in a sense which is not generally accepted. Hence, if it were proved—which it is not—that Bacteria were “cells” without a nucleus, it would be necessary to employ some other word than cytoplasm to designate their contents—for instance Van Beneden’s term “plasson,” or some such word. At present, however, there is no necessity to follow such a course. If one chose arbitrarily to call nucleus cytoplasm, and cytoplasm nucleus, one could easily make the astounding generalisation that cytoplasm was really not cytoplasm, but nucleus. Such, it seems to me, is the method of reasoning which is occasionally applied in considering the structure of Bacteria.

In addition to the foregoing considerations, I should like to emphasise another point. It is sometimes stated that the Bacteria show a peculiar kind of protoplasmic organisation in which nucleus and cytoplasm are not yet differentiated from one another—that Bacteria show, in fact, a primitive type of structure. Now it has never been proved—and indeed the evidence is against it—that Bacteria possess such a structure. It is obvious, therefore, that the assumption of a condition supposed to be primitive cannot be used as an argument in favour of the primitiveness of the group—as is sometimes done.

Having said so much with regard to the nucleus in general, I will pass on to an application of my reasoning to the experimental results.

I shall begin with a consideration of the Coccus forms of Bacteria. I have shown that certain Micrococci and Sarcinæ contain, in each cell, a single, centrally placed spherule. This body is a morphological feature common to every cell. When the cell divides, the spherule also divides—its division preceding that of the cell as a whole, and being characterised by the formation of a dumb-bell-shaped figure

during the process. There is therefore every reason to believe that the centrally placed body is a living constituent of the cell. It cannot be maintained that it is a non-living structure—for instance, a fat globule or metachromatic granule. Now on purely morphological grounds, on analogy with what is known of other cells, I think I am justified in calling this centrally placed body in cocci a nucleus. It corresponds as closely as could be desired with the structures which we are accustomed to call nuclei in other cells. If it is not a nucleus, then what is it? There is, I believe, only one possible answer to such a question—that it may be a structure, absent from other cells, which looks exactly like, and behaves exactly like, a nucleus, but is really not a nucleus. I think, therefore, that on morphological grounds it is completely justifiable to regard this body as a nucleus. Moreover, such a conclusion is considerably supported by the fact that the structure is stained red by Romanowski's method—the colour which is assumed by structures which are universally admitted to be nuclei.

The observations which I have made do not stand alone. They are supported by the quite independent observations of Nakanishi (1901) and Mencl (1910)¹—both experienced workers who employed reliable cytological technique. The organisms studied by Nakanishi, Mencl and myself, though all *Coccus* forms of Bacteria, are all different organisms, and the cytological methods used were different in each case. Both Nakanishi and Mencl, moreover, draw the same conclusion as I do—though not altogether from the same premisses. They both believe that the structures which they discovered are nuclei.

The contention of Meyer (1908), that the nuclei described by Nakanishi are really vacuoles, is hardly worth discussing;

¹ I should like to point out—though of course I do not claim priority in the discovery of nuclei in Cocci—that my observations were in no way influenced by the work of Nakanishi or Mencl. My own observations were made before I had seen Nakanishi's work, and two years before the publication of Mencl's paper.

for the same idea occurred to Nakanishi himself, and he brought forward good experimental evidence to show that this was not the case.

As a result of my researches I regard it therefore as certain that the Coccus forms of Bacteria contain a nucleus of the form which I have described in the earlier part of this paper.

And now let us consider the other Bacteria. I have pointed out already that I have investigated a large number of cocco-bacillar organisms which present every degree of form between typical Cocci on the one hand, and typical Bacilli on the other. With change in the external shape, the nucleus shows a corresponding modification. It becomes elongated with the elongation of the cell, and hence assumes the form of a filament. In round coccus forms, the nucleus is round. In slightly elongated cocci, the nucleus is in the form of a short rodlet, which may be curved or slightly bent. In still more elongated cocco-bacillar organisms, the nucleus may have the form of a zig-zag or spiral filament. These forms merge gradually into the forms of the characteristic spirogyra type.

I have not proved that the Cocci, spirogyra Bacilli and intermediate cocco-bacillar forms, which I have found living together, are genetically connected. A proof of this is immaterial for the present purposes. It suffices to know that all these forms occur. Morphologically considered, therefore, the spiral or zig-zag filament present in Bacilli of the spirogyra type is the equivalent of the spherical body which lies in the centre of the Coccus cells. Consequently, if it is agreed that the latter is a nucleus, it follows that the spiral filament of Bacilli of the spirogyra type is also a nucleus. This is a conclusion which is supported by the behaviour of the filament during cell-division and spore-formation, which I have described in detail in *B. spirogyra*. My study of this form indicates beyond a doubt that the filament is a living element of the cell, and not a metaplasmic structure.

A further point in support of the morphological equivalence of the spherical nuclei of Cocci and the filamentar nuclei of certain Bacilli is furnished by the observations recorded on p. 421. I have shown that the nucleus of certain Micrococci, when the cell is elongated during the process of cell-division, may be drawn out into a zig-zag or spiral filament. We see here directly, I believe, the way in which the filamentar nucleus of some Bacilli has been derived from the spherical nucleus of Micrococcus forms.

Again, staining reactions—so far as they go—support the interpretation of the filament in Bacilli of the spirogyra type as a nucleus.

At this point *Bacillus saccobranchi* must be considered. I have shown that this organism possesses at one stage in its life-history a nucleus of the characteristic spirogyra type—that is to say, a spiral or zig-zag filament which is the morphological equivalent of the nucleus of Coccus forms. Now this structure undergoes a remarkable transformation during the development of the organism. It becomes converted into the form which I have called the “irregular form”—assuming an appearance of an irregularly branching filament or network. This structure in turn breaks up to form a series of granules scattered diffusely through the whole cell—the “chromidial form.”¹ It follows, therefore, with absolute certainty, that if the spiral filament is a nucleus—as I have already shown is almost certainly the case—then the chromidial structures are also the morphological equivalent of a nucleus. They are developmental stages of the very same living constituent of the cell which is represented at other times by a spiral filament or irregularly branched filament or network. In *Bacillus saccobranchi*, therefore, there is every reason to believe that a nucleus in the form of scattered granules, or chromidia, exists at certain stages in the life-cycle.

¹ I have pointed out (p. 444) that it is possible that the changes in the nuclear structures may take place in the reverse order to that given above. It is immaterial to my argument in which direction the sequence of developmental changes takes place.

Again in this organism, staining results confirm the morphological interpretation.

Arguing now on analogy, it becomes highly probable that the scattered granules of Bacilli of the flexilis type—the chromidia, in other words—are of the same nature as the granules of *Bacillus saccobrauchi*. They are the only morphological elements distinguishable in the cells, and that they are living structures—not reserve material—appears to me quite certain from the part which they play during spore-formation. When it is further found that, in the course of spore-formation, the granules arrange themselves in the form of a spiral or zig-zag filament¹—like that of Bacilli of the spirogyra type—then the nuclear interpretation of the granules is not merely strengthened, but becomes almost a certainty. It appears to me that there is only one logical conclusion to be drawn from these facts—that the chromidia of Bacilli of the flexilis type represent the nucleus, being the equivalent (morphologically) of the spherical nucleus of Cocci and of the spiral filament of other Bacilli.

When we find that many smaller Bacilli show a structure which is essentially the same as that of the large Bacilli of the flexilis type, it is only natural to suppose that we see here, also, structures which are capable of a similar interpretation. The assumption is justified that the chromidia of small Bacilli constitute their nuclear apparatus.

In all these cases, moreover, staining reactions—so far as I have tried them—support the morphological interpretation.

If we now consider the structures which are present in the Bacilli of a modified flexilis form (from the newt and lizard—see p. 430) it becomes apparent that these structures also represent phases of the nuclear apparatus. The actual facts here are not so well established as in the forms which I have hitherto considered, but it is at least exceedingly probable that in these the nucleus exists, at some stages in the life-history, in the form of a few large globular masses.

¹ Discovered by Schaudinn (1902) in *Bacillus bütschlii*, and confirmed by me (1908) in the case of *B. flexilis*.

The aggregate of these masses in each cell is the morphological equivalent of the chromidia or the spiral filament.

Thus, we see here another modification of the nucleus which may exist in Bacteria.

I will now consider the spirillar forms which I have investigated. I have found that three different types of structure exist in these organisms. In one of these there is a minute spherical body present in each cell: it divides with a dumb-bell-shaped figure, its division preceding that of the cell (small *Spirillum* from *Stylopyga*). It is a living element—a morphological feature of each cell. In the second type, there is a filament of a zig-zag or spiral form, which also divides into two during cell-division (*Spirillum* from *Lacerta muralis*). Thirdly and lastly, there is a type of *Spirillum* whose characteristic morphological feature is a system of granules scattered through the cell (*Sp. monospora*, large *Spirillum* from *Stylopyga*). From a consideration of these spirillar forms alone we could, with considerable justification, reach the conclusion that these three different types of structure represent three different modifications of the nuclear apparatus—upon morphological grounds. When the analogy of these structures with the nuclei of *Cocci* and *Bacilli* is considered, however, it appears to me that only one logical deduction can be drawn, namely, that the single spherule, the spiral filament, and the chromidia of *Spirilla* are nuclei.

Staining, again, gives results consistent with this interpretation.

I believe my nuclear interpretation of the various structures discussed above is the only logical interpretation which can be given to the facts known to us at present. And of the accuracy of the facts which I have recorded, I have not the slightest doubt.¹

¹ Owing to the fugitive nature of the staining methods which I have frequently employed, it is now impossible to demonstrate many of my preparations satisfactorily. I have therefore at various times demonstrated my preparations to competent observers, in order that they

It remains now to consider how far these facts coincide with those recorded by others.

First of all, I would point out that my results are in agreement with those of Schaudinn (1902, 1903) and Guilliermond (1908)—both of whom made accurate cytological investigations of different organisms. Both these observers, however, examined Bacteria which possess a nucleus of the chromidial form; it is with my chromidial forms, therefore, that their results must be compared. Both Schaudinn and Guilliermond—though on different grounds—arrived at an interpretation similar to my own.

Quite recently, Guilliermond (1909) has recorded the existence of two species of *Bacillus* and a *Spirillum* which possess nuclear filaments like those which I have described in these forms. His observations appear to have been made quite independently of mine, and may therefore be taken as confirmatory.

I find it difficult to decide how far the results of Swellengrebel (1906, 1907, 1907A, 1909, 1909A) coincide with mine. He finds in *Bacilli* and *Spirilla* remarkable filamentar structures, usually in the form of an irregular or broken spiral. On account of the micro-chemical and staining reactions of these structures, he is led to interpret them as nuclei. They are not exactly like the filamentar structures which occur in *Bacilli* of the *spirogyra* type. In many cases they resemble certain of the nuclear modifications of *B. saccobranchi*. It seems to me possible that in some cases also the appearances are the result of imperfect fixation¹—the original spiral filament having been broken up in this process. Sometimes, also, the filaments may be really chromatin

could confirm my statements as to the existence of the structures which I have described—if their existence were called in question. Among those to whom I have shown one or other of my preparations may be mentioned Sir Ray Lankester, Prof. Adam Sedgwick, Prof. J. B. Farmer, and Prof. F. Vejdovský—all of whom have agreed with me as to the appearances presented.

¹ See footnote on p. 417.

granules connected by deeply stained cytoplasm—as maintained by Guilliermond (1908). As I have not myself made a study of the forms which Swellengrebel describes, and as his work has evidently been conducted with considerable care and thoroughness, I hesitate to make any more definite criticism of it at present.

The earlier observations of Bütschli (1890, 1896), Wahrlich (1890), Zettnow (1897), and others are in agreement with mine¹ if it be assumed—as appears highly probable—that they investigated only those forms of Bacteria which possess a chromidial nucleus. With Bütschli's interpretations, however, I cannot agree.

Nuclei in the form of a few small granules in each cell, described by Meyer (1897, 1899), and Preisz (1904), are probably of the same nature as chromidial nuclei, and the nuclei which occur in the Bacilli of modified flexilis type.

The facts and their interpretations, given by Nakanishi (1901) are—in many cases—closely parallel to my own. Nakanishi found filamentar nuclei in Bacilli (e.g. *B. anthracis*), and in *Spirilla* spherical and filamentar nuclei, which are very like the structures which I have myself observed in similar forms. After ably discussing his observations, Nakanishi arrived at an interpretation which agrees with mine.

How far the observations of Amato (1908) can be brought into line with my own I do not know. It is possible that the “nuclear” structures which he describes are really meta-chromatic granules—as suggested by Guilliermond (1910).

A point of considerable importance is to be found in the work of Schewiakoff (1893). In *Achromatium*, he found a number of minute chromatin granules scattered through the cytoplasm—in other words, he found a nucleus of the chromidial type. He observed that these granules undergo division—which is a further important piece of evidence

¹ So far as the actual morphology of some of the smaller Bacteria is concerned.

that they are living structures.¹ In Bacilli of the flexilis type the chromidia are too small for their division to be observed with accuracy, but I think they probably behave in much the same way as the larger chromidia of *Achromatium*.

The remarkable work of Mencl (1905) upon filamentous water Bacteria (*Cladothrix*, etc.) contains many observations which are in complete accord with mine. In the forms investigated—which are pleomorphic—he found nuclei of a spherical, filamentar, and chromidial form, with numerous intermediate forms. He was able to observe the division of these nuclei in the living cells—thus proving that they were really living structures, and not metachromatic or other non-living granules. He believes that the different nuclear forms occur, at different stages in the life-history, in the same organism. His results are therefore closely similar to mine.

The nuclear interpretation of the chromidial structures present in Bacteria—as upheld by Schaudinn, Guilliermond and myself—has been controverted by Růžicka (1909) on the grounds that the whole bacterial cell is itself the equivalent of a nucleus. Apart from the a priori absurdity of this view—which I have already pointed out above—I must emphasise the fact that the observations recorded in the present paper completely condemn such an interpretation. On the other hand, I believe the chromidial view is completely vindicated. The statement made by Ambrož, who follows Růžicka, that the chromidial view has been “reduced ad absurdum” by the latter, is therefore entirely erroneous.

The observations of Mitrophanow (1893)² seem to me to be capable of being brought into line with my own, when allowance is made for the difference in technique. I find it not always easy to comprehend Mitrophanow's meaning; his methods of fixation and staining also seem to leave much to

¹ Hinze (1903) made similar observations in the case of *Thiophysa*.

² This paper is an abstract only of a larger work in Russian. It is therefore possible that Mitrophanow's observations and views are more clearly given in the original—which is unfortunately inaccessible to me.

be desired. Nevertheless, he appears to have found organisms possessing nuclei in the form of chromidia, spiral filaments and spherical masses of chromatin. He also distinguishes between nuclei and "granulations," and points out the structural variability which the nucleus displays. On the whole, his observations—so far as I understand them—appear to be in agreement with mine.

Kunstler's (1887) observations upon the structure of *Spirilla* agree closely with what I have myself described in *Spirilla* with a chromidial type of nucleus. Also the chromatin structures described in the cholera *Vibrio* by Podwyssozki (1893) bear a strong resemblance in many cases to the nuclei which I have shown to occur in the small *Spirillum* from the gut of *Stylopyga orientalis*.

I believe the "chromatin" granules described in sulphur Bacteria by Hinze (1901, 1903) and Dangeard (1909) are—like Bütschli's findings in similar forms—to be interpreted as nuclei in a chromidial condition. The same interpretation will apply to the granules of *B. oxalaticus*, described by Migula (1894); and also to the iron- and phosphorus-containing granules found in *Beggiatoa* by Macallum (1899).¹

Rowland's (1899) results can easily be explained if it be supposed that the organisms which he studied possessed nuclei in the form of chromidia in addition to metachromatic bodies.

I think I may fairly claim, from what I have already pointed out in the preceding pages, that not only do my own observations furnish most conclusive evidence with regard to the nucleus in Bacteria, but that in almost every case in which careful investigation has been made by others, the results are not inconsistent with mine. In many cases they are, indeed, completely confirmatory. When good technique has been employed, and careful observations have been made,

¹ Certain points in connection with fixation are, moreover, not quite clear to me in the work of this author. It may also be pointed out that Macallum failed to find a nucleus in the yeasts—in which a typical vesicular nucleus certainly occurs.

I do not believe a single fact of any importance has been found which speaks against my results. In matters of interpretation, of course there is considerable difference of opinion already existing; but I am convinced that no interpretation, other than that which I have given, can be found which will fit all the facts known to us at present. How far such a conviction is justified further work alone can show.

So far I have considered only the Bacteria themselves, and I believe the evidence which I have given from this group alone is sufficient to establish the fact that Bacteria are nucleate cells. Considerable additional evidence may, however, be adduced from analogy with two other groups of organisms—the Protozoa and the Cyanophyceæ. In the Protozoa, a chromidial form of nucleus occurs in many different organisms, as a transient stage in the life-cycle. It may also occur as the normal vegetative condition. It is unnecessary to enter into this subject in detail here. The reader will find a condensed account of chromidia in a paper which I have previously published (see Dobell, 1909B). A nucleus in the form of irregular strands, networks, granules, etc., scattered through the cytoplasm, also occurs in Protozoa—especially in the Infusoria (cf. Dobell, 1909A).

In the Cyanophyceæ, analogous nuclear conditions probably obtain. It is impossible in the present paper to enter into a discussion of the vexed question of the nucleus in this group, but I should like to call attention to two recent contributions to the subject which have been made as a result of careful cytological work. I refer to the work of Gardner (1906) and Guilliermond (1907A). Gardner describes and figures nuclei in the form of networks, granules, and irregularly branched filaments. Guilliermond describes similar structures, and also nuclei in *Nostoc* which resemble those of *Micrococci*, and nuclear filaments, like those of *Bacillus spirogyra*, in *Rivularia*. If analogies were wanting for the structures which I believe to be nuclei in Bacteria, they could be found therefore without any great difficulty in the nuclei of other organisms.

Do Bacillar Forms with a Vesicular Nucleus exist?—I have already had occasion to note that Bacilli with a typical vesicular nucleus have never come under my observation. All the organisms which I found to be constituted in this manner have proved to be Fungi. Others, however, have described very definite instances in which vesicular nuclei occur, and the matter is of such moment that a brief discussion is here necessary.

In the accounts of the older observers, the observations are so incomplete, and the technique employed was so imperfect, that a discussion seems useless. This is not the case with some more recent work, however. I refer to the publications of the Bohemian investigators, Vejdovský, Mencl, and Raýman and Kruis.

There seems no doubt at all, from the very careful work of Vejdovský (1900, 1904) and Mencl (1907), that the organism which the former has named *Bacterium gammari* really possesses a vesicular nucleus, which divides mitotically.¹ The only point which requires to be settled is whether the organism really belongs to the Bacteria or not. Considerable discussion has already taken place regarding this. Some observers (e. g. Guilliermond, 1907, 1908, 1910) are inclined to regard it as a yeast-like fungus—not a *Bacterium* at all. The resemblance between certain yeast forms and this organism is certainly very striking (compare, for example, the figures of Mencl [1907]—figs. 4, 7, 10, etc. [pl. x]—with Wager's [1898] figures—figs. 45, 46, 47 [pl. xxx]—of *Saccharomyces pastorianus*). After my own experiences with *Bacterium*-like yeasts (see p. 455), I hesitate to express an opinion with regard to *B. gammari*. It is most important that further observations should be made upon this most interesting organism; and it is to be hoped that

¹ My friend Prof. Vejdovský has very kindly given me a preparation of this organism, so that I have been able to examine it myself. To my mind there can be no doubt as to the accuracy of the accounts which have been given of it.

before long someone to whom fresh material is accessible will reinvestigate the matter thoroughly.¹

Regarding Vejdovský's filamentar forms from *Bryodrilus*, my opinion is that they are really Fungi, similar to those which I have myself described. Guilliermond (1907, 1908) expresses a similar opinion—"nous sommes à peu près certains, après l'examen attentif de ses (i. e. Vejdovský's) préparations, qu'elle correspond à une moisissure. Nous n'avons trouvé en tous cas, dans cette espèce aucun des caractères des Bactéries" (1908, p. 37).

I think there can be no doubt that the Bacterium-like organisms, which I have already described (p. 455), are really Fungi, allied to the Saccharomycetes. The evidence for this is chiefly derived from two features of their life history—(1) the assumption of a characteristic yeast form, which reproduces by budding, (2) the formation of mycelium-like outgrowths.² Similar outgrowths have been observed in yeasts by other workers (cf. Janssens and Mertens, 1903). To this same group of organisms belong—I believe—two other forms which have recently been described, namely, *Kermincola*, a parasite of the body cavity of Coccid insects (Šulc, 1906), and *Bacillopsis stylopygæ*, from the cockroach (Petschenko, 1908). Both these forms appear to me to be indubitably Fungi, and not Bacteria (cf. also Vejdovský, 1906). The fact that my organisms, *Kermincola* and

¹ It is to be gathered from the discussion which has taken place regarding *B. gammari* (Guilliermond, 1907, 1910; Mencl, 1909) that Schaudinn—who saw Vejdovský's preparations at the Zoological Congress in Berne—at first expressed the opinion that the organism was a yeast. Later, however, he accepted Vejdovský's interpretation of it as a Bacterium—an opinion shared also by Schewiakoff.

² The formation of outgrowths is of course occasionally observable in true Bacteria (*Bacilli*, *Bacteria*, *Spirilla*). It is usually observed only in involution forms. Meyer (1901) interprets the outgrowths as a reminiscence of mycelium formation in the ancestors of Bacteria—believing them to be of fungal origin. For my own part, I do not believe that the Bacteria have anything whatever to do with the Fungi, and do not regard this as a correct interpretation of the phenomenon.

Bacillopsis, are all Fungi, indicates of course nothing regarding the existence or non-existence of true Bacteria with a typical vesicular nucleus.

The vesicular nuclei described in Bacteria by Mencl (1904, 1905, 1907) and Raýman and Kruis (1904) are, according to Guilliermond (1907, 1908, 1910), capable of a very different interpretation. According to him, the "nuclei" are really nothing more than various stages in the formation of transverse septa in dividing cells. This interpretation is vigorously attacked by Mencl (1909), who maintains that vesicular nuclei are actually present, and can be readily distinguished from the transverse septa. Mencl's figures certainly seem clear enough—as do the photographs of Raýman and Kruis. And I find it difficult to believe that so accurate and experienced an observer as Mencl could make such a mistake. Swellengrebel's (1907) results on *B. binucleatum* are also favourable to his interpretations. Yet a certain amount of uncertainty exists at present regarding these forms.

Finally I must say that it seems to me probable that Bacteria do exist which possess—at any rate during part of their life-cycle—nuclei of the vesicular form characteristic of the cells of "higher" animals and plants. It is certainly not legitimate to argue that because Bacteria have not been previously found which contain a vesicular nucleus, therefore that any form in which a vesicular nucleus can be demonstrated—e.g. *Bact. gammari*—does not belong to the Bacteria, but to the Fungi or some other group. This is simply begging the question. There is absolutely no reason, either from my own observations or from those of other workers, why typical vesicular nuclei should not occur in some Bacteria. The evidence, in fact, is in favour of the view that such nuclei do exist in certain Bacteria at certain stages in their lives.

Variability of the Nucleus at different Periods in the Life-cycle.—It will already be apparent to anyone who has read the preceding part of this paper, that the nucleus of any given bacterium is not necessarily constant in its form at

all stages in the life history. This point seems to me worth special attention.

In the case of *Bacillus saccobranchi*, I have pointed out that the nucleus may be in the form of a spiral filament, or in the form of chromidia, or in forms intermediate between these and characterised by having an appearance of irregular strands, granules or networks of chromatin. There can be no doubt that, in this *Bacillus* at least, the nucleus has a variable structure. There is, however, no evidence to show what relations these various nuclear modifications bear to the life-cycle as a whole. All that can be said at present is that these different nuclear forms exist.

When we turn to the *Bacilli* of the *flexilis* type, however, we have exact knowledge of the relations between the nuclear modifications and the phases in the life-cycle. From Schaudinn's (1902) study of *B. bütschlii* and my own researches on *B. flexilis* and allied forms it can be definitely stated that the chromidial stage represents the normal vegetative condition of the nucleus, existing throughout the greater part of life. A nucleus in the form of a spiral filament occurs as a transient stage connected with, and immediately preceding, spore-formation. In the spore itself a third nuclear modification is seen. The chromatin is in the form of a densely aggregated mass, which constitutes the chief part of the living substance of the spore. From this aggregated mass the chromidial condition is again assumed in the process of germination from the spore.

In the *Bacilli* which I have termed those of a "modified *flexilis* form," these three nuclear conditions are encountered in a modified form, but their relation to the phases of the life-cycle has not been determined.

In *Spirillum monospora* (Dobell, 1908), *Bacillus spononema* (Schaudinn, 1903) and many other *Bacilli* (Guilliermond, 1908) only two modifications of the nucleus have been established. During the vegetative condition the nucleus is in the form of chromidia. It then assumes the form of an aggregated mass, which enters into the formation

of the spore. These two different nuclear conditions therefore coincide very definitely with two different phases of the life-cycle.

In *Bacillus spirogyra* and allied organisms—as I have shown (1909)—two nuclear conditions are also found.¹ In the ordinary vegetative part of the life-history the nucleus is in the form of a filament. A part of this gives rise to a large, aggregated spherical mass of chromatin which enters into the spore. Here, again, the nuclear changes are correlated with definite stages in the life-history. I have not studied the young Bacilli which emerge from the spores in any organism of the *spirogyra* type. I cannot therefore state with certainty that the observed nuclear changes are the only ones which exist. On analogy with *B. saccobranchi*, it is quite possible that a chromidial condition of the nucleus occurs in Bacteria of this sort.

I have shown that three different nuclear conditions exist in three different species of *Spirilla* which I have studied. If one can argue on analogy in this case, it appears not improbable that these nuclear conditions are temporary, and that other phases in the nuclear structure exist in these organisms also. It is quite possible, for example, that the nuclear filament in the *Spirillum* from the intestine of *Lacerta muralis* may at other stages in the life-cycle—as in *Bacillus saccobranchi*—become modified into the chromidial form of nucleus which exists in such an organism as *Sp. monospora*.

My own belief is that the nucleus in Bacteria may display not one, but many forms during the whole life-cycle. Many of the nuclear structures which have been shown to exist in these organisms should, I think, be regarded as temporary stages rather than as permanent conditions. The different results which have been reached

¹ It may be emphasised also that the spiral filament itself in Bacteria of this type shows a wide latitude of variation in form. Whether these variations are correlated with special stages in the life-cycle is as yet unknown.

by different workers when working, apparently, upon the same species, may to some extent find an explanation in this circumstance.

I would call attention to the fact that Mencl¹—whose studies have been carried on with quite different Bacteria from those which I have investigated—has arrived at a similar conclusion. Many times Mencl has emphasised this point—a point which is, I believe, of fundamental importance for reaching a correct interpretation of the Bacteria. I am rejoiced that in this we are both agreed.

Pleomorphism.—Though I have no conclusive evidence to add to what has already been contributed to the hypothesis of the pleomorphism of Bacteria, nevertheless, I must point out that many of the facts recorded in the earlier part of this paper are consistent with such a view.

Whilst investigating the *Micrococcus*, *Cocco-bacillus* and *Bacillus* forms which I found in the gut of the lizard, I was often impressed by the apparent genetic relations existing between them. The same was the case with many of the different bacillar forms which I found in the blood of *Saccobranchus*. I have already pointed this out in previous pages, and although a direct proof of such genetic continuity is wanting, my observations are completely in accord with such an interpretation. This appears to me, in fact, the most probable hypothesis at present: otherwise it would be necessary to assume the existence of an almost inconceivably large number of species to account for the number of intermediate forms which occur.

For my own part, I believe—although this is a view which is not held by the majority of “bacteriologists”—that the greater number of Bacteria are pleomorphic. That pleomorphism does exist in many Bacteria, I think there can be no longer any doubt. Since the early work of Ray Lankester, Cienkowski, Zopf, Metchnikoff and others, an immense mass of evidence has been brought forward in favour of such a view. It is outside the limits of the present paper to enter

¹ See especially his studies on water Bacteria (Mencl, 1905).

into a discussion of this matter, but I should like to call attention to the exhaustive—but almost completely ignored—work of Billet (1890), and the remarkable researches of Mencl (1905) in this connection. Here will be found an immense collection of facts bearing upon the matter.

It appears to me probable that—just as in the case of their nuclei—the majority of Bacteria may possess a wide range of variation in their outward form at different stages in their life-histories. The matter can be decided, however, by further research only: but it offers a vast field for future investigation—investigation which is not only of a most fascinating nature, but of which the results also will be of the greatest biological interest.

Do Enuceate Bacteria Exist?—I wish to say a few words here about the belief which is often held, that the Bacteria are a group of organisms which possess no structure homologous with the nucleus present in the cells of other protists, animals or plants.

From a survey of the work which has been done upon the cytology of the Bacteria, I think it may be stated with absolute certainty that not a single bacterial species has been proved to be devoid of a nucleus. I do not say that a nucleus has been proved to be present in every bacterial species: but I do maintain that a nucleus has been demonstrated in a large number of species of Bacteria. The probability is, therefore, that all Bacteria are nucleate cells. That enuceate Bacteria may exist, is, of course, a possibility which cannot be denied; but at present there is absolutely not a vestige of evidence in favour of such a view.

I should like also to draw attention to a sort of statement about Bacteria which may be very frequently encountered in biological writings. The following quotation will serve as an instance of the sort of thing I mean: “It may be pointed out that it is in these low forms of life that we must look for a key to the secret of the origin of the cell nucleus, as well as for data to determine the morphological character of the

primal life organism" (Macallum, 1899, p. 439). This is one case in which this idea is definitely stated, but dozens of other passages in the works of other writers can easily be found in which a similar view is either formulated or tacitly assumed.

In statements of this sort two assumptions are made: first, that Bacteria are more simply organised than other living beings; secondly, that the more simply organised beings are phylogenetically the more primitive. There is no real justification for either of these assumptions. By calling Bacteria "low forms of life," it is easy enough to arrive at the conclusion that they occupy a position near the bottom of the phylogenetic tree. But this is nothing more than a *petitio principii*—a using of the conclusion at which it is desired to arrive as evidence for that conclusion. It is, of course, open to anybody to make the assumption that the Bacteria are like the most primitive forms of life; but the fact should not be lost sight of that this is at present an assumption, and nothing more.

"Fusiform Bacteria."—All the so-called "fusiform Bacteria" which I have examined possess a distinct nucleus, usually in the form of a spherical mass of chromatin—one in each cell. This nucleus divides previous to the division of the cytoplasm.

Nuclei, which divide by amitosis, were originally described in the fusiform organism ("Bacillus fusiformis") which occurs in the human mouth, by Mühlens and Hartmann (1906). This—so far as I am aware—was the first record of nuclei in these organisms. A detailed description of the nucleus was not given, and no figures were published.

Quite recently, Hoelling (1910) has given a detailed account of a fusiform organism—which he names *Fusiformis termitidis*¹—which occurs in the gut of termites (locality and species not stated). He also describes and

¹ Presumably a mistake for *termitis*. Hoelling proposes for all the fusiform organisms the generic name *Fusiformis* in place of the obviously inapplicable name *Bacillus*.

figures the fusiform organism from the human mouth, a form from fresh water, and a form from the cæcum of a mouse. In all these, he finds nuclei which are essentially the same as those which I have found in the various forms described in the preceding pages.

Hoelling describes the formation of long, multinuclear filaments by these organisms. He regards this as a degeneration phenomenon. The occurrence of these filamentar (unsegmented) forms lends, I think, some support to the view, which I have already expressed (p. 452), that the "fusiform Bacteria" are really Fungi.¹ At present there is no conclusive proof that this is so; but it should be noted also that there is no proof that these protists are Bacteria.

Whatever be the systematic position of the "fusiform Bacteria," I think there can be no longer any doubt that they possess a characteristic nucleus, in the form usually of a minute sphere or granule—one in each cell—which divides by a simple process of amitosis.

Affinities of the Bacteria.—This is not the place to discuss the affinities of the Bacteria in detail. Yet I believe we have now arrived at the beginnings of a correct interpretation of the structure and life-history of this group, so that a discussion of their affinities would be more profitable now than it would have been a few years ago.

Three chief views regarding the affinities of the Bacteria have been advanced: namely, that they are allied to the Fungi, to the Cyanophyceæ, or to the flagellate Protozoa. I have previously expressed the opinion that the Bacteria do not show affinities with the Fungi. The cytological studies recorded in this paper confirm this view completely. I believe there is not a particle of evidence to support the hypothesis that the Bacteria and Fungi are connected. The

¹ I would call attention to the resemblance which these organisms bear to a fungus described by Šulc (1910) from the body-cavity of *Chermes strobilobius*. This fungus—probably a yeast—which Šulc calls *Schizosaccharomyces chermetis strobilobii*, has a "caraway-seed shape," and the figures of it (fig. xv) certainly show a strong similarity to many "fusiform Bacteria" which I have observed.

name "Schizomycetes"—or "Spaltpilze"—is a complete misnomer. Similarly, with regard to the Protozoa, I see no real evidence at all which indicates that affinities exist between this group and the Bacteria. There is no real similarity between them.

There is, perhaps, rather more evidence of the affinities of the Bacteria with the Cyanophyceæ. Nuclear resemblances between the two groups certainly do exist, but on the other hand there are many important differences. The evidence is certainly very far from conclusive.¹

I believe that at present there is no clear evidence of the affinity of the Bacteria with any other group of organisms. For the present they must be regarded as a group of Protista which stands quite apart.

I believe, further, that amongst the Bacteria a number of forms are included which do not really belong—that the group Bacteria, as at present constituted, comprises a very heterogeneous assemblage of forms.

Similar views to these have already been expressed by Mencl (1907) and Guilliermond (1907), when considering the facts which were then known. I have myself also expressed the same views on a previous occasion, and I believe that they are now completely justified.

CONCLUSIONS.

I think, from the facts which have been given and analysed in the foregoing pages, the following chief conclusions are justified:

All Bacteria which have been adequately investigated are—like all other Protista—nucleate cells.

¹ I should like to point out here that the cytology of the Cyanophyceæ and sulphur Bacteria does not furnish us with anything more than analogical evidence regarding the structure of the smaller Bacteria (i.e. Bacilli, Spirilla, etc.). I believe many sulphur Bacteria are probably only distantly related to the majority of the smaller forms, and there is no clear evidence that the Cyanophyceæ have anything to do with them.

The form of the nucleus is variable, not only in different Bacteria, but also at different periods in the life-cycle of the same species.

The nucleus may be in the form of a discrete system of granules (chromidia); in the form of a filament of variable configuration; in the form of one or more relatively large aggregated masses of nuclear substance; in the form of a system of irregularly branched or bent short strands, rods, or networks; and probably also in the vesicular form characteristic of the nuclei of many animals, plants, and protists.

There is no evidence that enucleate Bacteria exist.

Finally, in addition to these purely morphological conclusions concerning the nucleus, I think another conclusion is rendered highly probable:

The Bacteria are in no way a group of simple organisms, but rather a group displaying a high degree of morphological differentiation coupled in many cases with a life-cycle of considerable complexity.

APPENDIX.

On the Alleged Autogamy of Bacteria.—In two earlier papers I have discussed the so-called "autogamy" of the disporic Bacteria in some detail. The actual facts regarding this process were recorded by Schaudinn (1902, 1903), and myself (1908). In a second paper (1909) I brought forward strong evidence to show that the so-called "autogamy" of Bacteria is not a sexual process at all, but has a much simpler explanation. It seems necessary, however, to refer to this matter once more, owing to the recent appearance of a very misleading article by Dr. Růžicka.¹

After mentioning Schaudinn's observations, the author

¹ V. Růžicka, "Ueber die experimentelle Autogamie der Bakterien," 'Arch. Entw.-Mech.,' Bd. xxx, Festschrift f. W. Roux, Teil. 1, p. 443, 1910.

proceeds (p. 443)—“Eine Bestätigung dieser Befunde ist bis jetzt nur von Dobell¹ eingelaufen, und zwar insofern, als er bei *Bac. flexilis* zum Teil ähnliche Bilder vorgefunden hat. Er bestreitet indes die Deutungen Schaudinn's, weil er die von diesem Forscher geschilderten und seine Deutung eigentlich bedingenden Plasmaströmungen nicht beobachten konnte.” And further (p. 445)—“Vielleicht ist der negative Befund Dobells damit zu erklären, dass er ohne vitale Färbung untersucht hat.”

Now if Dr. Růžicka had taken the trouble to read my first paper, he would have found that my results were essentially the same as Schaudinn's; that I accepted then Schaudinn's interpretation that the phenomenon was probably a sexual one; and that I did employ *intra-vitam* staining methods, and was unable to convince myself that streaming of the granules occurred in the living organisms on account of their motility.² It is in my second paper (1909)—which Dr. Růžicka completely ignores—that I have given what is, I believe, a definite proof that no sexual process occurs during spore-formation in the disporic Bacteria. There is very strong evidence that the “sexual” phenomena are due simply to a suppressed cell-division. I should like to point out that Dr. Růžicka's own observations, recorded in this paper, support my view. The “sexual act” which he invoked by growing his Bacteria upon abnormal and innutritious media may be quite simply explained by the fact—which he himself records—that the organisms divided imperfectly and then proceeded to form spores without developing typical colonies. Dr. Růžicka's incomplete observations and figures of the formation of disporic individuals add nothing to the facts observed and recorded by Schaudinn and myself. Disporic, or coupled monosporic, individuals have already been observed in many different Bacteria by many workers.

¹ Here follows a reference to my 1908 paper.

² But I have never used this as an argument against the sexual interpretation of the phenomenon. That some of the granules do pass to the ends of the cells I have, I think, helped to prove.

As Dr. Ružička has added no new facts regarding the method of spore-formation in these alleged autogamic forms, it is only his interpretation of the phenomena that I can dispute. But as I have already given my arguments against the view which he adopts, I can suffice with referring him to my second (1909) paper.

One or two other points appear worthy of mention. Dr. Ružička says (p. 443)—“Die Bakterien, bei welchen man bislang geschlechtliche Vorgänge festgestellt hat, waren als zufällige Gäste oder Parasiten anderer Organismen vorgefunden worden, ohne weiter und reingezüchtet worden zu sein. Das hätte Skeptikern als Punktum fixum dienen können, um ihre Zweifel an der Reihenfolge der Phasen des besprochenen Vorganges und an seiner Zugehörigkeit zu den sexuellen Erscheinungen weiter zu spinnen.” Now it may be noted, in the first place, that *B. sporonema* is a free-living form; and secondly, that phenomena continuously observed in organisms in their natural environment are of more, or at least equal, importance to those observed under abnormal conditions, in which many of the factors are unknown.

Dr. Ružička concludes his paper by stating (p. 458) that the facts of the alleged “autogamic” process are in accord with his interpretation of Bacteria as nuclei. It seems scarcely necessary to point out that such an opinion could be arrived at only by a complete confusion of ideas coupled with a misuse of words.

It seems to me unnecessary to discuss the speculative part of Dr. Ružička’s paper, since it is based—I believe—upon his misinterpretation of the facts. Until it can be proven that sexual phenomena occur, it is useless to construct further speculations upon the mere assumption. And at present I believe all the evidence speaks very definitely against the view that a sexual process occurs at any stage in the life-history of Bacteria.

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY,
LONDON. October 5th, 1910.

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EXPLANATION OF PLATES 16-19,

Illustrating Mr. C. Clifford Dobell's "Contributions to the Cytology of the Bacteria."

[All the figures are drawn from fixed and stained organisms under a Zeiss 2 mm. apochromatic oil-immersion (aperture 1.40) with the aid of compensating oculars 6, 8, 12, and 18. The magnification of all figures is the same, and is approximately 2000 diameters. The figures are in no way diagrammatic. They are accurate representations of the actual appearances observed.]

PLATE 16.

Figs. 1-20 are from wet film preparations of the blood of *Saccobranchus fossilis*, fixed with osmic vapour followed by absolute alcohol, and stained with Giemsa's stain.

Figs. 1-10, 12 and 14.—*Bacillus saccobranchi* n. sp.

Fig. 1.—Short Bacillus, with nucleus in the form of a slightly bent and varicose filament.

Fig. 2.—Two Bacilli with nuclei in the form of twisted zig-zag or spiral filaments.

Fig. 3.—Bacillus with nucleus in the form of fragments of a zig-zag filament.

Fig. 4.—Long Bacillus containing a long varicose zig-zag or spiral nuclear filament. (Nucleus of *spirogyra* type.)

Fig. 5.—Large Bacillus in which the nucleus is in the form of granules and irregular short, curved, bent, and branched filaments. (Irregular type of nucleus.)

Fig. 6.—Large Bacillus with nucleus partly in the form of an irregular zig-zag or spiral filament and partly in the form of irregular branched masses—connected with one another.

Fig. 7.—Two short Bacilli with irregular nuclei.

Fig. 8.—Large, slightly curved Bacillus, with nucleus in the form of a broken varicose zig-zag or spiral filament.

Fig. 9.—Bacillus with nucleus of irregular type. A part of the nucleus shows a very distinct reticular arrangement.

Fig. 10.—Bacillus with nucleus of chromidial type.

Fig. 12.—Bacillus with nucleus in the form of a thick varicose filament.

Fig. 14.—Bacillus containing a large and almost fully formed spore. Residual chromatin is seen lying in the cytoplasm outside the spore.

Figs. 11, 13, 15–20.—Smaller Bacteria, found in company with *B. saecobranchi*.

Fig. 11.—Chain of three individuals with nuclei of spirogyra type.

Fig. 13.—Short, thick Bacillus with nucleus in the form of short, thick, irregular rodlet, pointed at one end.

Fig. 15.—Bacillus with nucleus in the form of a varicose spiral or zig-zag filament.

Fig. 16.—Bacillus with nucleus in irregular masses.

Fig. 17.—Two Bacilli with nuclei in the form of short, irregular rodlets.

Fig. 18.—A similar organism, with nucleus undergoing division.

Fig. 19.—Three very small Bacilli with nuclei of spirogyra type.

Fig. 20.—Group of five small Bacilli with spirogyra type of nucleus.

Figs. 21–23.—Bacilli of flexilis type, from large intestine of *Mabuia carinata*. (Osmic acid 1 per cent., drop method; Leishman's stain.)

Fig. 21.—Ordinary individual, with chromidial nucleus.

Fig. 22.—Similar individual. The chromidia are smaller and more numerous than in the preceding.

Fig. 23.—Spore-bearing (disporic) individual. The spore-coats are stained blue, and a certain amount of residual chromatin material is seen in the cytoplasm.

Figs. 24–29.—*Sarcina* from large intestine of *Bufo melanostictus*. (Osmic acid 1 per cent., drop method; Giemsa's stain.)

Fig. 24.—Organism in two-cell stage. Small spherical nuclei (red) in each cell. The upper cell contains a refractile granule (white).

Fig. 25.—Four-cell stage. In the upper left-hand cell the nucleus has divided into two. The three other cells each contain a single nucleus. In each cell a single refractile granule is present.

Fig. 26.—Three-cell stage. The nucleus in the right-hand cell has divided into two, but fission of the cytoplasm has not yet occurred. The upper and lower left-hand cells contain dividing nuclei, of characteristic dumb-bell form. A single large refractile granule is present in the right-hand cell; the left-hand cells each contain a single and smaller refractile granule.

Fig. 27.—Three-cell stage. The left-hand cells each contain a single nucleus and a single refractile granule. The right-hand cell shows a nucleus undergoing division.

Fig. 28.—Four-cell stage. Each cell contains a nucleus and a refractile granule.

Fig. 29.—Four-cell stage. The lower right-hand cell contains a single resting nucleus. The three other cells contain dividing nuclei. The upper left-hand cell contains two small refractile granules—the three others one.

Figs. 30-40.—Large Bacilli of spirogyra type from large intestine of *Mabuia carinata*. (1 per cent. osmic acid, drop method; Leishman's stain.)

Fig. 30.—Long Bacillus, with nucleus in the form of a spiral or zig-zag filament.

Fig. 31.—A similar form to the preceding, but with a longer and more twisted nucleus.

Fig. 32.—Similar form, showing two loops in the nuclear filament.

Fig. 33.—Similar organism just completing division into two.

Fig. 34.—Shorter individual, with typical spirogyra type of nucleus.

Fig. 35.—Similar form with nucleus in the form of a straighter, varicose filament.

Fig. 36.—Short Bacillus, with nucleus clearly seen to be composed of chromatin granules, aggregated to form a spiral or zig-zag filament.

Fig. 37.—Bacillus containing finely granular cytoplasm and six large nuclear granules. Possibly a degenerate or developmental form of the preceding organisms.

Fig. 38.—Bacillus with nucleus in the form of a broken spiral filament. Degenerate or developmental form?

Fig. 39.—Spore-bearing individual of spirogyra type.

Fig. 40.—Degeneration form.

Fig. 41.—Long, slender Bacillus from large intestine of *Mabuia carinata*. Nucleus of chromidial type. (1 per cent. osmic acid, drop method; Leishman's stain.)

Figs. 42-44.—Micrococci from large intestine of *Mabuia carinata*. (1 per cent. osmic acid, drop method; Leishman's stain.)

Fig. 42.—Diplococcus form—each cell with a single nucleus.

Fig. 43.—Coccus with nucleus in the form of a short zig-zag filament.

Fig. 44.—Typical Micrococcus, with single nuclear granule.

PLATE 17.

Figs. 45-60.—Various Bacteria from large intestine of *Lacerta muralis*. (1 per cent. osmic acid, drop method; Giemsa's stain.)

Fig. 45.—Group of five Micrococci of different sizes. The nucleus is very obvious in each cell.

Figs. 46-49.—Four successive stages in the division of a *Micrococcus* similar to those seen in the preceding figure. Note the characteristic dumb-bell figure assumed by the nucleus during division. (Compare with figs. 24-29, Plate 16.)

Fig. 50.—Three coccobacillar forms. The nucleus is in the form of a filament, bent in a more or less spiral or zig-zag manner.

Fig. 51.—Group of short Bacilli, with nuclei of characteristic spirogyra form.

Fig. 52.—Chain of Cocci in which division is taking place. Note the zig-zag or spiral form assumed by some of the dumb-bell figures of the dividing nuclei. (This figure is drawn on a very slightly larger scale than the others.)

Fig. 53.—Small Bacillus with nucleus in the form of a short rod.

Fig. 54.—Similar organism to the preceding, in the act of dividing into two. The nuclear rod is completely divided into two parts.

Figs. 55-60.—Large Bacilli of spirogyra type.

Figs. 55-57.—Three individuals, showing three different arrangements of the nuclear filament.

Fig. 58.—Spore-bearing individual of same species. (A single terminal spore is formed—as in *B. spirogyra*.)

Fig. 59.—Dividing individual. The two halves of the nuclear filament are still joined by a very slender chromatin thread.

Fig. 60.—Another dividing individual. The nucleus—which is very much contorted (cf. fig. 59)—has already separated into two parts.

Fig. 61.—Three short Bacilli, with nuclei of spirogyra type, from large intestine of *Bufo melanostictus*. (1 per cent. osmic acid, drop method; Giemsa's stain.)

Fig. 62.—Long, curved Bacillus, with irregular varicose nuclear filament. Large intestine of *Bufo melanostictus*. (1 per cent. osmic acid, drop method; Giemsa's stain.)

Fig. 63.—Group of Bacilli from large intestine of *Lacerta muralis*. The nucleus is in the form of an irregular knotted rodlet. The lowest organism is undergoing division—the nucleus being already divided into two. (1 per cent. osmic acid, drop method; Giemsa's stain.)

Figs. 64, 65.—Slender Bacilli from large intestine of *Lacerta muralis*. Nucleus in the form of chromidia. (1 per cent. osmic acid, drop method; Giemsa's stain.)

Figs. 66, 67.—“Fusiform Bacteria” from large intestine of *Triton vulgaris*. The upper individual of the pair shown in fig. 66 is dividing. Note the nuclei—in the form of double granules. Fig. 67 is

a double form, with one nucleus (upper) appearing as a solid mass of chromatin, the other (lower) as a vesicular structure with a large karyosome. (40 per cent. formol, drop method, absolute alcohol; Giemsa's stain.)

Fig. 68.—“Fusiform Bacterium” (double form) from large intestine of *Lacerta muralis*. Each individual possesses a small spherical nucleus. (Dry film, absolute alcohol; Giemsa's stain.)

Figs. 69-78.—Bacilli from large intestine of *Mabuia carinata*.
(Osmic acid 1 per cent., drop method; Leishman's stain.)

Fig. 69.—Long slender Bacillus with nucleus of spirogyra type.

Figs. 70-73.—Smaller Bacilli of spirogyra type. Diverse forms and sizes.

Fig. 74.—Very small Bacillus with thick nuclear filament of spirogyra type.

Fig. 75.—A Bacillus, similar to that shown in fig. 72, undergoing fission.

Fig. 76.—Slender Bacillus with nucleus of chromidial type.

Fig. 77.—Slender Bacillus with large central nuclear mass (possibly a plasmolysed form?).

Fig. 78.—Bacillus with nucleus in the form of a short, irregular, and slightly bent rod-like filament.

Figs. 79-82.—Bacilli of modified *flexilis* form from large intestine of *Triton vulgaris*. (40 per cent. formol, absolute alcohol; Giemsa's stain.)

Fig. 79.—Bacillus of *flexilis* form, with chromidial nucleus.

Fig. 80.—Individual with finely granular, darkly staining cytoplasm, and large nucleus-like masses of chromatin, eight in number.

Fig. 81.—Long, sporulating individual, bearing a large chromatin spore-rudiment at each end. (The organism is normally disporic, like *B. flexilis*.)

Fig. 82.—Long individual similar to that shown in fig. 80. The cytoplasm is alveolar, and the chromatin in the form of large nucleus-like masses.

Figs. 83, 84.—Long and short individuals respectively of Bacillus of *flexilis* type from large intestine of *Lacerta muralis*. Nuclei of chromidial form. (Osmic vapour [wet film], absolute alcohol; Giemsa's stain.)

Figs. 85-90.—Bacilli of modified flexilis form from large intestine of *Lacerta muralis*. (Dry film, absolute alcohol; Giemsa's stain.)

Fig. 85.—Long individual, containing three large nucleus-like masses of chromatin.

Fig. 86.—Short individual, with curious arrangement of the chromatin.

Fig. 87.—Large individual, somewhat similar to the preceding.

Fig. 88.—Short individual with a single, centrally placed, nucleus-like body.

Fig. 89.—Long, sinuous individual, with chromidial nucleus of characteristic flexilis type. Many of the chromidia are conspicuous by their large size.

Fig. 90.—Long, straight Bacillus, with chromatin mainly in two large masses. Possibly a plasmolysed or degenerate form.

PLATE 18.

[All the figures, unless otherwise stated, are drawn from wet film preparations fixed with Schaudinn's sublimate-alcohol, and stained with Heidenhain's iron-hæmatoxylin.]

Figs. 91-95.—Bacilli of spirogyra form from large intestine of *Lacerta muralis*. Various forms of nuclear filament are shown. The organism depicted in fig. 92 is dividing.

Figs. 96-108.—Large *Spirilla* from large intestine of *Lacerta muralis*.

Fig. 96.—Short individual, showing large cytoplasmic alveoli and nucleus in the form of a short rod-like filament at one end of the cell.

Figs. 97, 98.—Dividing forms. Note nuclear filaments.

Fig. 99.—Short individual, with nucleus in the form of a short and somewhat zig-zag or spiral filament.

Fig. 100.—Short individual with long, varicose nuclear filament.

Fig. 101.—Longer individual, with long spiral or zig-zag nuclear filament.

Fig. 102.—Very long individual, with long nuclear filament similar to that of the preceding.

Figs. 103-105.—Shortest individuals (*Vibrio* form) with nuclear filaments.

Fig. 106.—A form similar to fig. 99, but with a longer nuclear filament.

Fig. 107.—Longer organism, with short, centrally placed nuclear filament.

Fig. 108.—Long individual, in which the nuclear filament has divided into two preparatory to cell division.

Fig. 109.—Group of five small Bacilli with darkly staining nucleus-like bodies—similar to those shown in fig. 53 (Pl. 17). (These "nuclei" are possibly spore-rudiments.) From large intestine of *Lacerta muralis*.

Figs. 110–112.—Large Spirilla from the hind gut of *Stylopyga orientalis*. The cytoplasm has an alveolar structure, and the nucleus is of the chromidial type.

Figs. 113 and 114.—"Fusiform Bacteria" from the large intestine of *Lacerta muralis*. Each cell shows a single spherical nucleus.

Fig. 115.—"Fusiform Bacterium," of double form, from large intestine of *Bufo vulgaris*. (Fixation: corrosive sublimate and acetic acid.)

Fig. 116.—"Fusiform Bacterium," of double form, from large intestine of *Stylopyga orientalis*. The lower nucleus in dividing.

Figs. 117 and 118.—*Bacillus spirogyra* from large intestine of *Bufo vulgaris*. Note the nuclear filaments. (Fixation: corrosive sublimate and acetic acid.)

Figs. 119 and 120.—*Bacillus flexilis* from large intestine of *Bufo vulgaris*. Note the alveolar structure of the cytoplasm (rather indistinct) and the nucleus in the form of chromidia. The organism shown in fig. 120 is undergoing division.

Figs. 121–132.—Small Spirilla from the hind gut of *Stylopyga orientalis*.

Fig. 121.—Small *Vibrio* form with terminal nucleus.

Figs. 122–124.—Small individuals with centrally situate nuclei.

Figs. 125 and 126.—Longer individuals with dividing nuclei. Note the characteristic dumb-bell figure which the nucleus assumes. (Compare with *Micrococci* and *Sarcina*.)

Fig. 127.—Individual in which nucleus has divided into two, though fission of the cytoplasm has not yet occurred.

Figs. 128 and 129.—Dividing organisms.

Fig. 130.—Long individual with centrally placed, undivided nucleus.

Fig. 131.—Small *Vibrio* form. Central nucleus.

Fig. 132.—Smallest *Vibrio* form. Central nucleus in the form of a minute chromatin granule.

Figs. 133 and 134.—Bacilli of *flexilis* type from large intestine of *Lacerta muralis*. Chromidial nuclei. Fig. 134 shows a dividing individual. Same forms as those shown in figs. 83, 84 (Plate 17).

Figs. 135 and 136.—Bacterium-like organism from large intestine of *Boa constrictor*. (Wet film, absolute alcohol; Delafield's hæmatoxylin.)

Fig. 135.—Bacterioid forms—a chain of four.

Fig. 136.—Zymoid forms. A free single individual and another, which has formed a bud.

PLATE 19.

[All figures are of the nucleated Bacterium-like organism (or its developmental forms) found in the large intestine of *Boa constrictor*. (Dry film preparations: fixed absolute alcohol, stained Giemsa.)]

Figs. 137-141, 144.—Bacterioid forms.

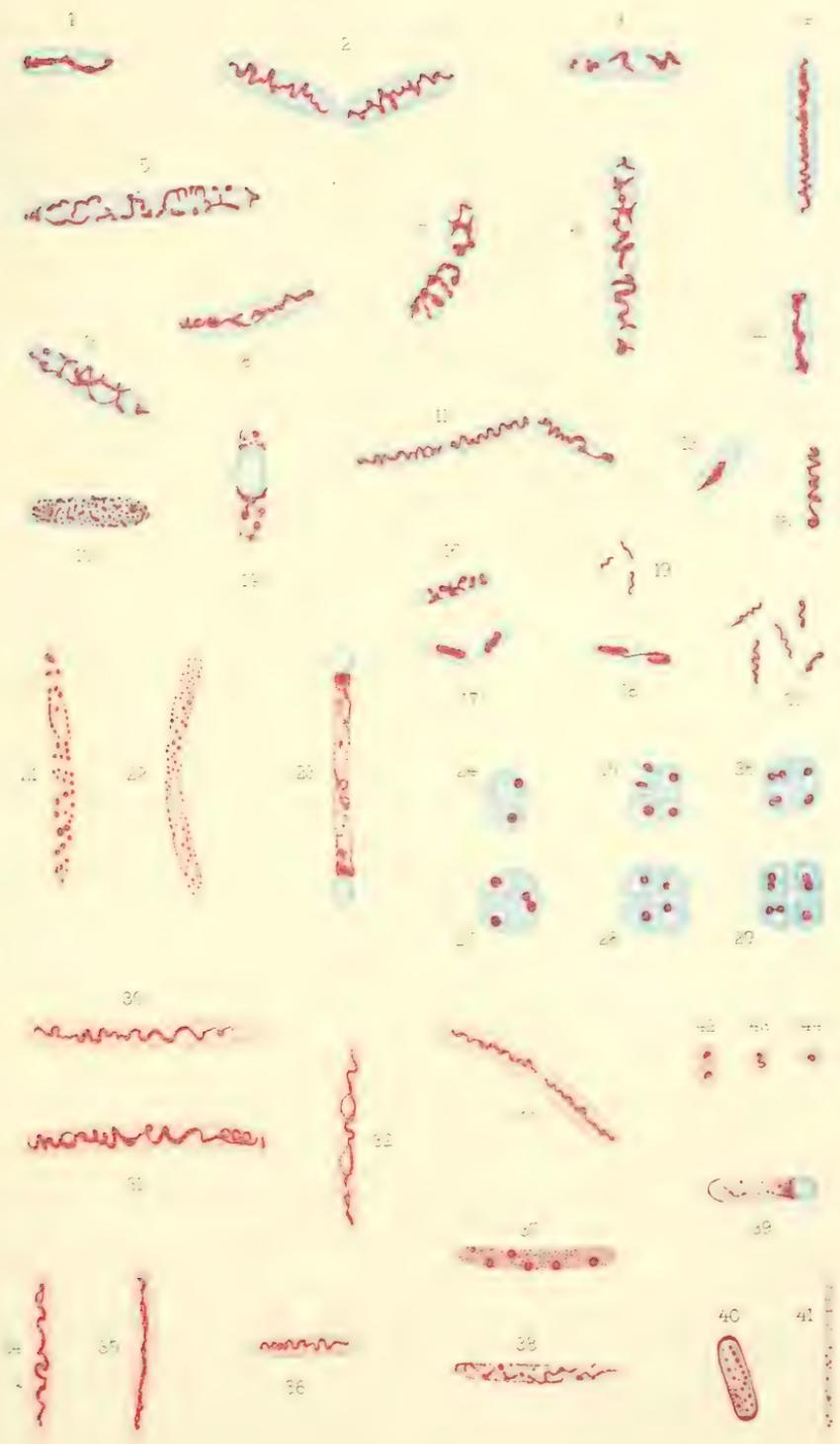
Fig. 142.—Form intermediate between bacterioid and zymoid form.

Figs. 143 and 145.—Zymoid forms.

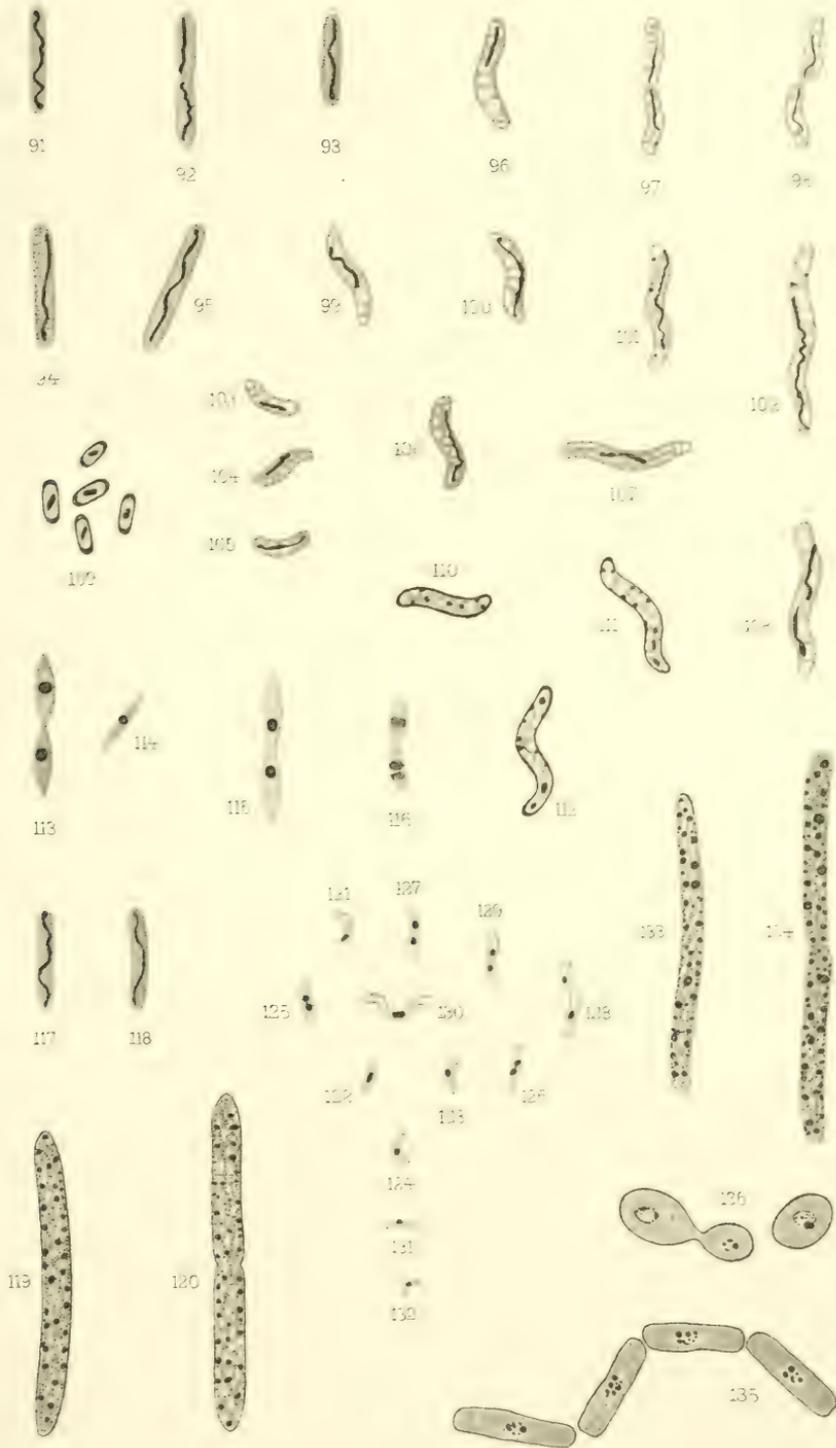
Figs. 146, 147, 149, 150.—Zymoid forms, producing outgrowths. In fig. 150 the outgrowth has divided off as a more or less bacterioid cell.

Fig. 148.—Four bacterioid forms in a chain—the two middle individuals producing outgrowths.

Fig. 151.—A chain composed of both zymoid and bacterioid individuals.









On *Cristispira veneris* nov. spec., and the
Affinities and Classification of Spirochæts.

By

C. Clifford Dobell.

Fellow of Trinity College, Cambridge; Lecturer at the Imperial
College of Science and Technology, London.

With Plate 20 and 2 Text-figures.

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INTRODUCTION.

DURING the last few years many memoirs have made their appearance in connection with the remarkable group of Protista which may be conveniently collected under the common name "Spirochæts." Of these organisms the most divergent descriptions have been given, and consequently the most divergent views have been held regarding their affinities with other organisms. Many workers consider that

the Spirochaets are allied to the flagellate Protozoa; many consider that their proper systematic position is among the Bacteria. As I have devoted a considerable amount of study to both these groups—as regards their cytology and life-histories—I have naturally been anxious to extend my studies to the Spirochaets. The present paper represents a part of the researches which I have made upon these organisms, with the conclusions derived from them. I may state at the outset that my own observations have led me to believe that the Spirochaets are really neither Protozoa nor Bacteria, but a group of Protista which—for the present—must be held to stand apart.

In the present paper I shall describe some researches which I made upon the Spirochaets of Molluscs—to one species of which I have devoted special attention. With the exception of the work of Schellack (1909) and Gross (1910), almost all the observations which have been made upon these forms are, I believe, marred by incorrect interpretation. My own observations and interpretations—made quite independently, and upon different material—correspond in many ways with those of Schellack and Gross.

I shall adopt the generic name *Cristispira* Gross to denote the flexible, spiral organisms which occur in the crystalline styles of so many Lamellibranchs. It is obvious that the name “*Spirochaeta*,” which has now for some time been applied to them, is no longer applicable (see p. 534).

MATERIAL AND METHODS.

The organism with which the present paper is chiefly concerned is a large species of *Cristispira* which inhabits the crystalline style of *Venus (Meretrix) casta* Chem. As no *Cristispira* has previously been recorded from this mollusc, I propose to name the new organism *Cristispira veneris* n. sp.

The discovery of this organism is due to Dr. Arthur Willey, F.R.S., who called my attention to it when I was

visiting Ceylon in 1909, during my tenure of the Balfour Studentship of Cambridge University (cf. Dobell, 1910). As the organism is of large size, I took the opportunity of investigating its structure and life-history as far as possible.

All the specimens of *Venus casta* which I examined were taken from Tamblegam Lake,¹ in the Eastern Province of Ceylon. These molluscs—together with others—were collected for me by Dr. Willey, at Niroddumunai, and sent thence to me in Trincomalee—about eight miles distant. With Dr. Willey's assistance I also examined some of the molluscs at Niroddumunai soon after they had been captured. I take this opportunity of again thanking Dr. Willey for his kind collaboration.

A crystalline style was present in 50 per cent. of the specimens of *Venus casta* which I examined. In every instance in which a style was present it was found to be infected with *Cristispiræ*. Examination of the contents of the œsophagus and stomach of individuals possessing no crystalline style was in every case negative; but only a few of these individuals were carefully examined. The *Cristispiræ* were usually present in large numbers, and were always very actively motile when first removed from their host.

After studying the living organisms, I made a number of permanent preparations in order to investigate finer details of structure. The method employed was the same as that which I have frequently used with success in studying similar forms, Bacteria and blood-inhabiting Protozoa. I made moist films of the substance of the crystalline style, fixed them by exposure to osmic vapour followed by absolute alcohol, and then stained them by Giemsa's or Leishman's modification of Romanowski's stain. The films were then washed in water and allowed to dry, or previously differentiated in weak alcohol. They were examined under an immersion in cedar-wood oil. This method gives, I believe, very accurate results when properly employed. The fixation with osmic vapour

¹ Tamblegam Lake is a salt-water lake, connected with Koddiiyar Bay. All my observations were made in September, 1909.

must be done with care; if this is the case, harmful effects do not result from subsequent drying after staining. Minchin¹ has found a similar method suitable for studying the structure of trypanosomes. I have also obtained excellent preparations of these and hæmogregarines, etc., in this way.

I also made dry film preparations, fixed in absolute alcohol and stained by Giemsa's method in the usual way. This method gives rise to most misleading appearances in the organisms, but these are of considerable interest for comparison with those in other preparations which have been properly fixed.

The appearances observable in different preparations will be considered later, but it may be noted here that they vary according to the length of time during which the osmic vapour is allowed to act, and according to the degree to which the stain is extracted with alcohol after staining.

During my stay in Trincomalee, I was unable to use other methods of fixation and staining. But from my previous experience of the method I employed, I believe that my preparations are trustworthy, and give reliable information regarding the structure of the organisms. Comparison of my results with those of others has served to strengthen this opinion.

In all cases the films were made from the crystalline style immediately after removal. *Cristispiræ* usually undergo degenerative changes soon after they have been removed from their host, and therefore exhibit a structure which is very different from that of normal individuals. It is true that they will often live in carefully made preparations for several hours. But their motility, as a rule, diminishes rapidly, and their internal and external structure becomes modified by degenerative changes.

¹ E. A. Minchin. "The Structure of *Trypanosoma lewisi* in Relation to Microscopical Technique," 'Quart. Journ. Micr. Sci.,' vol. 53, 1909, p. 755.

OCCURRENCE OF MICRO-ORGANISMS IN THE CRYSTALLINE STYLES
OF LAMELLIBRANCHS.

In addition to making an examination of the crystalline style of *Venus casta*, I searched for *Cristispiræ* in the styles of eight other species of Lamellibranch. All these were also obtained from Tumblegam Lake. I found *Cristispiræ* present in the style of only a single species—*Soletellina acuminata* Desh. Only three individuals out of eleven examined harboured the parasites, though a crystalline style was present in every individual. In one style, all the *Cristispiræ* were dead and degenerating when I found them.

These *Cristispiræ* in the style of *Soletellina acuminata* had been previously discovered by Dr. Willey (cf. Dobell, 1910). On account of the small amount of material which I obtained, I was unable to make any extensive observations upon these organisms. The *Cristispiræ* of this species are small, and resemble *C. interrogationis* Gross. I found similar forms—possibly identical with these—sometimes inhabiting the style of *Venus casta*, in company with the large *C. veneris*. As they were found in relatively small numbers I have not been able to make a careful study of them. I believe, however, that there can be little doubt that they belong to a separate species, and are not developmental forms of *Cristispira veneris*. The occurrence of more than one species of *Cristispira* in the same style has already been described by Schellack (1909) in several Lamellibranchs (*Ostrea*, *Tapes*, etc.), and by Gross (1910) in *Pecten*.

In some of the other molluscs which I examined, I found that the crystalline style was infected with Bacteria. These were not present simply as a few organisms—derived from the gut contents—on the surface of the style, but permeated the whole of its substance. In fact, the whole style appeared to be a pure culture of the particular organism which was inhabiting it. As far as I am aware, this has not been

observed previously in the styles of other Lamellibranchs, and I will therefore devote a few words to a description of my observations.

Out of nine individuals of *Circe gibbia* Lam. which I examined, five possessed a crystalline style, and three of these were heavily infected with Bacteria—two being uninfected. The Bacteria all appeared to be of the same species. They were non-motile *Vibrio*-like organisms of small size, and many dividing forms were present.

A single individual of *Cyrena impressa* Desh. which I examined contained a style heavily infected with a *Bacillus*.

Seven individuals belonging to the species *Psammotæa variegata* Wood were found to possess crystalline styles. Five of these contained large numbers of a *Bacillus*.

I examined ten specimens of *Arca* (*Scapharca*) *rhombæa* Born, and found a style present in six of these. Four out of these six styles were filled with curious branching filaments, whose nature was not determined. I found the same sort of filaments in the style of one specimen of *Soletellina acuminata*. In the living state, the filaments look like fungal growths, and after staining by Giemsa's method they are seen to contain a large number of deeply staining granules. Owing to an unfortunate accident—a heavy thunderstorm which overtook me when I was returning to Trincomalee with my preparations—my slides of these organisms were much damaged, so that I can give no further particulars regarding these peculiar growths.

The window-pane oyster (*Placuna placenta*), of which I examined a few specimens, was always found to possess a very long and well-developed style. No parasites were found inhabiting it. Dr. Willey has also examined a number of styles of this mollusc, and always with the same negative results.

I give the results of my examination of the eight species of Lamellibranch referred to in the following table :

Mollusc.	No. of individuals examined.	No. in which a style was present.	Observations.
<i>Area</i> (<i>Scapharca</i>) <i>rhombea</i> Born.	10	6	4 styles were infected with filaments; 2 uninfected.
<i>Circe gibbia</i> Lam.	9	5	3 styles were infected with Bacteria; 2 uninfected.
<i>Cyrena impressa</i> Desh.	1	1	Style infected with Bacteria
<i>Placuna</i> pla- centa L.	3	3	All styles uninfected.
<i>Psammotæa vari-</i> <i>egata</i> Wood	7	7	5 styles infected with Bacteria; 2 uninfected.
<i>Solen</i> (Ensis) <i>regularis</i> Dunk.	1	0	—
<i>Soletellina acu-</i> <i>minata</i> Desh.	11	11	2 styles infected with a small <i>Cristispira</i> ; 1 with dead <i>Cristispira</i> ; 1 with filaments.
<i>Venus</i> (<i>Dosinia</i>) <i>cretacea</i> Reeve	2	1	Style uninfected.

I take this opportunity of thanking the Rev. A. H. Cooke for very kindly identifying these Lamellibranchs for me.

A good deal has already been written about the function of the crystalline style of the Lamellibranchiata. It has been suggested that it is a body of a secretory or excretory nature, that it is a reserve supply of food material, and that it is a mechanical device for catching and conglomerating food particles. Mitra¹ has shown that it contains a proteid substance—which he showed to be a globulin—and that an amylolytic ferment is present in it. He therefore regards it as a body which is primarily connected with the digestion of

¹ Mitra, "The Crystalline Style of Lamellibranchia," 'Quart. Journ. Micr. Sci.,' vol. 44, 1901.

food. Hornell,¹ from his own observations on the style of the oyster, regards the style as a food-catching apparatus, as was maintained earlier by Barrois. Pelseener² states that "the product of its solution forms a sort of cement which encrusts any hard substances that may have been ingested and thus protects the delicate walls of the intestine from injury."

This is not the place to discuss these and other views which have been put forward regarding the functions of the crystalline style. But as this is of some importance in connection with the organisms which inhabit it, the structure itself cannot be ignored. It appears to me most probable—from the observations recorded by others—that the crystalline style serves both to catch food particles and prepare them mechanically for digestion and also to assist in the digestion of the amyloid constituents of these particles.

In some Lamellibranchs—e. g. in *Pecten* (Gross, 1910)—the *Cristispiræ* are found in the stomach and intestine, and only rarely in the crystalline style. It therefore seems to me probable that *Cristispira* is really a gut parasite, which often happens to find the substance of the crystalline style a suitable culture medium. The same is also suggested by the occurrence of Bacteria in the style. The latter contains some 12 per cent. of globulin, with about 1 per cent. of salts and 88 per cent. water.³ It might therefore well serve as a culture medium for many micro-organisms which reach it accidentally. I do not think any deeper significance need be attached to the association of Protista with the crystalline style.

CRISTISPIRA VENERIS, N. SP.

I will now record my observations upon the structure

¹ Hornell, "Report on the Operations on the Pearl Banks during the Fishery of 1905," 'Ceylon Marine Biological Reports,' Part II, June, 1906.

² Pelseener, "Mollusca," in Lankester's 'Treatise on Zoology,' London, 1906.

³ Mitra, loc. cit.

and mode of division of *Cristispira veneris*—the large “mollusc spirochaet” which I found inhabiting the crystalline style of *Venus (Meretrix) casta* Chem. in Tanglegam Lake. I shall here give my own observations only—reserving an analysis of my own results and those of other workers for the next section (p. 527).

(1) Structure.

Cristispira veneris is one of the largest members of the genus, resembling *C. balbianii* Certes and *C. pectinis* Gross. The average length is 50–60 μ , the average breadth—in fixed and stained specimens—about 1.5 μ . A certain amount of variation in the breadth of different individuals is observable in fixed and stained organisms—the narrowest being slightly over 1 μ , the broadest approximately 1.9 μ . Dried films stained with Giemsa not uncommonly possess a width of almost 2 μ . The longest undivided individual which I have measured was 74 μ in length.

Living individuals appear to be of approximately the same width, though it is almost impossible to make accurate measurements of them on account of their great motility. The differences in width observable in stained individuals are due, I believe, to the greater or less degree of flattening which takes place in the organisms in making the preparations. It can be seen in the living organisms that they are cylindrical—that is to say, they are circular and not band-like in optical transverse section. In the process of making films, the cylindrical shape is modified by flattening to a band-like shape, thus making the individuals appear broader. Thus, if the diameter of the cylinder constituting the organism were 1 μ , the circumference would be $\frac{22}{7}$ μ . If complete flattening of the cylinder occurred, the breadth of the organism would appear to be $\frac{1}{2} \times \frac{22}{7}$ or approximately 1.6 μ . According to the amount of flattening which occurred, different individuals

might therefore display any breadth between $1\ \mu$ and $1.6\ \mu$. If the breadth of *C. veneris* is therefore a little more than $1\ \mu$ —that is, about $1.2\ \mu$, subject of course to slight individual variation—then the different breadths observed in stained specimens are easily accounted for by the different degrees of flattening which different individuals have undergone in the process of making the preparations. I believe, therefore, that the body of *C. veneris* is cylindrical, and has an actual uniform diameter of approximately $1.2\ \mu$ in the living organism.

I have already described a similar apparent variation in breadth—due, I believe, to the same causes—in the case of Bacteria (see Dobell, 1910 A). The apparent variability in the breadth of different individuals of *Cristispira* is a point of some importance when considered in relation to the method of division (see p. 526).

As in other members of the genus, the body of *C. veneris* possesses a spiral, corkscrew-like shape. The number of complete turns in a full-grown individual is approximately four. The number is greater than this in dividing individuals (five or six), and less in newly divided individuals (two or three).

In the living organisms, I have not been able to distinguish any structure in the protoplasm of the cell, which appears homogeneous under the highest magnification which I was able to employ (Leitz $\frac{1}{12}$ in. oil-immersion \times ocular 5, using direct sunlight for illumination). A few small refractile granular inclusions were usually to be seen in the protoplasm.

The ends of the organism are bluntly pointed (see fig. 1, Pl. 20), being less rounded than the ends of *C. balbianii* and less pointed than those of *C. anodontæ*. The body usually tapers very slightly towards the two ends. The structures called "polar caps," described in *C. balbianii*, *C. pectinis*, etc., are not observable in *C. veneris*. They appear to be confined to the species which possess rounded ends.

Neither in living nor in fixed and stained specimens can any structures comparable with flagella be seen.

Like other *Cristispiræ*, *C. veneris* has a flexible body. It may be noted, however, that in living and actively moving individuals the body is kept relatively rigid—flexibility being chiefly observed in slowly moving (? abnormal) individuals, and indicated by the irregular spiral conformation often observable in fixed and stained organisms. I believe that bending movements occur very seldom in normal active individuals. The ordinary movements of *C. veneris* are similar to those of *C. balbianii*, which have already been described by Perrin (1906).

The two most important characteristics of the *Cristispiræ* are the crista and the structure of the protoplasm. I will now describe these in detail in *C. veneris*.

The *Crista*.—This structure, formerly called the “undulating membrane” on account of its supposed homology with the undulating membrane of trypanosomes, has hitherto been correctly interpreted—I believe—by Gross alone. The name *crista*, or *crest*, which he has proposed for it, appears to me a convenient and suitable one. I shall therefore adopt it.

A *crista* is present in every individual which I have examined in the living condition or in properly fixed and stained preparations. In dried Giemsa preparations, it may be torn and distorted and sometimes appear completely lacking, but this is due to the drying which has taken place before fixation, and is therefore not a normal condition. In all cases in which proper fixation with osmic vapour has been effected, the *crista* is present and presents the same characteristic appearance.

The *crista* is in the form of a narrow band, radially situate on the surface of the organism, and spirally disposed (see fig. 2). It does not as a rule reach the extreme ends of the organism, and appears to me to be a simple prolongation of the membrane which clothes the body. At the ends it merges gradually into this, and no structures comparable with basal granules or blepharoplasts are present. It is homogeneous

throughout, and shows no fibrillar structure in living or properly fixed specimens. It is stained a pink or violet colour by Giemsa's method, in marked contrast with the general blue colour of the body (see fig. 2, etc.). There is no thickened, chromatic edge to the crista. In fact, it does not in any way resemble the undulating membrane of a trypanosome.

In macerated individuals the crista may present a very different appearance (fig. 3). It becomes greatly enlarged and distorted, and shows a very definite fibrillar structure. This is an artifact, and though it may indicate that the crista is really composed of fibrils arranged longitudinally, it must not be forgotten that in normal individuals it appears absolutely homogeneous. This fibrillar appearance has often been described as the normal structure of the "undulating membrane" of *Cristispira*—which it certainly is not.

The crista of *C. veneris* is therefore a delicate, uniform, band-like appendage, wound spirally round the body, and extending almost to the ends. It is always present, and has no resemblance to the undulating membrane of a trypanosome. It serves, apparently, as a rigid lateral fin-like extension of the body, in the performance of the screw-like movements of the organism. Some further account of some of the previous interpretations of this structure will be found on p. 528.

Structure of the Protoplasm.—As I have already noted, the protoplasm of the living organisms appears homogeneous. In stained specimens, however, it has a distinct and highly characteristic structure. This structure has been observed by Schellack and Gross, though the interpretations of these two observers differ.

If a *Cristispira* be fixed by exposure to osmic vapour for about thirty seconds, then transferred immediately (without any drying being allowed to take place) to absolute alcohol for ten minutes, then stained by Giemsa's method, and examined in the manner already described (p. 509), it is seen to possess a structure like that of the individual shown in fig.

1, Pl. 20. This organism is from a moist film preparation of a crystalline style which was so treated. The whole organism (fig. 1) shows a protoplasmic structure consisting of a single row of chambers or alveoli. The walls of these chambers are stained a deep blue, their contents a uniform pale blue. The relative dimensions of these chambers are not always constant; they may vary not only in different organisms, but at different points in the same organism—being sometimes square, sometimes oblong (cf. figs. 7, 8, etc.). The alveolar walls separating adjacent chambers from one another appear as transverse septa in optical section (see figs. 1, 2, etc.). At the point where the transverse septum joins the wall of the cell a dark purple granule can be seen. The whole organism thus appears to contain a series of paired purple granules, united by blue transverse lines—representing the alveolar walls (fig. 1). This appearance is always presented by individuals treated in the manner described. If the exposure to osmic vapour has been limited to about thirty seconds, and no overstaining has taken place, then the appearances are constantly encountered. The difference in size observable in the purple granules should be noted—also the fact that they always lie at the edges of the organism, and never centrally (figs. 1, 7).

If the osmic vapour be allowed to act for a longer period of time—i. e. for several minutes—then the organisms present a different appearance after Giemsa staining. The granules appear much smaller, and are stained a deep blue (figs. 2, 8). The chambers are easily visible, but the granules have dwindled to tiny dark blue points. In some cases they cannot be distinguished with precision at all levels in the body (cf. fig. 6).

Organisms which have been dried previous to fixation, fixed in absolute alcohol, and then stained by Giemsa's method in the usual way, often present appearances which are quite different from those seen in osmic-fixed organisms. They show, in fact, all the remarkable "chromatin" configurations which have been described by Perrin and others. The

chambers are often indistinctly seen, or absent. Vacuoles are not infrequently present. Red "chromatin" structures of varying form are seen in different individuals and at different points in the same individual. Fig. 10 shows some of the "nuclear" structures observable in dried organisms. It is drawn from a part of a *Cristispira* which was dried before fixation, fixed in absolute alcohol, and stained by Giemsa's method. It will be seen that the "chromatin" is in the form of spiral or zig-zag filaments, rods, granules, "tetrads," etc. These arrangements of the "chromatin" are found side by side in the same organism at the same time.

The appearances which are observable in organisms which have undergone plasmoptysis are instructive. Such an individual is shown in fig. 3. The whole organism is filled with red granules, of variable size and irregular distribution. At the points where the cell membrane has burst, the protoplasm has flowed out, and it can be seen that it consists of two different substances—a bluish or lilac coloured substance and a denser dark-red substance.

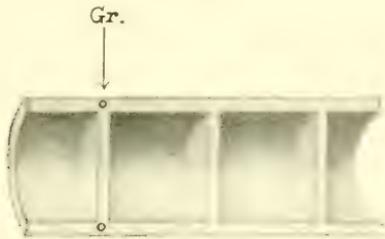
How are all these different appearances to be interpreted? I believe the correct interpretation is as follows: The structure of *Cristispira* may be compared with that of a bamboo stem. The whole body is in the form of a hollow cylinder, divided into a single series of chambers by means of a series of transverse disc-like partitions like the nodes of a bamboo rod. The cytoplasm forming the walls of the cylinder and the disc-like partitions is dense and deeply stainable; the cytoplasm which fills the chambers is less dense and less deeply stainable. Text-fig. 1 illustrates diagrammatically the structure of a portion of a *Cristispira* which is supposed to have been split longitudinally, so as to divide the body into two equal parts. When viewed from inside, an appearance such as is shown in Text-fig. 1 would be seen.

The tube forming the body is divided into cylindrical chambers by transverse disc-like partitions—only half of each disc and chamber being seen, of course, when the other half is split off. In a *Cristispira* all the solid structures dia-

grammatically represented in Text-fig. 1 are composed of the denser part of the cytoplasm, the chambers—represented empty in the diagram—being filled with the less dense cytoplasmic matter.

Now I believe that the only other morphological con-

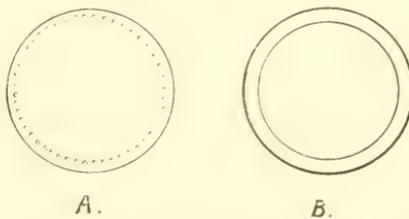
TEXT-FIG. 1.



Explanation in text.

stituents of the cell are a number of small granules, which are arranged round the circumference of the disc-like partitions—in the dense cytoplasm which lines the cell. The position of these granules—which I suppose to form a ring when a partition is seen in a transverse section of the whole

TEXT-FIG. 2.



A.

B.

Explanation in text.

cell—is shown in the diagram (text-fig. 1, *Gr.*). A transverse section of the cell, passing through a partition, would present an appearance similar to that shown in Text-fig. 2 A. In this diagram it will be seen that the granules are arranged in the form of a ring round the circumference of the disc. A

transverse section of a cell, passing through the middle of a chamber, would present an appearance like that shown diagrammatically in Text-fig. 2 B. The appearance is that of a tube—the wall composed of dense cytoplasm, the inside filled with less dense and more lightly staining cytoplasm.

Now I think that those individuals which have been exposed to osmic vapour for several minutes and then stained with Giemsa's stain, present appearances which must be interpreted as representing a structure such as I have just described. The chambered structure of the cytoplasm, with the ring of small granules round the circumference of each partition, is quite clearly seen in these individuals. In optical section, of course, only a single pair of granules is seen—lying at the point where the partition joins the cell wall. The granules are of very small size, and are therefore visible under the highest powers only, and after correct differentiation. It is difficult to be absolutely certain that a ring of granules is present round each partition, but I believe that this can often be demonstrated. As the bodies of *Cristispiræ* treated in this way remain cylindrical—or undergo only a very slight flattening—in the process of fixing and staining, it is necessary to suppose such an arrangement of the granules to account for their constant appearance at the edges of the organism—at the points where the septa and cell-walls unite (cf. figs. 2, 8, etc.).

As I have pointed out, the organisms which have been fixed by exposure for a shorter time to osmic vapour show pairs of much larger purple granules situated at the points where the partitions join the sides of the cell. A ring of granules is not present in these forms. I believe the correct interpretation of such organisms (figs. 1, 7) is as follows: In the course of making the preparation the organisms have become flattened, as a result of drying following upon inadequate fixation. Exposure to osmic vapour for about half a minute is not sufficient to fix the organisms properly. As they dry on the slide the granules run together into small masses at the edges of the organism, and so give rise to the appear-

ances which I have described. It is easy to understand why the granules—in reality masses of granules—appear to be of different sizes in such organisms (fig. 1), and why they always appear at the edges of the organism, which we know to be really cylindrical when alive.

When no fixation previous to drying occurs, the cell undergoes plasmolysis and complete flattening on the slide. The small granules run together in various ways, giving rise to the various “nuclear” figures which have been described (cf. fig. 10). It is easy to understand how the flowing of the granules through the walls of the chambers, and their massing together in various ways, can give rise to the appearance of transverse bars, spirals, tetrads, etc., of “chromatin.” It will hardly be necessary to describe in detail the several ways in which such appearances may be caused.

The staining reactions of the substance of which the granules are composed require a brief consideration. I have already noted that the granules stain a deep blue after a long exposure to osmic vapour, purple after a brief exposure, red when osmic fixation is omitted, and only absolute alcohol is employed after previous drying. I believe these differences are directly due to the action of the osmic vapour—prolonged action of which so changes the granules that they are unable to take up the red-staining element in the Romanowski stain. I have observed this action of osmic acid in the case of Bacteria and many Protozoa, and I believe it must have been noticed by many other workers who employ Romanowski staining after osmic fixation. A short exposure to osmic vapour permits the granules to stain red—as they do when not acted upon by it. A longer exposure permits them to stain red to a less extent, and gives rise to a purple coloration. Still longer action of the osmic vapour renders the granules incapable of taking up the red element in the stain, and they therefore appear blue—the blue element alone being capable of staining.

There can be no doubt, I believe, that the granules are composed of a substance which is different from that of the

cytoplasm. It is a substance, moreover, which may be stained red with Giemsa's stain (cf. figs. 3, 10). From this it may perhaps be inferred that the granules are composed of a chromatin substance, and are therefore of a nuclear nature. This consideration, however, does not really justify the conclusion that the granules constitute the nuclear apparatus. Further evidence of the behaviour of the granules during other phases of the life-history is required before their true significance can be settled. Yet for the present, I regard the nuclear hypothesis as the most probable, and believe that the granules represent a chromidial nucleus somewhat similar to that which occurs in many Bacteria (see Dobell, 1910A), and some Protozoa.

To summarise my interpretation of the protoplasmic structure of *Cristispira*: The whole body is composed of a single series of cylindrical chambers or alveoli, separated from one another by disc-like partitions. These structures are composed of a denser cytoplasm constituting their walls, and a less dense cytoplasm which fills the chambers. Very small granules—probably constituting, as a whole, a nucleus of a chromidial form—are arranged round the circumference of each disc-like partition. Various appearances—such as a series of pairs of large granules, tetrads, transverse bars, spiral filaments, etc., of chromatin—which are often encountered, and have been frequently described by others, are artifacts.

One more point in the protoplasmic structure of *Cristispira veneris* requires consideration. It often happens that here and there, in the body of an individual, certain chambers appear more darkly stained than the remainder. This appearance is well seen at the point marked *a* in the individual depicted in fig. 6. At other times the partitions between the chambers appear thickened (see fig. 8, *b*, etc.), and appearances which are intermediate between a darker chamber and a thickened septum are also to be seen (see fig. 9, where this is shown in two places). Similar appearances have been figured by Gross and others.

The explanation of these appearances is, I believe, quite simple. As will be shown in the next section of this paper (vide infra), the method of multiplication is by transverse fission. The daughter-individuals which arise from the transverse division of a long individual are therefore short—being only half the length of the original organism. Before they undergo a subsequent division they must grow in length, and must therefore form new chambers. I believe that these new chambers are formed at various points in the body, and arise by the gradual thickening of a partition and its subsequent hollowing out. Thickened partitions therefore correspond to the points where new chambers are beginning to be formed—more darkly stained chambers are newly formed chambers. Successive stages in the formation of chambers in this way are shown in fig. 8 (where a thickened septum is seen at *b*), fig. 9 (which shows the hollowing of the septa at two points), and fig. 6—where a darkly staining (newly formed) chamber is seen at *a*.

(2) Division.

Although I have not been able to observe every stage in division in the living organism, I have encountered a number of dividing forms in my stained preparations which leave no room for doubt as to the essential features of the process. Division is transverse, and is effected in the manner described by Gross in the case of *C. pectinis*. I have never seen any indications of a longitudinal division, and all the observations which I have made speak strongly against the view that such a method of multiplication occurs in these organisms.

The long individuals which are about to divide into two transversely are in the form of spirals consisting of five or six complete turns. Before dividing, they bend themselves double—the two halves becoming intertwined (see fig. 4). This phenomenon has been described in *C. pectinis* by Gross, who calls it “incurvation.” The transverse fission of the organism begins when it is in this condition. It occurs

in the middle of the incurved individual, at a point where a transverse partition separates two adjacent protoplasmic chambers from one another (cf. fig. 4). The partially divided organism then untwists itself—passing out of the condition of incurvation to the original form of a simple spiral (fig. 6). In this condition fission is completed, and the two daughter-individuals separate from one another. The latter are, of course, short individuals in the form of spirals consisting of two or three turns.

In the division of the body the crista is also involved. It divides with the rest of the body, in the manner shown in fig. 7. This figure shows the middle region of a dividing *Cristispira* which is just straightening itself after being in the state of incurvation.

The whole process of division is extremely simple, and resembles—apart from the incurvation—the process of division which can be seen in many *Spirilla* and other Bacteria.

I think there can be no doubt at all that the incurved individuals are not really stages in a longitudinal division—as they seem frequently to have been interpreted by other workers. The crista does not split longitudinally. I have never seen partially longitudinally split individuals; the transverse division of the looped end of the incurved organism is often very easily seen; the number of turns in the spiral in a newly divided individual is half that of the undivided individual; and finally, the width of all individuals—when allowance is made for the differences due to technique (see p. 515)—is fairly constant. These facts indicate most clearly that division is transverse and not longitudinal, as Schellack and Gross have maintained in the case of other species of *Cristispira*. I believe, with these two observers, that all cases of longitudinal division which have been described in *Cristispira* are due to misinterpretation of the observed appearances.

Formation of gametes, conjugation and encystation I have never encountered. These phenomena—first described by

Perrin—have been said to occur by several observers, but their statements are based, I believe, upon a wrong interpretation of the facts. This has already been pointed out by Schellack and others, so I will therefore omit further discussion of the matter here.

THE MORPHOLOGY, AFFINITIES AND CLASSIFICATION OF SPIROCHÆTS.

In the following pages I shall discuss the most important features in the morphology and life-history of the *Cristispiræ*, or, as they are commonly called, "mollusc Spirochæts." A discussion of these features is necessary in order to arrive at conclusions regarding the affinities of this remarkable group of organisms, and of Spirochæts in general.

Two excellent contributions to this subject have recently been made—that of Schellack (1909) and that of Gross (1910). Both these workers employed good cytological methods, and made careful detailed observations on the forms which they investigated. As they have both discussed the earlier work at some length, and entered fully into the literature on the subject, I will confine myself chiefly to pointing out wherein my results agree with or differ from those of these two workers.

The Cell Membrane.—The body of a *Cristispira* is bounded by a cuticle-like covering, which I shall call the cell membrane. This membrane is usually termed the "periplast"—a name originally applied to it by Perrin, who believed the organisms to be Trypanosomes. The use of this special word for the cuticular covering in these two groups of organisms—Spirochæts and Trypanosomes—appears to have led many people to believe that the cell membranes are so similar to one another, and different from other cell membranes, as to indicate affinities between the two groups. The only real similarity between the cell membrane of a *Cristispira* and that of a *Trypanosoma* is that the same word is used for both. Both are, of course, modified forms of mem-

brane which bound the protoplasm of the body; but such membranes are found in the majority of Protista, only they are not usually called "periplasts." I shall therefore avoid using this term, as I believe it leads to a confusion of ideas; and I shall speak of the cuticular covering of a *Cristispira* as the "cell membrane," or simply as "the membrane."

A membrane certainly exists in *Cristispira*. Unless this were present, it is difficult to see how the contours of the body are preserved. The appearance of burst individuals also indicates that a membrane of some sort is present (see fig. 3). Moreover, the presence of a membrane is clearly demonstrated when the organisms undergo plasmolysis. This has been clearly shown by Swellengrebel (1909) in *C. balbianii*.

It has frequently been stated that the "periplast" of *Cristispira* possesses a fibrillar structure, which can be seen when the organisms are macerated. I have seen many individuals of *C. veneris* which show the appearances which have been thus interpreted, and I believe the fibrils are derived in all cases from the crista (see fig. 3). The cell-membrane itself possesses no structure. Schellack (1909) states that "bei den grossen Spirochæten¹ ist ein fibrillärer Periplast sicher nachgewiesen; er kann künstlich aufgefasert werden." I believe this is incorrect. My own view is the same as that expressed by Gross—"Der Periplast existirt gar nicht. Die Cristispiren haben einfach eine ziemlich starke, aber färberisch nicht differenzirbare Zellmembran." As Gross has discussed the matter fully I will say nothing further about it—merely pointing out that my interpretation agrees with his.

The *Crista*.—Schellack (1909) interprets the crista as an artifact—"als ein durch künstliche Veränderung des Periplasts hervorgerufenen Gebilde." I believe this interpretation to be quite incorrect. The crista is easily visible in slowly moving, living organisms, and is constantly present in properly fixed specimens. It is homogeneous and possesses

¹ I.e. *Cristispira*.

no chromatic border. It is totally different from the undulating membrane of a Trypanosome, to which most previous workers have likened it. My interpretation of this characteristic structure is the same as that of Gross (1910). "Die Crista ist ein Organell sui generis."

A deeply staining ("chromatic") edge to the crista and a fibrillar structure can only be seen in macerated organisms, or organisms which have been imperfectly fixed. Such structures must therefore be regarded as artifacts. The normal crista of *C. veneris* stains pink or violet with Giemsa's stain, but this does not necessarily indicate that it contains chromatin.

Flagella.—Flagella or cilia, such as occur in flagellate Protozoa or Bacteria, are not present in *Cristispiræ*. The matter has been fully discussed by Schellack (1909) and Gross (1910), who have both come to this same conclusion. Further discussion will therefore be superfluous.

Protoplasmic Structure.—The chambered structure of the protoplasm, which I have described in *C. veneris*, has already been clearly recognised in other *Cristispiræ* by Schellack and Gross. I am convinced, with these two observers, that the various nuclear figures (spiral filaments, transverse rodlets, tetrads, etc.) described by Perrin and others are really artifacts. Moreover, Perrin's account (1906) of the relations existing between the various nuclear figures and the longitudinal division of the organism must be discarded. For the nuclear figures are artifacts, and longitudinal division does not occur.

The interpretations of the appearances observed by Schellack and Gross differ from that which I have given in preceding pages. It will therefore be necessary to discuss their views briefly.

Schellack's (1909) interpretation of the protoplasmic structure of *Cristispira* is somewhat similar to mine. His description of the structure of the chambers is in close agreement with my own description. In one point, however, Schellack's interpretation differs from mine. He believes

that chromatin granules are scattered through all the walls of the chambers, whereas I believe that—in *C. veneris*—the granules are confined to the circumference of each transverse disc-like partition. Schellack thus regards a *Cristispira* as containing a nucleus of a kind of chromidial form.¹

Gross's (1910) interpretation is peculiar. Although he appears to have observed the same structures as Schellack and myself, he comes to the conclusion that the protoplasm is really structureless, and there is no nucleus of any sort present. The chambers are artifacts, because they can be seen neither in the living organisms nor in organisms fixed with Flemming's fluid and stained with iron-hæmatoxylin. Gross always found the chambered structure present after fixation with corrosive sublimate, but he attributes this structure to the action of the fixative.

I believe that another explanation is correct. I believe that the invisibility of the chambered structure after fixation with Flemming's fluid is the direct result of the action of the fixative. It is often exceedingly difficult to obtain good differentiation of the internal structure of Bacteria after they have been fixed with Flemming's fluid, and I believe that this is due to the action of the fluid upon the cell-membrane and the protoplasm. Every cytologist must have experienced, at some time or other, a difficulty in staining cells after fixation in Flemming's fluid. At all events my own experience leads me to believe that this must be so. I would also point out that, in the case of *C. veneris*, not only does a prolonged action of osmic vapour—in the course of fixation—cause a change in the staining reactions of the granules, but it also gives

¹ This statement requires some qualification. For although Schellack describes the chromatin as being in the form of granules ("Die Kammerwände scheinen aus einer festeren Substanz zu bestehen und es sind ihnen Körnchen aufgelagert," p. 400), he seems inclined in another place to regard the nucleus as being constituted by the whole of the substance of the chamber walls. He says: "Die Gesamtheit der Waben in einer normalen Spirochäte bildet einen ziemlich fest in sich haltbaren, kompakten Stab, den sogenannten Kernstab. Die Periplasthülle liegt ihm direkt auf," etc. (p. 401).

rise to a less precise staining of the cell as a whole. *Cristispiræ* which have been subjected to osmic vapour for many minutes tend to take up a more diffuse blue stain, and show the chambered structure less distinctly in consequence. But although this is the case, the chambers can always be seen. They never disappear completely, though they do become fainter after more prolonged fixation. That the chambered structure cannot be seen in the living organism I do not regard as any proof of its non-existence. For the width of the cell is small (less than $2\ \mu$): the cell-membrane is fairly thick and possesses a considerable degree of refractivity: and the difference in refractivity between the protoplasm forming the walls of the chambers and that which fills them is probably not very great in the living organism. The chambered structure appears with such constancy in organisms fixed with osmic acid or corrosive sublimate that it will require a good deal more evidence than that furnished by Gross to prove that it does not exist.

Swellengrebel's (1907) original account of *C. balbianii* differs in some ways from his later description (1909), in which he records appearances which are consistent with my interpretations. The transverse bars of chromatin which he describes are, I believe, similar to the transverse bars which I have frequently seen, and are produced in precisely the same way—by imperfect fixation. Swellengrebel states that he fixed the organisms in formaldehyde (1907, p. 19), but he appears to have overlooked the fact that fixation in the way he describes is inadequate unless employed in conjunction with after-treatment with alcohol—a point which I have already had occasion to point out elsewhere (Dobell, 1910A).

It is apparent from the foregoing, therefore, that whereas Schellack appears to regard the body of a *Cristispira* as being chiefly composed of a nuclear structure, Gross regards it as enucleate, and I regard the nucleus as being in all probability represented by chromidial structures arranged in the manner described in previous pages (see p. 521).

Plasmolysis.—Swellengrebel (1909) has proved that

Cristispiræ are plasmolysable. The phenomenon is so often seen in organisms which have been dried, or are drying, in a drop of sea-water, that it is almost inconceivable that anyone should have stated that the organisms are implasmolysable. I think there can be no doubt whatever that plasmolysis may be caused in these organisms, and that it is similar to that which may be seen in many Bacteria.

Division.—My own conclusions regarding division are completely in accord with those of Schellack and Gross. Division is transverse, and not longitudinal. The errors of interpretation which have led many workers to believe that longitudinal division occurs have been fully discussed and elucidated by Gross. Further discussion of the matter therefore appears to me unnecessary.

Polarity.—A point of considerable importance, but one which has received hardly any attention from those who have discussed the affinities of the *Cristispiræ* and similar organisms, lies in connection with what I may term the "polarity" of the cell. All flagellate Protozoa possess an antero-posterior differentiation—that is to say, they show by their movements that one end of the body is the front end, the other the hind end. It is therefore correct to speak of their movements as backward or forward movements. The front end is usually the end which bears the flagellum. Now in the Bacteria no such differentiation can be observed. Spirilla and Bacilli cannot correctly be said to move backwards or forwards, because neither end is definitely differentiated as anterior or posterior. In other words, either end is a facultative anterior or posterior end.

In this respect *Cristispira* and the other so-called Spirochaets are similar to the Bacteria, and stand in sharp contrast with the flagellate Protozoa.

The point is not one to be ignored. For it is evident that a differentiation of this sort must involve the organisation of the whole organism, and must therefore be of profound significance.

Flexibility.—It has more than once been urged that

Cristispira and its allies, being flexible and not rigid organisms, show affinities with the Protozoa and not with the Bacteria in consequence. I do not know who is responsible for the original statement that all Bacteria are rigid organisms, but it is certain that such a statement cannot be accepted. Many Bacteria of large size are flexible to a considerable extent. I have shown this to be the case in *Bacillus flexilis* (Dobell, 1908) and a number of allied forms (Dobell, 1910A). It is therefore manifest that flexibility cannot be used as a criterion for judging whether the Spirochæts are to be ranked among Protozoa or Bacteria.

Conjugation.—The organisms described as “gametes” by Perrin and others, and the stages said by them to represent conjugation stages, are all quite arbitrarily so designated. I believe there is absolutely not a vestige of evidence that conjugation occurs in these organisms. Neither Swellengrebel, nor Schellack, nor Gross, nor myself could find any indication of sexual phenomena in this group. Both Schellack and Gross have discussed the matter more fully, and I am in complete agreement with their conclusions.

Encystment.—Whether *Cristispiræ* encyst or not is a point which is still undetermined. I believe the “cysts” described by Perrin and others are really to be regarded as the results of degeneration or plasmoptysis. Schellack and Gross both appear to be of the same opinion. At all events, it may be said with justice, I believe, that no clear case of encystment has yet been described in *Cristispiræ*.

Affinities and Classification.

Having now briefly noted the more important features in the structure and life-cycle of the *Cristispiræ*, it is possible to discuss the affinities and classification of these most remarkable organisms.

At the present moment it is customary to assemble under the common name “Spirochæts” three different groups of unicellular organisms. These are (1) the *Cristispiræ*,

parasitic in Lamellibranchs, (2) the much smaller parasitic organisms like "*Spirochæta*" *pallida*, "*S.*" *buccalis*, the organisms of relapsing fevers, etc., (3) the free-living forms *Spirochæta plicatilis* and its allies.

Now the name *Spirochæta*¹ was introduced by Ehrenberg in 1833 for the free-living organism *S. plicatilis*. It must therefore be applied to this and similar organisms. The structure of *S. plicatilis* has been described by Schaudinn (1905, 1907). According to him there is an undulating membrane and a nucleus in the form of a longitudinal filament surrounded by chromidia—these two elements corresponding respectively to the kinetic and trophic nuclei of a trypanosome. Reproduction occurs by multiple transverse fission.

Quite recently these organisms have been more carefully studied by Zülzer (1910), whose observations differ greatly from those of Schaudinn. She interprets the axial filament as an elastic body—not part of the nucleus. The latter is represented by large, regularly arranged chromatin granules. There is no undulating membrane. If this description is correct,² it is obvious that *S. plicatilis* is a very different organism from *Cristispira*. Anyone who has observed living specimens of *S. plicatilis* would, I should think, be impressed by their dissimilarity to *Cristispiræ*—both as regards movements and general appearance. This, at all events, is my own impression. The bodies of both are flexible and spirally wound, but beyond this there is no great resemblance. The differences are at least sufficiently great to justify the bestowal of different generic names upon the two organisms. As Gross has introduced the name *Cristispira* for the mollusc Spirochaets it should henceforth be adopted.

The smaller parasitic Spirochaets—such as the syphilis

¹ The correct spelling of this name is *Spirochæta*, and not *Spirochæte*, as adopted by Doflein (1909) and numerous other writers.

² I have every reason to believe it is, as I had an opportunity of conversing with Fr. Dr. Zülzer and seeing some of her preparations at the International Zoological Congress in Graz this year (1910).

organism, the organisms of relapsing fevers, etc.—differ not a little from *Cristispira* and *Spirochæta*. In the forms which I have been able to study myself,¹ I have never been able to make out any definite structure—chiefly on account of their very small size. I believe that no protoplasmic structure similar to that of either *S. plicatilis* or *Cristispira* is visible. I also regard it as exceedingly doubtful that a crista is present. The method of division is, I believe, in all probability always transverse. Although the facies of these organisms is very similar to that of *Cristispiræ*, I think it is advisable to keep the two groups of organisms in separate genera for the present.

Regarding the generic name which must be applied to these organisms, it is obvious that as neither *Spirochæta* nor *Cristispira* can be used, some other name must be selected. The name *Spironema*, proposed by Vuillemin (1905) for the syphilis organism, is pre-occupied—having been used by Kiebs for a flagellate. Schaudinn (1905A) therefore proposed the name *Treponema*—a name which must stand, according to the rules of nomenclature. If it be allowed that the small parasitic *Spirochæts* are similar to the syphilis organism,² it therefore follows that they must all be placed in the genus *Treponema*. It appears to me advisable to adopt this system.

For the three groups of organisms which are included in the common name “*Spirochæts*” there are therefore three generic names already in existence. On the assumption, then, that these three groups are sufficiently akin to one another to justify their being collected into a common class—an assumption which appears to me to be justified in our present state of knowledge—I propose to classify the *Spirochæts* as follows :

¹ These are especially forms from the gut of the frog and toad (Dobell, 1908), from termites (Dobell, 1910), and “*S.*” *buccalis* and “*S.*” *dentium* (unpublished observations).

² I see no valid reason for drawing a generic distinction between *Treponema pallidum* and such forms as “*Spirochæta*” *recurrentis*, “*S.*” *duttoni*, “*S.*” *dentium*, etc.

Spirochætoidea.

Genus 1.—*Spirochæta* Ehrenberg. Free-living forms, freshwater or marine. Examples: *S. plicatilis* Ehrenberg, *S. gigantea* Warming.

Genus 2.—*Treponema* Schaudinn. Parasitic in animals (Vertebrates and Invertebrates). Examples: *T. pallidum* Schaudinn, *T. recurrentis* Lebert, *T. dentium* Koch, *T. gallinarum* Blanchard, etc., etc.

Genus 3.—*Cristispira* Gross. Parasitic in Lamelli-branchia. Examples: *C. balbianii* Certes, *C. anodontæ* Keysselitz, *C. pectinis* Gross, *C. veneris*, etc., etc.

The exact classificatory value to be attached to the group *Spirochætoidea* cannot at present be accurately determined. The name stands for a group of Protista which, like several other groups (e. g. Bacteria, Mycetozoa, Myxobacteria), cannot at present be regarded as a "class," "order," or any other sort of subdivision of another group, but must be regarded as an independent group of unicellular organisms which show very little affinity to any other group.

This last statement requires some further qualification. Many workers regard the Spirochæts as showing affinities to other Protista. It has been suggested that there are resemblances between them and the flagellate Protozoa, the Bacteria, and the Cyanophyceæ.

Schaudinn was the first to express the opinion that the Spirochæts are allied to the Trypanosomes, and hence to the flagellate Protozoa. Krzysztalowicz and Siedlecki (1908) go so far as to place them in a group *Spirilloflagellata* among the Mastigophora. Dofflein (1909) places them in a group—*Proflagellata*—between the Bacteria and the Mastigophora. Now I think that I am completely justified—from what I have already pointed out in the preceding part of this paper—in stating that there is not one character of

importance which is common to Spirochæts and Flagellates—save that both are unicellular. It is, to me, most remarkable that anyone can see any real resemblance between a Spirochæt and a Trypanosome. The nuclear and cytoplasmic structures are wholly different: a Trypanosome has a flagellum, a Spirochæt has none:¹ the crista is not like an undulating membrane: the cell-membranes are not similar: and moreover, the method of division is quite different in the two groups of organisms. As regards conjugation, nothing has been proved either in Trypanosomes or Spirochæts, so that its occurrence or non-occurrence can furnish no grounds for discussion of affinities between the two groups. The flexibility of Spirochæts also, as I have pointed out, affords no criterion for determining their protozoal or bacterial affinities.

Many workers regard the Spirochæts as Bacteria. Novy and Knapp (1906) place them in the genus *Spirillum*. Swellengrebel (1907) places the Spirochæts and Spirilla in the same family (*Spirillaceæ*) among the Bacteria. Schellack (1909) suggests that the Spirochæts are related to the Cyanophyceæ by way of *Spirulina* and similar forms. Gross (1910) finally places *Spirochæta* with the Cyanophyceæ, and *Cristispira* and *Treponema* with the Bacteria. Zülzer (1910), however, who has made a special study of *S. plicatilis* and the spiral forms of Cyanophyceæ (*Spirulina*, *Arthrospira*), has shown that there is no real similarity between these organisms. Affinities between Spirochæts and Cyanophyceæ appear therefore not to exist.

Now beyond a certain superficial similarity of form between certain Spirochæts and Spirilla, there is really no reason for regarding Spirochæts as Bacteria. The points of similarity are chiefly these—both possess the same sort of cell polarity (see p. 532), both divide transversely, both are plasmolysable.

¹ The "flagella" of various species of *Treponema* are probably—as has often been pointed out already—merely the drawn-out ends of organisms which have just resulted from the transverse division of a longer organism. They have nothing to do with the flagella of Protozoa or Bacteria.

But the same might be said of many other Protista. Two most important characters of the Bacteria—the formation of endospores and the possession of flagella—are not encountered in the Spirochæts. The structure of the cell, especially as regards the nucleus, in *Cristispira* and *Spirochæta* is quite different from that of *Spirilla*. With regard to the latter, I would refer the reader to my work on the cytology of the Bacteria (Dobell, 1910A). There is, in fact, no real reason for regarding Spirochæts as Bacteria.

There seems to be a curious tendency on the part of many workers to reason thus: Spirochæts are not Protozoa, therefore they are Bacteria; or conversely, they are not Bacteria, therefore they are Protozoa. The premisses are both correct, I believe, but the deductions are both wrong. Spirochæts are neither Protozoa nor Bacteria; they are a group of Protista which stands alone. They certainly have a few characters in common with Bacteria, but the differences greatly outweigh these.

In conclusion, I will summarise the results to which my work has led me. They are as follows:

The organisms commonly called Spirochæts may be conveniently collected into a single group, for which I propose the name Spirochætoidea. This group comprises three different sets of forms, which may be correspondingly classified in three different genera—*Spirochæta*, *Treponema*, *Cristispira*. These three groups of organisms, whilst showing certain resemblances to one another, possess no definite relations with Protozoa, Bacteria, or Cyanophyceæ. The Spirochætoidea should therefore be regarded—for the present—as a group of Protista which stands apart.

IMPERIAL COLLEGE OF SCIENCE AND
TECHNOLOGY, LONDON.
November, 1910.

ON CRISTISPIRA VENERIS NOV. SPEC.

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EXPLANATION OF PLATE 20,

Illustrating Mr. C. Clifford Dobell's paper "On *Cristispira veneris* nov. spec., and the Affinities and Classification of Spirochaets."

[All figures are drawn from stained preparations of *Cristispira veneris* n. sp., from the crystalline style of *Venus* (*Meretrix*) *casta* Chem., taken in Tamblegam Lake, E. Province, Ceylon. The drawings were made under a Zeiss 2 mm. apochromatic oil-immersion, with compensating oculars 6, 12, and 18. The magnification of the figures is approximately 2000 diameters.]

Fig. 1.—An average-sized individual, in optical section. The general form of the body is well seen. Note also the chambered structure of the cytoplasm and the arrangement of the purple-stained granules. (Osmic vapour 30 secs. absolute alcohol; Giemsa's stain.)

Fig. 2.—A somewhat extended individual, showing the disposition of the crista and the structure of the protoplasm. The body is seen in optical section, but the crista is shown as it appears when focussed carefully at different levels. (Osmic vapour [several minutes]; absolute alcohol, Giemsa.)

Fig. 3.—An individual which has been macerated in a drop of sea-water, allowed to dry, then fixed in absolute alcohol and stained by Giemsa's method. The organism has undergone plasmoptysis, and the crista shows a fibrillar structure.

Fig. 4.—A dividing organism in the stage of incurvation. (Osmic vapour, absolute alcohol, Giemsa.)

Fig. 5.—Part of an almost completely divided organism in incurvation stage. The upper end corresponds with the upper end of fig. 4, being the point at which fission occurs. (Slightly more highly magnified than the other figures.) (Osmic vapour, absolute alcohol, Giemsa.)

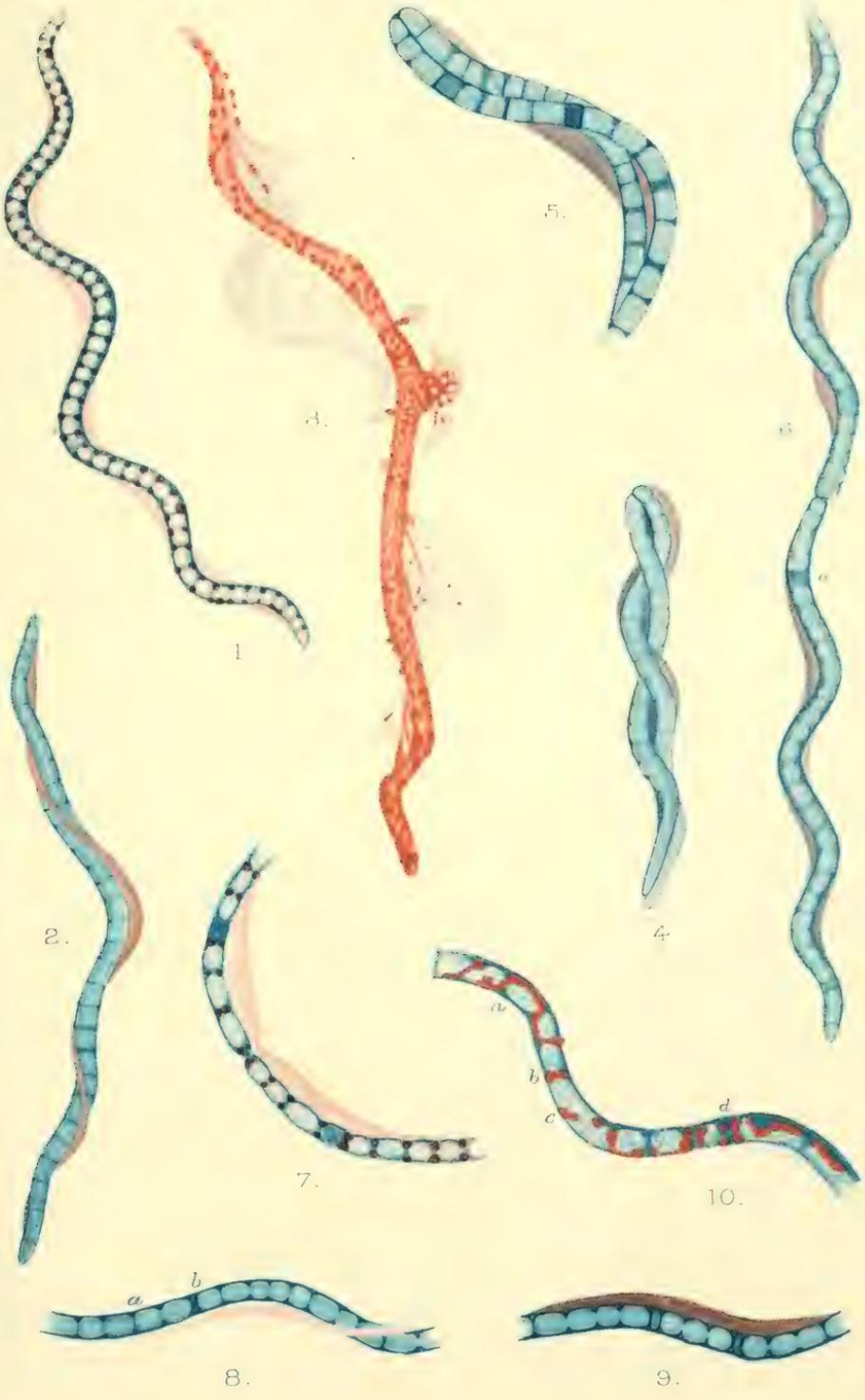
Fig. 6.—An individual which is almost completely divided into two. Stage following incurvation. At *a*, a darkly stained chamber. (Osmic vapour [several minutes], absolute alcohol, Giemsa.)

Fig. 7.—Middle region of an individual which is opening out after incurvation. Division of body and crista is seen. (Osmic vapour 30 secs., absolute alcohol, Giemsa.)

Fig. 8.—Part of the body of an organism which has been fixed by exposure for several minutes to osmic vapour, then treated with absolute alcohol, stained with Giemsa, and differentiated in alcohol. At *a* the dark blue granules are distinctly seen; at *b* is seen a thickened partition.

Fig. 9.—Part of another organism, treated like the preceding, but more deeply stained. The granules are not sharply differentiated from the walls of the chambers. At two points new chambers are being formed.

Fig. 10.—Part of a dried organism fixed in absolute alcohol and stained with Giemsa. "Nuclear" structures in the form of a spiral or zig-zag filament (*a*), a transverse bar (*b*), granules (*c*), a tetrad (*d*), etc., are seen.



On the Development and Structure of the Trochophore of *Hydroides uncinatus* (Eupomatus).

By

Cresswell Shearer, M.A.,
Trinity College, Cambridge.

With Plates 21-23 and 29 Text-figures.

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I. INTRODUCTION.

WHILE working at Naples some years ago, I was led to investigate the early development of the Annelid *Eupomatus* with a view to determining the origin of the mesoblast bands and their relation to the head-kidneys. This species is common at Naples and breeds throughout the year. The blastulæ and gastrulæ are very hardy, and development is normal under the adverse conditions of heat and impure sea-water incidental to their study under laboratory conditions. Fertilisation takes place quickly when the ripe generative

products are brought together, and material can be easily obtained of any stage. The trochophores can be readily reared to the adult worm in small jars of sea-water to which sufficient food is added from time to time, in the form of cultures of the common Diatom *Nitzschia closterium*. On this they rapidly grow, and soon attach themselves to the sides of the culture jars and form their tubes.

The minuteness of the egg is a serious disadvantage, however, in following the changes that lead up to the establishment of the trochophore. The fully formed larva barely measures 65μ in diameter, and the pre-trochophoral stages are very small, and the cells of the blastulæ and gastrulæ are unusually minute. In following the origin and growth of the head-kidneys one is forced to depend almost wholly on sections, and sectioning larvæ of this size is a tedious proceeding.

In the Serpulid *Pomatoceros* I soon found a more suitable object in which to trace the development of the head-kidneys. The egg is larger and more deeply pigmented. In the arms of the "cross-cells" this pigment quickly becomes segregated on development, where it affords a ready means of orientation. For these reasons I early abandoned the study of *Eupomatus* for that of *Pomatoceros*, on which I hope shortly to complete my "Studies on the Development of Larval Nephridia," by publishing a full account of the origin and growth of these organs in this animal.

The present notes dealing with *Eupomatus*, although incomplete, I have thought worthy of publication, as they deal with the formation of the trochophore and the appearance of the coelomesoblast. They derive some importance from the fact that on this Annelid, Hatschek (17) conducted his classical investigations on the development of the mesoblast bands—investigations which have played so prominent a part in all our speculations concerning the mesoderm. Any revision, therefore, of the subject on the same material as that studied by him is not without interest.

In the following account I have incorporated some drawings and notes of *Hydroides pectinata*, kindly placed at my disposal by Prof. E. B. Wilson, which I believe were made by him some years back.

One object I have kept in view has been that of following the changes leading from the gastrula to the formation of the trochophore. In the numerous careful accounts of the development of Annelids that have been published few attempts exist to connect the cell regions of the early stages with the organs of the trochophore. Many of the early embryologists, as Kowalevsky (23), Agassiz (1), Hatschek (18) and Salensky (30) seem to start their studies only with the young larva, when the rudiments of the larval organs have already appeared. On the other hand, many of the more recent investigators, commencing with the unsegmented egg, frequently fail to carry their studies far enough when they stop short at the end of gastrulation, and before the definitive organs of the larva have appeared.

Some considerable confusion has arisen through taking the conditions found in relation with the mesoderm at relatively late stages, and considering these same relations to hold in the early phases. This is seen in the work of Hatschek (18) and Fraipont (12) on *Polygordius*; and has resulted in some error with regard to the head-kidney rudiments, larval and cœlomesoblast.

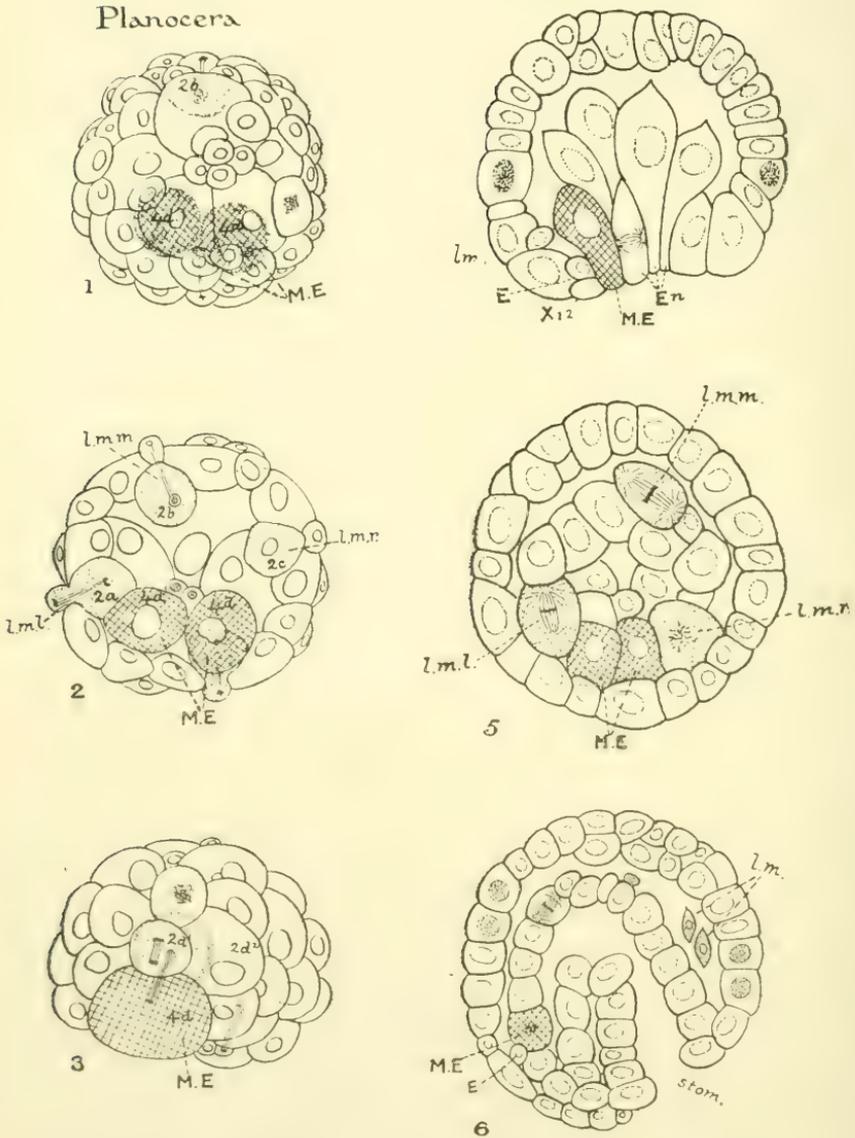
In all Annelids with a free-swimming larva such as that of *Eupomatus* there is always a considerable interval between the end of gastrulation and the assumption of the full trochophoral condition. This period, for the sake of convenience, I shall refer to as that of the pre-trochophoral stage. It is the period of which we know the least in the development of Annelids.

The excellent papers of Woltereck (52) on *Polygordius* and Torrey (41) on *Thalassema* have done much to advance our knowledge. The early cell-regions have here been traced clearly to their ultimate fate in the organs of the trochophore. Woltereck has shown that the head-kidneys arise early and

TEXT-FIGS. 1-6.

Podarke

Planocera

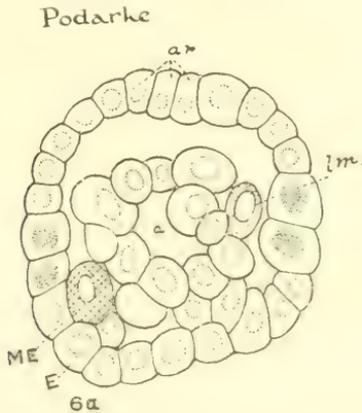


Text-figs. 1-3.—Early segmentation stages of *Planocera* (Surface).
 Text-figs. 4-6.—*Podarke* (Treadwell). *E.* 1st endoderm cell budded off from $4d_2$. *En.* Entomeres. *l.m.l.* Left portion of the ectomesoblast. *l.m.m.* Median portion of the same. *l.m.r.* Right portion of the same. *M.E.* Cælomesoblast. *Stom.* Stomodæum. $X_{1,2}$. Anal cell.

before the mesoblast bands. They are already functional before the bands have appeared, for the pole-cells so conspicuous in Hatschek's figures have no existence at this stage.

It is true that Meyer (27), from the study of late stages, came to the conclusion that larval mesoblast was a structure apart from the cœlomesoblast, but I doubt if the evidence derived from the study of these late stages in *Polygordius* alone is sufficiently convincing.

TEXT-FIG. 6A.



Section through a late gastrula stage of *Podarke* (Treadwell).
a. Archenteron. *a.r.* Apical rosette. *E.* First endoderm cell budded off from $4d_2$. *l.m.* Larval or ectomesoblast.
ME. Cœlomesoblast.

Within the last twenty-five years a large literature has grown up with regard to the question of the mesoderm, and embryologists have held many opinions regarding its origin and significance. These conflicting views are roughly reducible, however, to two groups, each of which has been advocated with more or less success. To the first belong those who consider the mesenchyme (larval mesoblast, ectomesoblast) and mesothelium (definite mesoderm or cœlomesoblast) as one and the same structure; to the second belong those who consider them as two separate structures.

The first consider they have a common, while the second claim they have a separate origin.

Hatschek, as the result of his studies on *Polygordius* (18), *Echiurus* (16), *Eupomatus*, and *Teredo* (17 and 15), many years ago pointed out the difference between the irregular scattered cells of the mesenchyme and the definite cells of the mesoblast bands. He claimed, however, to have observed the origin of the mesenchyme cells from the mesoblast bands. In his opinion mesenchyme and mesothelium arise from a common foundation. This was followed by Wilson's (48 and 47) work on *Hydroides*, *Polygordius* and *Lumbricus*, where he found a complete gradation from the stellate cells of the mesenchyme scattered through the blastocœl to the round fixed cells of the anterior ends of the germ bands. Many other observers have pointed out more or less the same thing, as, for instance, Roule (29) in *Euchytræoides*, Fraipont (12) in *Polygordius*, Bürger (5) in *Nepheleis*, *Hirudo* and *Aulastoma*, Hacker (13) in *Polynoe*. The common nature of both mesenchyme and mesoderm at one time gained wide acceptance through its adoption and elaboration by the brothers Hertwig (19) in their well-known 'Cœlomtheorie.'

On the other hand, the majority of those embryologists who have recently investigated the development of Annelids and Molluscs hold that these structures are both ontologically and phylogenetically distinct; that the mesenchyme has an origin apart from the cœlomesoblast, that it arises in a peculiar fashion from the ectoderm; hence they have sought to denote this in the name they have applied to it, i.e. that of ectomesoblast. The cœlomesoblast, on the contrary, is usually segregated in a single large cell seen in the ventral lip of the blastophore.

Kleinenbergh (21) was perhaps the first to lead the way towards this conception of the nature of mesoderm and mesenchyme, in his paper on the development of *Lopadorhynchus*, where he pointed out that the mesoderm arises as a membrane between the two primary layers, and, as he

thought, direct from the ventral side of the ectoderm. This was followed by the work of Whitman (45) on Clepsine, Bergh (3) on Lumbricus, Vejdovsky (44) on Oligochaets. Schimkewitsch (32) in Dinophilus described a separate origin of the mesenchyme in the anterior end of the larva from the definite mesoderm of the posterior region. Finally the separate nature of mesenchyme and cœlomesoblast has been most ably championed in the very extensive researches of Meyer (27) on the mesoderm of Annelids.

In the work of the cell-lineage investigators, however, the distinction between larval and cœlomesoblast has been most definitely brought to light. In all Annelids, Lamellibranchs, and Gasteropods studied by them, with one exception, the cœlomesoblast invariably arises from a large cell in the ventral side of the blastophore (*4d*). The one exception is the Annelid *Capitella*, where, according to Eisig (11), it arises from the third and fourth quadrants of the third quartette. Here the cell *4d* contains a little larval mesoblast, but the main portion contains ectoderm. In Molluscs, according to Conklin (7), *4d*, while containing the cœlomesoblast, is more than half endoderm. In the Annelid *Podarke*, according to Treadwell (42), *4d* divides and then sinks in, and takes up its position in the endoderm of the archenteron (Text-figs. 4, 5, 6). Here at a later stage it gives rise to the cœlomesoblast.

At the time *4d* is being invaginated, or even before, irregular ectoderm cells are given off into the interior of the blastocœl; these are the larval mesoblast cells. They migrate inwards and scatter throughout the cavity. Their origin has been determined in a large number of forms, first by Lillie (25) in *Unio*, and then by Conklin (7) in *Crepidula*, Treadwell (42) in *Podarke* (Text-fig. 5, *l. m. r.*, *l. m. l.*, *l. m. m.*), Wierzejski (46) in *Physa* (Text-fig. 11, *l. m. r.*, *l. m. l.*), Torrey (41) in *Thalassema* (Text-figs. 8 and 9, *l. m. r.*, *l. m. l.*, *l. m. m.*). The mode of origin of the ectomesoblast, therefore, is distinctly in opposition to that of the cœlomesoblast. In *Unio* it arises asymmetrically, and only afterwards takes

up a bilateral position. In *Thalassema* it arises from the first and third quartettes. In some thirty Annelids it can be said definitely that the coelomesoblast arises from the posterior cell of the fourth quartette, while the larval mesoblast arises from the first and third. This title of "larval mesoblast" does not mean necessarily that it is confined alone to the organisation of the larva, for the greater part of it enters into the structure of the adult. The same has been shown to be the case in a number of other Annelids, as in *Polygordius*, *Podarke*, and *Thalassema*.

Meyer long ago put forward the theory that the mesenchyme of higher forms corresponds with the mesoderm of the lower; that the larval mesoblast of Annelids and Molluscs is to be homologised with the adult mesoderm of Platodes. Wilson (50) has shown in *Leptoplana* that the mesoderm in this Polyclad arises from the second and third quartettes, while in Annelids the larval mesoblast, as I have mentioned above, takes its origin from the same quartettes. He has established that here the large cell $4d$ is almost entirely entoblastic. The early development of the Polyclad *Planocera* has been studied by Surface (40): "At the forty-four cell stage the posterior cell of the fourth quartette ($4d$) buds a single large cell into the interior of the embryo; both of these subsequently divide bilaterally (Text-fig. 3). Of these four cells the two upper and inner (Text-fig. 3, $2d$) give rise to a portion of the mesoderm, and possibly a small part of the endoderm (Text-figs. 1-3, $4d$). The lower pair lying on the surface of the embryo give rise practically to all the endodermal part of the alimentary canal." Thus the history of this cell ($4d$) in this Polyclad shows a remarkable resemblance to its homologue in Molluscs and Annelids. "A portion of the mesoderm, chiefly that part lying round the pharynx, is derived from cells of the second quartette, and thus corresponds with the secondary mesoblast or larval mesenchyme of Annelids and Molluscs (Text-fig. 1, $2b$). In the spiral cleavage the segregation of the ectoblast in three quartettes, the formation of a large portion of the mesoderm

from $4d$, the formation of the apical cells, and in many other details, the development corresponds to that of Annelids and higher forms."

In *Platodes* the mesoderm has a radial origin, and this is also the case in a number of Annelids with regard to the larval mesoblast. I have also mentioned in a number of Annelids and Molluscs that a small portion of $4d$ is entoblastic. The condition in Polyclads, where the greater part of $4d$ is entoblastic, is suggestive of a more primitive condition than that found in Annelids. If the germ-cells in the Polyclad arise from the $4d$ portion of the mesoderm, then the homology of this cell with the cell $4d$ of Annelids would be complete. The history of the posterior cell of the fourth quartette in Polyclads, Annelids, Lamellibranchs, and Gastropods has a remarkable resemblance in all these forms, and the relation it shows with the endoderm of the gut points clearly, as Wilson (50) has said, to the way in which teloblasts have arisen by progressive specialisation from a purely endodermic origin of the cœlomesoblast as retained in an unaltered condition in the Echinodermata to-day. As he says, it is difficult to explain these facts otherwise than on the grounds "that cell outlines represent definite boundaries of differentiation areas in the developing embryo."

Child (6), on the contrary, claims that no importance can be attached to resemblances of this nature, and that in the case of the cell $4d$ they are purely cœnogenetic, and have to do with the formation of the larval body from a growing region at its posterior extremity, and the resulting segregation of material at this point. I think this cannot be said of all cases where there is a similar segregation of the cœlomesoblast. The growth of the adult from the *Glochidium* larva is different in many respects from the growth of the adult worm from the trochophore, yet in both we get a marked segregation of the cœlomesoblast.

With the high degree of specialisation shown by eggs that give rise to a free-existing larva, the cœlomesoblast, which primitively arose as diverticula from the gut, became restricted

in the course of time to certain cells in the wall of the archenteron, and as development became progressively specialised their origin became localised in the posterior cell of the fourth quartette.

It is from the wider and more definite facts of comparative anatomy rather than from those drawn from development alone that the real value of Meyer's theory lies. It is evident that the germ-cells—the foundation of the later cœlomesoblast—are much older in the phyletic sense, as Kleinenbergh (21) and Meyer (27) long ago pointed out, and as Eisig (11) has recently stated, than the two primary germ layers, and that they were differentiated long before the ectoderm and endoderm had been evolved as separate structures, as is the case to-day in *Volvox*. Meyer's theory has been recently considered by Lang (24) and Eisig (11) so exhaustively that it is unnecessary for me to go into it here. No theory certainly accounts for so many facts or has been so widely supported by evidence, both anatomical and embryological.

Thus the separation of the mesoderm into two definite portions is a characteristic feature of the development of Polyclads, Annelids, and Molluscs. It remains to be mentioned that in a few Molluscs a larval or ecto-mesoblast has not been observed or is apparently wanting. This would seem to be definitely the case in *Aplysia*, the late stages of which have recently been studied by Carr Saunders and Miss Poole (31). In *Umbrella*, Heymons (20) has been unable to find this structure, but he suggests that possibly in stages later than those he studied ectoderm cells may migrate into the interior of the larva and form mesoderm. In *Neritina* Blochmann (4) also fails to figure it; but both Heymons and Blochmann's work was done at a time when the importance of the larval mesoderm was hardly recognised, and ultimately it may prove to be present in these forms. Its absence in *Aplysia*, however, seems to be clearly established. It is hard to understand why this should be the case, as the majority of Molluscs possess a larval mesoblast, and one is present in *Fiona*.

Korschelt (22) has called attention to the relation of the ecto- and cœlomesoblast in *Physa*. If we take a section of such a stage of *Physa* as is shown in Text-fig. 11, he points out that the ecto- and cœlomesoblast between them form a complete ring round the blastopore. He thinks this condition points to the conclusion that in Annelids and Molluscs ecto- and cœlomesoblast were originally one structure, which has been divided and specialised as the result of larval development. In *Phoronis* and the other great group of animals of the Deuterostomia type this has not taken place. *Phoronis* is undoubtedly closely related to the Annelids in the Actinotrocha stage, with its solenocyte-bearing nephridia and ciliated rings, but shows no segregation of the cœlomesoblast into pole-cells.

From the work of De Selys Longchamps (34) we know that the mesoderm consists of a large number of irregular cells scattered throughout the blastocœl. I have shown (36), and it has also been clearly demonstrated by the work of other investigators, that these cells arise in the region of the blastopore, or from the line along which the blastopore has already closed. The cells resemble the larval mesenchyme of Annelids more than the cells of the Annelid cœlomesoblast.

In Brachiopods the mesoderm is also of the irregular variety, and arises from the cœlom, which is here a direct outgrowth from the anterior end of the primitive archenteron, as Conklin (7) has recently described in *Terebratulina*. No division into ecto- and cœlomesoblast can be distinguished, and it is purely cœlomesoblastic.

There would thus seem to be a sharp division between *Phoronis* and Brachiopods on the one hand, and Annelids and Molluscs on the other. In one we get a sharp division of the mesoderm into two portions, while in the other there is no such division. Korschelt (22) thinks that without a more definite knowledge of how the cœlomesoblast arose in the hypothetical Annelids, we cannot reconcile these two types of mesoderm formation.

It appears to me, however, that in *Phoronis*, or at least in the early stages of the *Actinotrocha* larva, we have exactly the same thing as in Annelids.

I have said that in *Phoronis* the mesoderm arises in the region of the blastopore as a number of irregular cells, which are budded off into the blastocœl. These scatter throughout the cavity, where they give rise to the mesodermic structures. According to De Selys Longchamps (34), some of these cells in the trunk region collect to form a rather imperfect cœlomic sac about the rectum or posterior portion of the stomach. I was of opinion, however (36), that the cells that gave rise to this sac had their origin in the gut wall, but of this I was by no means certain. In any case, in *Phoronis* we have the mesoderm showing a specialisation into a cœlomic portion, forming the primitive cœlomic sac, and the irregular mesodermal cells scattered throughout the blastocœl. Whether we regard the cœlomic portion as arising from the gut wall or not, it seems to me we have here the two forms of mesoderm as in Annelids, and that *Phoronis* is intermediate between Annelids and animals in which the mesoderm is entirely cœlomic. Korschelt (22) sums up the mesoderm formation under five heads, which are worth reviewing in this connection.

1st. Mesoderm band formation from teloblasts or pole-cells, as in Annelids and Molluscs.

2nd. Secondary mesoderm band formation, a modification of the above process, and re-multiplied in Arthropods and Cephalopods.

3rd. Formation of mesoderm from gut pouches.

4th. Formation of mesoderm from solid out-growths of the gut.

5th. The mesenchyme cells alone give rise to the cœlom and all the mesodermic structures.

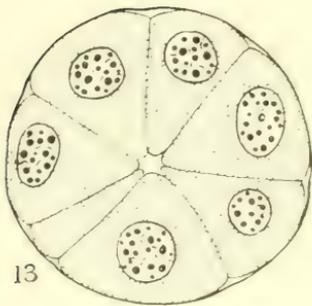
To the trochophore originally described by Hatschek (17) in *Eupomatus* undue importance has perhaps been attached, for such a trochophore is possessed by only a limited number of Annelids, and is almost exclusively confined to the group

of the Serpulids. Our text-books frequently cite it as a typical trochophore, although most Annelids possess a trochophore quite different. The trochophore characteristic of the majority of Polychaets is one such as that of *Sabella* or *Nereis*, and not that of the Serpulids. This possesses no head-kidney, and the mesoderm bands develop under conditions that modify their growth as compared with those of *Eupomatus*. The blastocœlic cavity in these is always greatly reduced or entirely obliterated, and gastrulation is usually epibolic; while in the Serpulid larvæ there is always a large blastocœlic cavity, and gastrulation is by invagination. The egg in the majority of the Serpulids, again, is small and contains very little yolk, although forms like *Spirorbis* and *Sabella* contain a considerable quantity. It is hard to make any fast distinctions, however, for larvæ occur in the same family, and even in the same genus, which differ entirely in this respect. The principal cause of this great diversity of form is due in most cases to the modification undergone by their locomotor organs, as the result of their adoption of different life-habits. Frequently closely related larval forms differ greatly in this respect. If they live a free-swimming pelagic existence, or the contrary, their locomotor organs are correspondingly developed or reduced. *Terebella conchilega*, leading a pelagic life, possesses strongly developed ciliated rings, and is a powerful swimmer, while *Terebella meckelii*, for the most part spending its larval existence in the jelly-like mass in which the eggs are deposited, is uniformly ciliated, and lacks these structures.

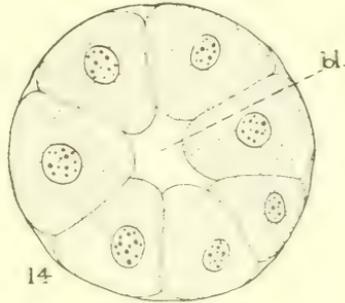
Thus the tuberculous Polychaets can be divided roughly into two classes on the basis of their possession or non-possession of a trochophoral stage. The first of these, including *Eupomatus*, *Pomatoceros*, and *Psygmodranchus*, possess typical free-swimming larvæ with well-developed prototroch and ciliated rings; while a second group, including some of the *Terebellids*, *Aricia* and *Arenicola*, do not possess a free-swimming stage, are often uniformly ciliated, and are poor swimmers. In addition, we

TEXT-FIGS. 13-18.

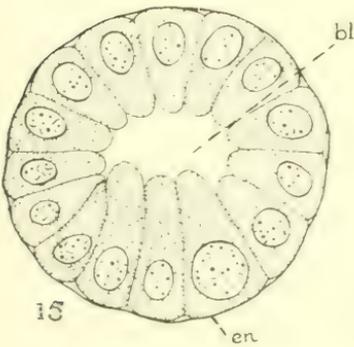
Eupomatus



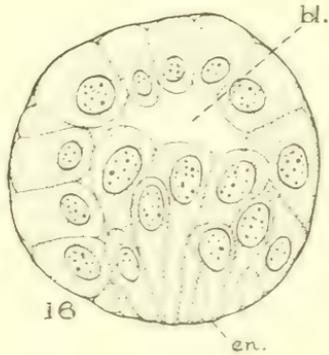
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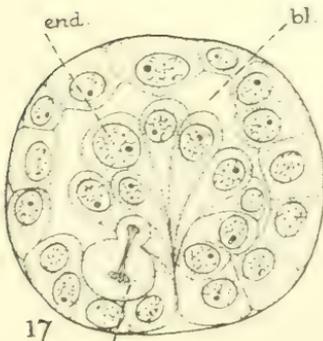
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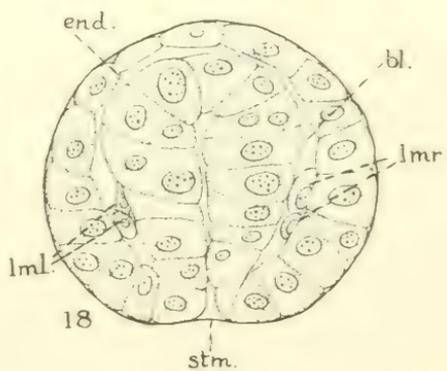


16



17

ME



18

stm.

Sections of segmentation and gastrulation stages of *Eupomatus*.
bl. Blastocœl. *end.* Endoderm. *ME.* Cœlomesoblast. *lml.*
 Left portion of ectomesoblast. *lmr.* Right portion of same.

find a very large number of errant forms, which have undergone so much modification that in many cases it is difficult to say to which group they belong. In the first class of the pelagic type we have the larvæ of *Nereis*, *Phyllodoce*, and *Aphrodite*, while in the second we have forms like *Diopatra*, *Ophryotrocha*, and many of the Eunicid larvæ. Probably the most difficult to class of all are these last, on account of their great variation (Häcker, 13).

Without some knowledge, therefore, of the mode of life of the primitive ancestors of the Polychæts, and the conditions under which they existed, it is difficult to decide which of these various larval types is the most primitive. It is probable that the free-swimming type has been recently evolved, and is a more highly modified one than the uniformly ciliated type, that the trochal has been derived from the atrochal form. And this is borne out by the fact that in its most perfect form, as in *Eupomatus*, it is found in so relatively few Annelids.

2. REVIEW OF LITERATURE.

The early development of the Serpulid Annelids has been studied by a number of investigators. The earliest account is that of Stossich (39) in 1878, who described in some detail the development of *Serpula uncinatus* (*Eupomatus*) and *S. glomerata*. It is clear from his figures that many of his larvæ were abnormal. I have obtained many similar larvæ during the hot months in Naples, when the temperature of the Laboratory sea-water was unusually high. Through the study of these larvæ Stossich came to many erroneous conclusions.

Salensky (30), in 1883, studied the development of *Psygmobranchus* and *Terebella*. In these forms the presence of a considerable quantity of yolk and the absence of a true trochophoral stage considerably modify the course of development. He arrived at no certain conclusions regarding the origin of the mesoderm, although he observed the teleoblast cells of the mesoderm bands.

Conn (9) pointed out that in *Serpula* the egg-chorion is never thrown off, but remains as the cuticle of the larva. The gastrula has three noticeable features. The blastopore is not round but slit-like, and arranged round its margin is a circular band of locomotor cilia. Right opposite the blastopore is the apical thickening, bearing a tuft of hair-like cilia. The growth of the gastrula is not accompanied by elongation in the main axis, but obliquely to this in such a way as to pass through one end of the slit-like blastopore. One end of the blastopore is thus carried backwards away from the other, which remains more or less fixed. The blastopore becomes an elongated slit, the lips of which meet in the middle and close, forming the rudiment of the future gut. For a short time the digestive tract remains attached to the ectoderm throughout the length of the blastopore, but after a little it only retains this connection at either end. With further growth the embryo is converted into the trochophore. The digestive tract becomes hollow and acquires two openings to the exterior at the two points of its previous connection with the ectoderm. That near the ciliated band becomes the mouth, while the other becomes the anus.

“Just before the formation of the anus a number of ectodermal cells near the region of the future anus become separated from the rest of the digestive tract and form a mass of cells lying outside the alimentary canal in the body-cavity. These cells form the mesoderm. Some of these cells increase in size and form stellate mesenchyme cells, and finally a few of them stretch across the body-cavity near the anus, forming a membrane which separates a small portion of the body-cavity from the rest, forming the anal vesicle. Occasionally another partition grows across it, separating it into two smaller divisions.” Certain other mesoderm cells form the true mesoderm. “They multiply quite rapidly, and soon give rise to the mesoderm bands. One of the eye-spots develops much before the other” (p. 671).

Von Drasche (10), in 1884, gave an account of the development of *Pomatoceros*, but the early stages and the forma-

tion of the trochophore were very briefly studied. He did not observe the origin of the mesoderm cells.

Hatschek (17), in 1885, studied the development of *Eupomatus* at Trieste. He supplemented these observations by the examination of a small trochophore found in the Pantano, at Faro, Sicily. The identity of this larva he did not definitely establish. The eggs studied at Trieste were fertilised by the addition of ripe sperm, and were studied in the living state. Segmentation is equal, and of the spiral type characteristic of many Polychæts. In the resulting blastula the cells from which the germ layers form are already differentiated. The greater part of the lower hemisphere of the blastula produces endoderm. Two cells here larger than the rest give rise to the primitive mesoderm cells, or teloblasts. The region where they lie corresponds to the anal end of the larva. At this time the pre-oral band of cilia makes its appearance as an equatorial circle of cilia. Shortly afterwards the apical cilia appear. The endodermic part of the blastula invaginates about nine hours after fertilisation. The two mesoderm cells at the same time move to the interior of the segmentation cavity and detach themselves from their connection with the other cells. The invaginated portion of the endoderm forming the gut then bends towards the anal side of the larva, and fuses with a slight depression of the ectoderm and produces the anus and proctodæum. At the same time the blastopore has become narrowed to a slit, which gradually closes from behind forwards. At the place where the last trace of the blastopore remains the ectoderm invaginates and forms the œsophagus. At the same time the two primitive mesoderm cells divide, giving rise to the mesoderm bands, while other cells near the pole-cells of the bands give rise to the head-kidneys; these increase greatly in length and become hollow. The head-kidney then extends from the pole-cells in the region of the anus to the wall of the œsophagus, to which they are attached by a thin protoplasmic strand, while another runs up in the apical region. They open, according to Hatschek, on the exterior on either side of the

anus. The eye-spot is located in a cell in the apical region. There is a peri-anal circle of cilia.

E. B. Wilson (48) briefly studied (1890) the segmentation of the egg of a species of *Hydroides* found at Naples. The order and direction of the early cleavage planes coincide very closely with those of *Eupomatus*, and segmentation is of the equal spiral type. The spiral symmetry would seem to be retained until a late stage. He did not definitely observe the cell $4d$ or follow its history. In his early paper (48) on the origin of the mesoblast bands of Annelids he was of opinion that the bands gave origin to the mesenchyme cells. He did not observe the pole-cells of the bands as described by Hatschek. He pointed out that the head-kidney probably opened into the proctodæum.

The later development of *Psygmobranchus* has been studied by Meyer (27), who made some important observations on the mesoderm. He pointed out that in the young trochophore it can be divided under three headings: First, the mesoderm bands, which are closely applied to the ventral surface of the endoderm; secondly, a collection of irregular cells attached to both ecto- and endoderm, which we can call the embryonic mesenchyme; thirdly, a row of functional primary larval muscles. The mesoderm bands appear as a paired plate of cells converging on one another posteriorly, each ending in a pole-cell—the so-called teloblasts. The plates extend forward into the oral region. The cells of the mesoderm bands can be clearly distinguished from the irregular cells of the mesenchyme by their polygonal outlines and their dark-staining nuclei. The larval mesenchyme cells, on the contrary, are irregular in outline, and their nuclei stain less deeply than do those of the bands. The mesenchyme does not form a compact structure, but is somewhat irregularly arranged into masses on the inner wall of the ectoderm or the wall of the gut. It is divided into a median and a lateral portion, which is again divided into a trunk and head portion.

The lateral trunk mesenchyme lies on either side of the

inner surface of the body-wall, and commences behind the teloblast cells of the mesoderm bands, and runs forward in the region of the oral ciliated ring. In the pre-oral region one finds a number of these mesenchyme cells under the body-wall, where they form a portion of the head division of the mesenchyme in relation with the prototroch and apical organ.

The median trunk mesoderm begins behind in front of the anal vesicle, and is continued forward in the median line under the gut into the region of the stomatodæum between the mesoderm bands. The functional larval muscles of the mesenchyme type consist of a ventral and dorsal longitudinal set and the pre-oral circular muscles of the prototroch. With the growth of the larva the greater part of the larval mesenchyme is converted into the definite musculature of the adult. The mesoderm bands in no instance give rise to mesenchyme cells, and the two can be sharply distinguished throughout the course of the larval development.

The development of *Spirorbis borealis* has been briefly described by Schively (33). There is a very small blastocœl, and the blastopore is a median ventral slit. It closes from the posterior end forwards until nothing remains but a small aperture at the anterior end, which becomes the future mouth. The endoderm on invagination forms the archenteron. The mesoderm can be traced to the left posterior macromere, which sinks into the segmentation-cavity, giving rise by a bilateral division to the primitive mesoderm cells. No mention is made of the larval mesoblast.

The early development of *Serpula infundibulum* has been studied by Soulier (38) in 1902. The main outcome of his work has been to confirm very closely Hatschek's results for *Eupomatus*. The mesoderm cells are recognisable as two large cells in the endoderm at the time of invagination. They arise at the point of union of ecto- and endoderm, and pass into the segmentation-cavity, where they give rise to the mesoderm bands. Their relation to the irregular cells of the mesenchyme was not determined.

Apart from the Serpulids, the development of *Thalassema*, *Podarke*, and *Polygordius* closely resembles that of *Eupomatus* in its essential features. The cleavage in these is of the equal spiral type of that of *Eupomatus*, and, in fact, the early cleavage of *Podarke*, *Thalassema*, and *Eupomatus* are almost similar cell for cell until the time of gastrulation. In each the gastrula is formed by invagination, and a well marked blastocœlic cavity is present. In the later stages of gastrulation *Thalassema* and *Polygordius* more closely approach *Eupomatus* than does *Podarke*. This is possibly due to the fact that the trochophore of *Podarke* is somewhat modified, apparently not having any head-kidney. *Polygordius*, with its large blastopore, represents possibly a more primitive condition than do the others. *Thalassema* in the pre-trochophoral stage approaches nearer *Eupomatus* than do the others, for in *Polygordius* the head-kidneys form some time before the mesoderm bands. The details of the resemblance between these four will be considered further on.

3. MATERIAL AND METHODS.

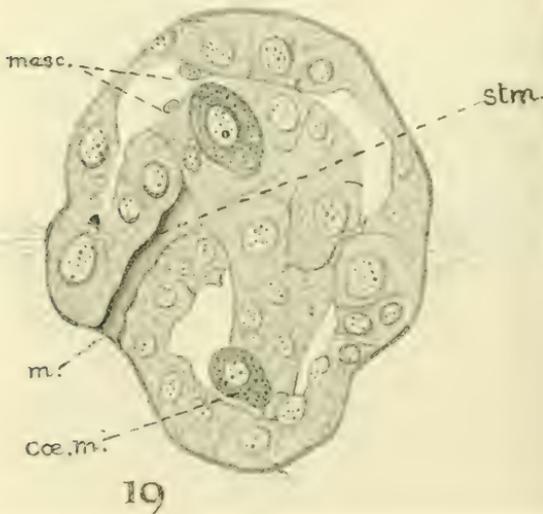
When the sexual products are ripe in *Eupomatus* it is an easy matter to distinguish the sexes from the colour of the body. The female is bright yellow, while the male is white. In Naples they grow in dense colonies attached to stones, the sexes being evenly proportioned, although the males and females show a slight tendency to occur together in separate spots in the colony. Their tubes stand upright, being attached by one end.

In effecting fertilisation under artificial means, it is unnecessary to wait until the eggs are deposited as in many Annelids, as *Nereis*, *Podarke*, *Phyllodoce*. The ripe eggs cut from the body-cavity fertilise as readily as those laid in the normal manner.

The egg of the Neapolitan *Eupomatus* seems to be more opaque than that studied by Hatschek at Trieste, for I have

been unable to follow the fate of the invaginated cells during gastrulation in surface views of the living egg as he was able to do. In the following work I have relied entirely on the evidence of sections. By means of the combined celloidin-paraffin method of embedding, one is able to obtain good sections of small gastrulæ such as these. For fixing I have found sublimate acetic and Flemming solution give satis-

TEXT-FIG. 19.

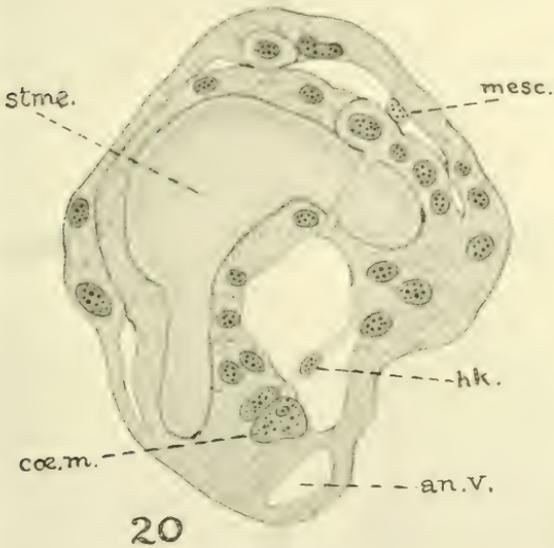


Section of early trochophore of *Eupomatus*. *coe.m.* Celomesoblast. *mesc.* Mesenchyme. *m.* Mouth. *stm.* Stomodæum.

factory results. From these sections I have followed the formation of the gastrula cell by cell. No mistake can be made, therefore, in the position of these cells, as is frequently done in the study of surface views alone, and one does not get flattening and distortion from the pressure of the cover-glass, as is invariably the case in the study of living preparations. The fertilised egg measures about 55μ in diameter. The eggs laid under normal conditions are almost spherical,

but those obtained from the body-cavity are always flattened and lenticular in shape. On being placed in sea-water, after a short time they fill out and become spherical and regular in outline. They are covered by a thin membrane which remains attached throughout segmentation and gastrulation,

TEXT-FIG. 20.



Section through a trochophore of *Eupomatus* three days old.
an.v. Anal vesicle. *coe.m.* Coelomesoblast. *hk.* Head-kidney.
stmc. Stomach.

becoming the cuticle of the trochophore. This in the living egg is smooth and transparent, but shrinks and becomes considerably wrinkled under the reaction of reagents, especially sublimate. This renders the study of fixed material difficult, as the cuticle has a strong affinity for stains, obscuring the underlying cells and adding to the uncertainty of orientation.

This cuticle has been noticed by Stossich (39), Conn (9), and Hatschek (17); the first of these investigators observed that it became the cuticle of the larva. A similar though somewhat thinner membrane surrounds the eggs of *Podarke*. In *Serpula* it is even thicker than in *Eupomatus*, where at the animal pole it leaves quite a space surrounding the polar bodies. A smaller space is found in *Eupomatus*, in which two dark polar bodies are seen. There is no micropyle, and the sperm seems to be able to penetrate the membrane at any point.

It must be remembered that the type of cleavage of such widely separated forms as *Hydroides*, *Thalassema*, *Podarke*, and *Lepidonotus* resemble one another on account of their possession of a trochophore. They all possess a free-swimming stage of considerable duration, and as the initial size of the blastomeres stands in direct relation to the size of the part to which they give rise, as pointed out by Lillie (25), the resulting cleavage conforms to the same type.

4. SEGMENTATION AND GASTRULATION.

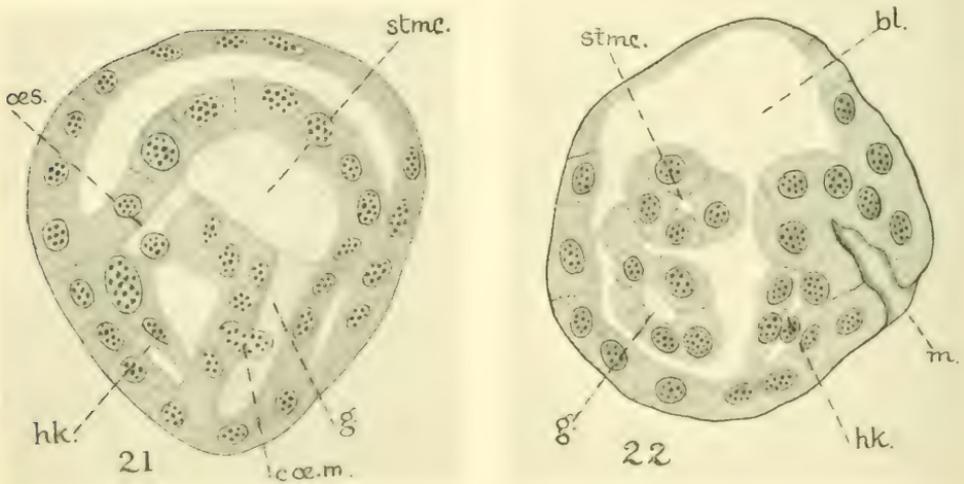
Segmentation begins about one to two hours after the sperm have been added to the eggs. The rate of development naturally varies greatly, being increased with any rise and decreased with any fall in the temperature. With the hot weather in Naples during the summer months, development quickly becomes abnormal unless precautions are taken to keep the water cool in the culture dishes. Segmentation is rapid and regular once it has set in, and results in a blastula containing a segmentation-cavity of variable dimensions. It is of the equal type, and resembles very closely that of the Annelid *Podarke*, which is remarkable for the fact that the spiral symmetry is retained almost complete up till an unusually late stage. The first cleavage furrow cuts through the egg, sinking in more rapidly at the upper than at the lower pole, and produces the two-cell stage. The first cleavage is

exactly equal; the two nuclei of the cells are opposite one another, and show no tendency to rotate as Conklin (7) has described in *Crepidula*. The subsequent divisions follow in rapid succession. With the third cleavage four slightly smaller upper cells are separated by a dextrotropic division from the lower macromeres. In the fourth cleavage the micromeres of the second group are of the same size, and are very slightly smaller than the macromeres.

Invagination produces a typical gastrula. Gastrulation usually commences about seven or eight hours after fertilisation, and consists of a sinking in of the ventral ectoblastic plate, all the entomeres of which are alike during the early stage of the process. Gastrulation is of the modified embolic type, with considerable preparatory flattening of the ventral plate. The cells about to sink in elongate, and their nuclei take up a position at their inner swollen ends. While this flattening is taking place the apical portion of the gastrula is rounding out, the apical tuft of cilia commences to appear, and the endoderm cells sink in till they come in contact with the inner wall of the ectoderm, in the region of the "rosette cells." At first there is a complete obliteration of the segmentation-cavity, the endoderm folding up close against the ectoderm; but in the immediate filling out of the gastrula, which takes place almost simultaneously, the ectoderm is again drawn away and the segmentation-cavity reappears (Text-fig. 18). At this stage a number of viscid protoplasmic threads are seen connecting the two layers, and one blastomere with another. They have been observed in *Podarke* by Treadwell (43), in *Serpula* by Soulier (38), and in *Thalassema* by Torrey (41); I have already drawn attention (35) to them in *Eupomatus*, and pointed out that they are probably similar to the filose strands first described by Andrews (2), and considered by him as cell connections. Prof. Loeb has suggested to me, however, that they are rather more in the nature of the fine cytoplasmic strands so frequently seen in membrane formation during fertilisation than definite cell communications.

The blastopore at first lies exactly in the middle of the ventral plate, and is marked out behind by two large cells, which, as in *Nereis*, probably belong to the X group (fig. 11). When fully formed it is an elongated slit, somewhat enlarged at its anterior end. This end never completely closes, but after the formation of the stomach becomes the future mouth. The posterior portion closes completely, the anus breaking

TEXT-FIGS. 21 AND 22.



Sections through early trochophores of *Eupomatus*. *g*. Gut. *oes*. Oesophagus. *m*. Mouth. *hk*. Head kidney. *cœ.m.* Cecomesoblast. *stmc*. Stomach.

through almost immediately at the point where the last portion of this part of the blastopore disappears. Thus the closure of the blastopore in *Eupomatus* is essentially the same as in *Polygordius*, although the different steps in the process are not so evident. In the majority of Annelids the blastopore usually closes completely, as in *Capitella*.

5. THE ECTOMESOBLAST.

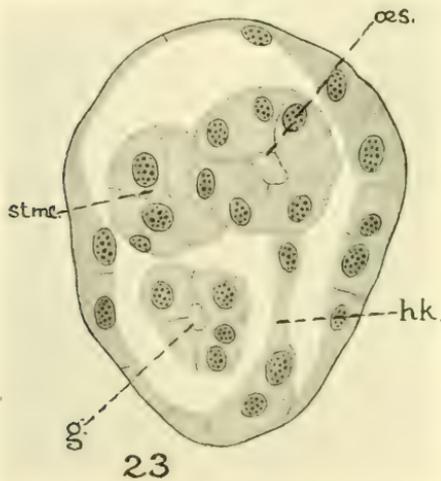
Towards the end of gastrulation some irregular cells are

seen in the segmentation-cavity. Their origin I have not succeeded in observing. They are shown in Text-fig. 18, *Imm.* and *Iml.* I believe they arise from cells of the third quartette, but as I have not followed the cell-lineage carefully, I am by no means certain of their exact origin. They sink into the cleavage-cavity during gastrulation, and take up a bilateral position on either side of the blastopore, as shown in Text-fig. 18. They immediately divide, giving rise to some irregular small cells that apply themselves closely to the wall of the stomodæum, and later form larval muscles. One large cell on either side gives rise to a string of cells, which enter into close relation with the cœlomesoblast. From their mode of origin and their subsequent behaviour I think there can be no doubt that they represent the larval or ectomesoblast of Podarke and Thalassema. In addition to these cells, some mesenchyme cells are also constantly seen in slightly later stages (figs. 9 and 10) in the apical region under the "cross-cells." Whether they arise by migration of some of the cells from the stomodæum, or by the sinking in of ectoderm cells in the apical region, which last I think is more likely, I have not determined. As in Podarke and Thalassema and molluscs, therefore, the larval mesoblast can be divided into the median, the portion under the apical organ, and the right and left portion on either side of the blastopore. These cells (*Imm.* and *Iml.* of Text-fig. 18) would correspond with the right and left parts of the ectomesoblast of Podarke and Thalassema.

It is worth repeating the description of these structures in these forms. In Thalassema, Torrey (41) states, "The most important source of functional mesenchyme, in Thalassema, are the three cells* from the third quartette, namely, $\beta d_{2 \ 2 \ 2 \ 1}$, $\beta c_{2 \ 1 \ 2 \ 1}$, and $\beta a_{2 \ 2 \ 2}$. The first two sink into the cleavage-cavity, just before gastrulation, and lie at first close to the cœlomesoblast cells. They soon migrate laterally, and bud off simultaneously small cells towards the mesoblast cells, dividing like teloblasts, but in the reverse of the ordinary direction. So close is the connection of these cells

with the cœlomesoblast (see Text-fig. 9, *1ml.*) that one would certainly be led to think that they formed part of these bands, unless their cytogeny had been carefully followed" (p. 223). They have been described as follows in *Podarke* by Treadwell (43). They arise as in *Thalassema* from the *3d*, *3c*, and *3a*, and sink into the segmentation-cavity, where they arrange themselves symmetrically, forming bands of three or

TEXT-FIG. 23.



Oblique corneal section through early trochophore of *Eupomatus*.
Lettering as in fig. 22.

more cells. "Since the posterior end of each band lies very close to the definitive mesoblast, the effect is that of a well-developed mesoblast band, lying in the usual position in the segmentation-cavity" (p. 427).

The median portion of the ectomesoblast in *Eupomatus* retains its position untransformed into larval musculature until a very late stage, when the trochophore becomes segmented. It is shown under the apical organ of the early trochophore in figs. 9, 10, 16 (*mesc.*). In the fully formed trochophore it is shown in figs. 2, 3, and 6 (*mesc.*).

I will now describe in detail the changes undergone by these cells. In a late gastrula stage such as that shown in this text-figure (Text-fig. 18) these cells have already divided; the division usually is an unequal one, in which one of the daughter-cells is much smaller and more irregular in shape than the other. They seldom divide simultaneously on both sides, but the right usually precedes the left. If we refer to fig. 9, we see the larger of these cells attached to the ventral wall of the œsophagus (*hk.*). The smaller seems to give rise to some of the mesenchyme cells that are attached to the wall of the œsophagus. These are very irregular in shape and size. At this early stage they are only seen with difficulty, as they are few in number, and are closely pressed against the surface of the œsophagus. Although the stage represented in fig. 9 has already assumed the shape of the early trochophore, it is but slightly older than the late gastrula stage represented in Text-fig. 18.

The head-kidney strand is derived from the division of the large cell (*hk.*) seen in fig. 9. This divides once, and then by a second division of one of the daughter-cells a band of three cells is formed (Text-fig. 23). The nuclei of these cells so arrange themselves that two remain in the end of the strand attached to the œsophagus, while one moves to the distal end, which abuts against the anal end of the gut. This stage is represented in fig. 12. In fig. 10 the head-kidney cell has divided, forming two daughter-cells, one of which is applied close to the wall of the œsophagus, while the other rests against the inner lower surface of the larval hemisphere. In fig. 12 the two distal nuclei of this band have moved apart, one resting against the anal end of the gut, while the other remains close to the œsophagus.

In Text-figs. 21 and 22 the strand of cells forming the head-kidney is shown in sections. In Text-fig. 21 (*coe. m.*) is shown part of the nucleus of the cœlomesoblast cell. This figure is almost in the median plane, while the plane of section of Text-fig. 22 is quite oblique, showing only a portion of the stomach and gut-wall. The cell boundaries disappear, so

that the head-kidney strand consists of a thin thread of cytoplasm, at either end of which are the nuclei. A fine lumen begins to appear in the middle about the second day; this increases in size and works its way towards either end, and by the middle or end of the third day the organ becomes functional as the head-kidney, having acquired an opening into the proctodæum.

6. THE CŒLOMESOBLAST.

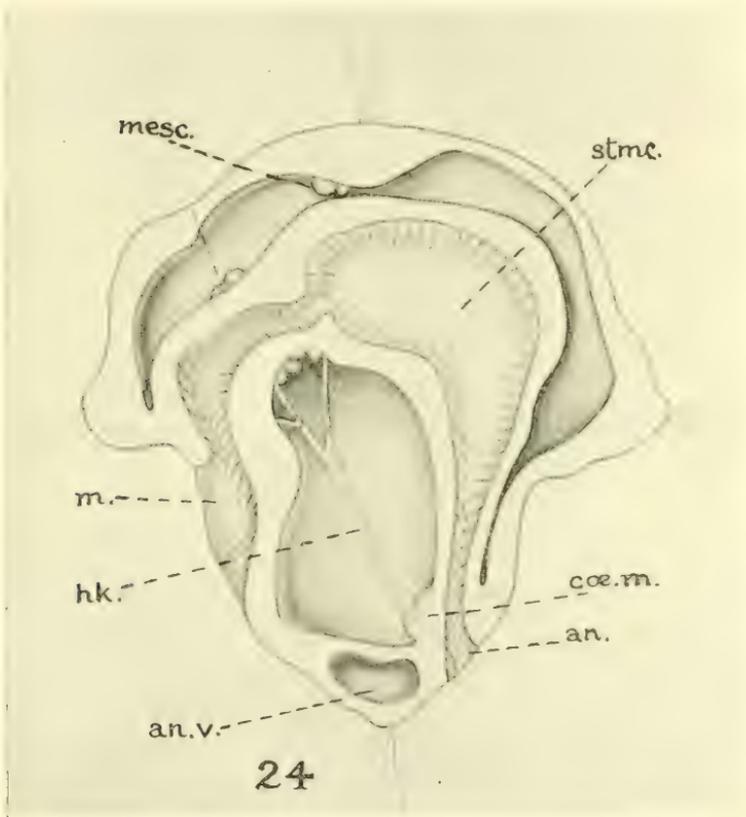
Towards the end of gastrulation, and after the period when the ectomesoblast has already appeared in the blastocœl, two large cells are seen side by side in the ventral lip of the blastopore. In surface views they seem to lie more in the ventral ectodermic plate than in the endoderm. From sections, however (Text-fig. 17, *me.*), they are seen to be part of the endoderm at its point of junction with the ventral plate. They are not free in the segmentation-cavity, and during the course of invagination they come to lie in the wall of the primitive archenteron. They finally rest in the anal end of this structure, where, at much later stages, by a series of rapid divisions, they give rise to a number of cells which push out into the blastocœl and form the mesoderm bands. They are, therefore, the cœlomesoblast cells.

In the stage represented in section in Text-fig. 17 they are usually seen in the ventral lip of the blastopore undergoing division. The fate of the smaller of the resulting daughter-cells I have been unable to determine, but I believe they represent the small cells forming part of the wall of the archenteron in *Podarke*. The larger of the two cells becomes the cœlomesoblast. As development advances they are carried back in the wall of the archenteron, and do not lie free in the blastocœl till a later stage. In late stages they are seen in the anal end of the archenteron as in Text-figs. 19-25 (*ca. m.*); here they always project slightly from the gut-wall.

After their division, as shown in Text-fig. 17, the various

steps by which the larger of the two cells is shifted back into the anal region are somewhat difficult to follow. Sometimes they do not appear to differ greatly from the surround-

TEXT-FIG. 24.



Diagrammatic figure of an early trochophore of *Eupomatus* before the formation of the mesoblast bands, and showing the opening of the head-kidney into the proctodæum. *coe.m.* Cœlo-mesoblast. *an.* Anus. *an.v.* Anal vesicle. *mesc.* Mesenchyme or ectomesoblast. *hk.* Head kidney. *m.* Mouth. *stmc.* Stomach.

ing cells, but they can usually be distinguished by their greater affinity for stains; and in late stages they can always be recognised by the way they are wedged into the

gut wall above the anal vesicle. Close examination of the sections shows them first as two cells in the ventral wall of the stomach, and then the gut. The change that has to do mostly with bringing this about is the great increase in the dorsal surface of the gastrula and the consequent narrowing of the blastoporal surface, changing the large ventral to a small ventro-lateral surface. At a time when the anal opening of the gut has not been established they occupy about the mid-region of the archenteron. At the period when the anus breaks through they have already moved into the anal end.

The blastocœl during this time is still small, and has not undergone the great increase it shows shortly after this period, as only a trace of it can be seen between the gut and the ectoderm. This adds somewhat to the difficulty of determining how the various steps in the process take place. The primitive trochophore about this time begins to assume its typical shape; up to this the round shape of the gastrula has been retained. During early gastrulation before the division of the cœlomesoblast cell, as shown in Text-fig. 17, I have been quite unable to distinguish it from any of the other endoderm cells. No conspicuous cell is seen forcing its way into the segmentation-cavity as shown by Hatschek (17) and Soulier (38), and I believe that both these investigators have been mistaken in their identification of the cœlomesoblast cell. The cell shown in Hatschek's figs. 25-36, and in Soulier's figs. 25-27 and 33 and 34, and identified by them as the cœlomesoblast, are really the right and left portions of the ectomesoblast. At a later stage they give rise to the head-kidneys. The real cœlomesoblast at this period still lies in the gut-wall, and not free in the blastocœl.

In the late gastrula stages the right and left portions of the larval mesoblast appear as shown in Text-fig. 18. In all respects these cells answer to the mesoderm cells of Hatschek's figs. 25-37, fig. 9 of this paper corresponding to Hatschek's fig. 33. By a comparison of figs. 9, 10, 12, 13, 15, 16, the various changes will be seen by which these cells are transformed almost entirely into the head-kidneys. In fig. 16 the

mesoblast bands have not appeared. In the young trochophore shown in fig. 1. they are just appearing as they grow out from the gut-wall. As these cells just mentioned are converted into the head-kidneys before the mesoblast bands have appeared, it is fair to assume that they do not represent the coelomesoblast cells as Hatschek and Soulier claim. It must also be recalled that both these investigators have not followed the cell lineage, and therefore they have no definite grounds of cytological importance on which to substantiate their claim as to the nature of these cells.

In the early stages of invagination it is certain that the coelomesoblast cells cannot be distinguished, as these investigators state, by their conspicuous size and the manner in which they force their way into the segmentation-cavity. In fact, I have been only able to distinguish them satisfactorily in early stages by following their development backwards from a stage when these are clearly recognisable in the anal end of the gut to a stage towards the end of gastrulation; prior to this I cannot see that they differ from any of the other endoderm cells.

In fact, the condition in *Eupomatus* is simply a more marked type of that found in *Podarke*. In this Annelid, according to Treadwell (43), at the sixty-four-cell stage the fourth group of micromeres have just formed. They are all alike, but shortly one of them divides bilaterally, thus aiding substantially in the establishment of the bilateral symmetry. Then each buds off a small cell ventrally; these small cells form a part of the wall of the archenteron. During the course of gastrulation the mesoblast cells lie in the wall of the archenteron, with which they are carried inwards, finally coming to lie in the anal region. They protrude considerably, and in sections that pass (Text-fig. 6A) a little to one side of the sagittal plane they seem to lie actually free in the segmentation-cavity.

The condition in *Podarke* and *Eupomatus*, again, is only a more marked state of that found in *Crepidula*, where the greater portion of the primary mesoblast cell

contains endoderm, remaining a mesendoblastic cell for eight divisions before the mesoblastic is separated from the endodermic portion. The endodermic part enters into the wall of the archenteron. In *Eupomatus* and *Podarke* the greater part of the mesoblast cell (*Ad*) is mesoblastic, while in *Crepidula* only a small part of it is mesoblastic. In *Amphitrite*, Mead (26) represents the mesoderm cell similarly giving off a small cell. The spindle of this division, as in *Eupomatus*, lies in the short diameter of the cell, which at this moment is compressed between the ventral wall of the ectoderm and the main mass of the invaginated endoderm. The axis of the spindle is in the direction of greatest pressure.

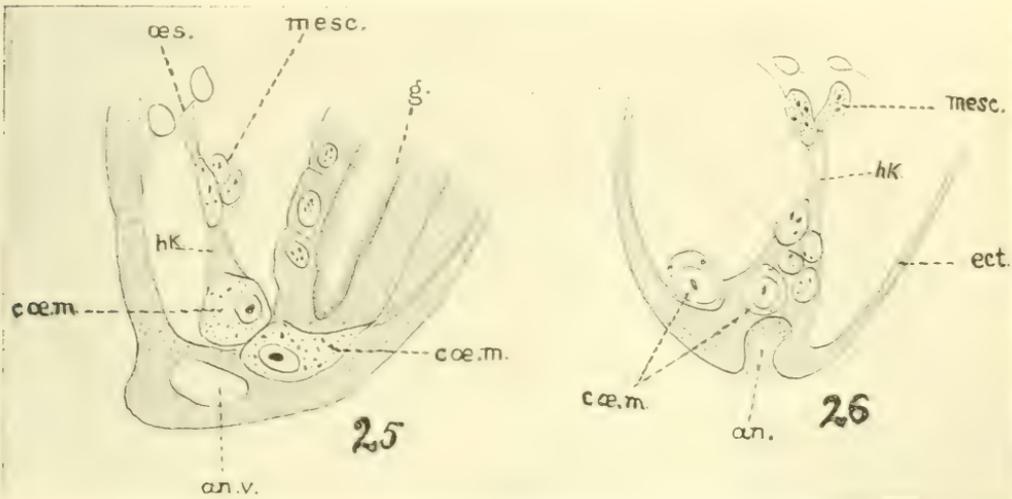
In *Thalassema* the mesoblast cells, which at first are pressed together under the ventral lip of the blastopore, separate and move apart towards the sides, lying well up towards the prototroch (Text-fig. 9, *me.*). As they move apart they divide rapidly, each giving rise to a group of five or more cells, which form the mesoblast bands as in Annelids. They are quite free in the blastocœl, and enter into close relation with the right and left portions of the larval mesoblast, from which they can be distinguished, as in *Eupomatus*, by their different staining reaction. Thus *Thalassema* represents a condition midway between that of *Eupomatus* and *Physa* and other Molluscs, where the mesoderm cell lies free in the blastocœl from the time of invagination.

In *Polygordius* I have shown (37) that the head-kidneys form early and before the mesoderm bands have appeared; that the rudiments of these organs are first recognisable as two cells in the ventral plate of the ectoderm. They grow out into the blastocœl, and by division give rise to a string of cells, as in *Eupomatus*, that run up to the œsophagus. They fuse together and become one strand of cytoplasm, with three or more nuclei. This then hollows out, develops a flagellum, and becomes functional as a head-kidney, at an age when the mesoderm bands are represented by a few cells on either side of the anal opening.

I have advanced reasons for believing that the head-kidney

strands in *Polygordius* are in many ways comparable to the lateral portions of the larval or ectomesoblast of *Thalassema* and Annelids. The condition in *Polygordius*, where the ectomesoblast arises and becomes functional so much earlier than the cœlomesoblast, shows that the head-kidney strands do not form from the bands, and this point is borne out by the cell-lineage as worked out by Woltereck (52). In *Eupomatus* the formation of the cœlomesoblast follows so

TEXT-FIGS. 25 AND 26.



Sections through the anal ends of early larvæ of *Eupomatus*.

an. Anus. *an.v.* Anal vesicle. *cœ.m.* Cœlomesoblast. *hk.*

Head kidney. *ect.* Ectoderm. *g.* Gut. *oes.* Œsophagus.

closely on that of the ectomesoblast that this difference is not so marked.

To sum up: the cœlomesoblast in *Eupomatus* is not recognisable until a relatively late stage in gastrulation, and the cells described by Hatschek and Soulier as the mesoderm cells are probably portions of the larval or ectomesoblast. At the time the ectomesoblast is represented by two cells on each side of the mouth, the cœlomesoblast is represented by a cell in either side of the gut-wall above

the proctodæum. Only in the trochophoral stage does the cœlomesoblast divide, giving rise to the mesoblast bands, which gradually grow up the head-kidney ducts to the region of the œsophagus. There is relatively a considerable period during the trochophoral stage, when the larva is without mesoderm bands, and the rudiments of the bands are represented by a single cell on either side of the gut-wall in the anal region.

7. THE EARLY TROCHOPHORAL STAGES.

In part the early trochophoral stages have been considered in the foregoing section. Before the completion of gastrulation the larva begins to assume the shape of the trochophore. Figs. 9, 10, 12, 13, 15 and 16 show the shape of the early larvæ; of these probably fig. 12 is the most typical. In these figures the upper and lower larval hemisphere is dome-shaped and rounded, as compared with the pointed and more conical appearance of the mature larvæ shown in figs. 1, 2, and 3. The apical cilia, cilia of the mouth, prototroch and paratroch, are, for the sake of simplicity, not shown in these figures, which are drawn from fixed material, and are therefore more granular looking than the living larvæ. These stages are derived from the gastrula about the twentieth to the thirtieth hour of development. At this time there is a great thinning out of the tissues, and the larva rapidly increases in size. In the region of the prototroch a very active proliferation of the cells is taking place, by which the gastrula is lengthened out into the conical dome-shaped larva. The primitive archenteron becomes sharply divided into the cylindrical œsophagus, cubical stomach, and narrow gut. The cells of its walls are seen dividing rapidly. The inner surface of the œsophagus secretes a cuticle, as in *Thalassema* (Text-fig. 19). The archenteron is lined throughout with strong cilia. Those of the œsophagus are remarkably long and powerful. The inner wall of the stomach is covered uniformly with fine cilia, which keep the food contents in constant motion. The cilia of the

gut are somewhat longer and more powerful than those of the stomach. Immediately above the proctodæum the lumen of the gut is narrowed down by a projecting ridge. This is well shown in the trochophore of *Hydroides pectinata* (fig. 18). Below this constriction the gut opens into the proctodæum, which, like the stomodæum, also secretes a fine cuticle. At first the cells of the archenteron are uniformly cubical in appearance, but those of the œsophagus and the gut soon thin out, while those of the stomach alone retain their primitive appearance.

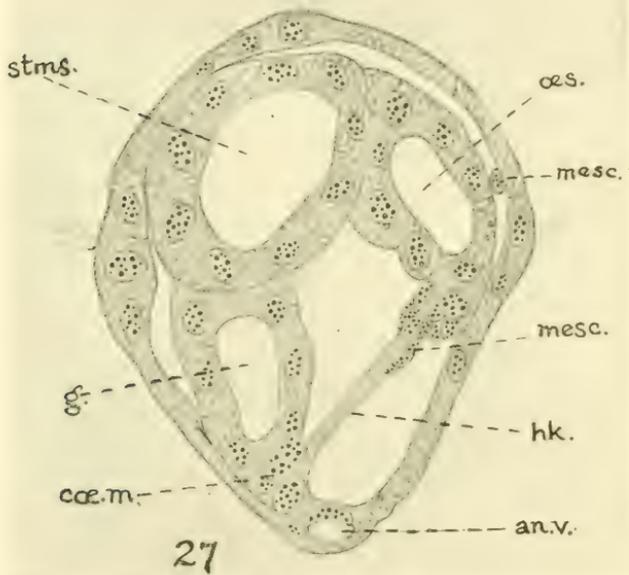
The larva at this time has the shape represented in figs. 9, 10, and 12. The anal vesicle begins to appear as a small vacuole in one of the ectoderm cells of the anal region. This at first connects with the exterior by a small duct, but this soon closes, and the vesicle increases rapidly in size. The cytoplasm of the cell stretches so that a thin envelope alone is left which surrounds the vesicle. It then becomes constricted into two portions, as shown in fig. 14. The original nucleus of the cell is seen projecting into the blastocœl from the upper wall of the vesicle.

On either side of the gut, just above the anal vesicle, a large conspicuous nucleus is seen embedded in the wall. This is the nucleus of the cœlomesoblast cell. In the stages represented in figs. 9 and 10 it is not so prominent as in the later stages shown in figs. 12, 13, 15, and 16. As development proceeds it is pushed out more and more into the blastocœl. In Text-fig. 19 it appears to be free in the blastocœl, but examination of the subsequent sections of this series clearly shows it to lie in the gut-wall. As I have mentioned, it is of somewhat different staining reaction to the surrounding cells, and this contrast is shown somewhat in this text-figure, which is from a camera drawing of an actual section. The section passes a little to one side of the median line, and is slightly oblique, as the mouth and œsophagus are cut in the median plane, while the section passes through the lateral wall of the stomach and the gut. In Text-fig. 20 is shown a section of an older stage in which

the head-kidney has formed, and the mesoderm cell is seen wedged in between the anal vesicle and the head-kidney.

The growth of the bands from these cells is not that of a true teloblastic one; when the cœlomesoblast cells start to divide they do so quite irregularly. The bands at first consist

TEXT-FIG. 27.



Section through trochophore of *Eupomatus* older than those of the foregoing figures. *an.v.* Anal vesicle. *cœ.m.* Cœlomesoblast. *g.* Gut. *hk.* Head kidney. *mesc.* Mesenchyme or ectomesoblast. *stms.* Stomach.

of groups of three or four cells; they divide in all directions, so that after the first division it is not possible to speak of a pole-cell, the divisions always being equal. Hatschek's rather elaborate account of the origin of the bands by teloblastic growth conveys quite an erroneous impression of the process. The cœlomesoblast cell first divides into two equal cells, and

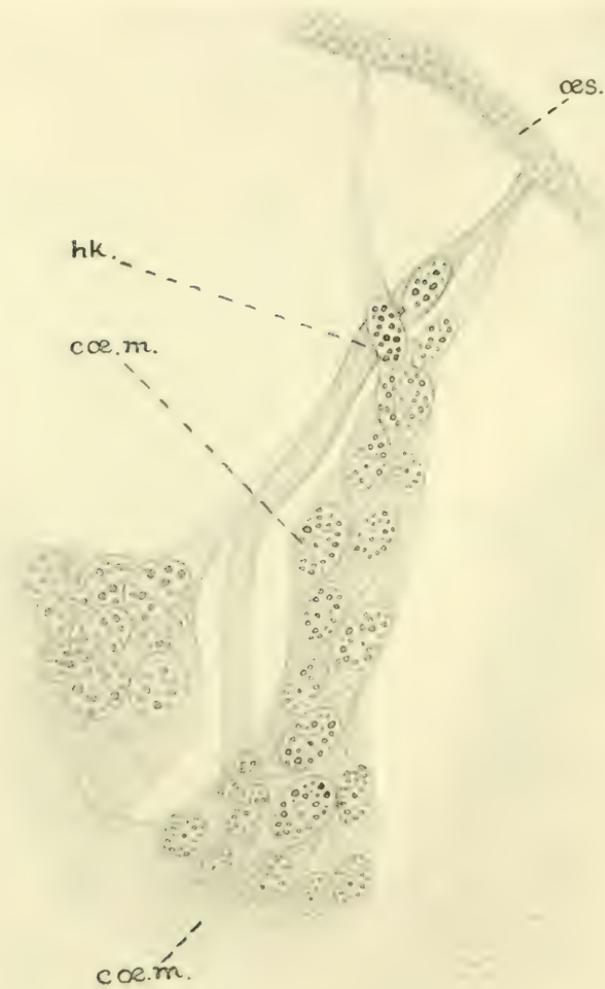
these, again, in turn divide equally. One cell remains attached to the gut-wall, as shown in a late stage in fig. 17 (*pm.*), but this cell does not divide in this stage, and the growing point of the bands is not here, but towards the ends nearest the œsophagus. The position of this cell alone gives it the appearance of being a pole-cell.

According to Wilson (48) there is a complete absence of teloblastic growth in the species of *Hydroides* studied by him, for he states: "I have carefully studied the development of *Hydroides dianthus* (a form nearly allied to *Eupomatus*) by following the cleavage of the living ovum, by examination of stained and cleared embryos, and actual sections. The cleavage is in every detail identical with that of *Eupomatus*, the gastrulation takes place in essentially the same manner, and the trochophore is of quite the same type. Yet I have been unable to identify the teloblasts at any period. They are certainly not present at a stage when the mesoblast bands consist of not more than four or five cells each. At this period each band ends posteriorly in a group of about three cells, two of which are not perceptibly larger than the others, are joined by a narrow bridge of protoplasm stretching across in the angle between the proctodæum and the wall of the anal vesicle" (p. 215).

In *Thalassema*, Torrey (41) has not been able to find a teloblastic growth of the bands. "It is a fact," he says, "as far as I know, without exception, that in all forms where there is a trochophore stage of long duration (as in the case of all Annelids with equal cleavage), the two cœlomesoblast cells do not, in the earlier stages at least, bud like teloblasts" (p. 222).

As the bands grow out from the gut-wall in *Eupomatus*, they keep quite apart from the mesenchyme cells of the blastocœl, nor have I been able in any of the stages I have studied to observe the origin of these cells from the ends of the bands. This is a very debated point in Annelid embryology. Are not the numerous mesenchyme cells of the blastocœl in part derived from the ends of the bands? So

TEXT-FIG. 28.



28

Head-kidneys and mesoblast-bands in a late larva of *Eupomatus*.
cœ.m. Cœlomesoblast. *hk.* Head kidney. *oes.* Œsophagus.

recent an investigator as Treadwell (43) is of opinion that they have such an origin. He, however, did not trace the bands in *Podarke* beyond a stage when they were represented by a few cells, so he obtained no definite information on this point.

In *Eupomatus* it is clear that the anterior ends of the bands never give off cells into the blastocœl as Hatschek has described. They can be plainly observed throughout the course of their growth; they are always a compact mass of cells, clearly distinct from the larval mesenchyme. The larval mesenchyme cells enter into close relation with the cells of the bands, as may be seen in Text-figs. 25 and 26, and in part overgrow them, but even in the living condition they can usually be distinguished. In sections in which the fixation has been rapid they can readily be separated on account of their different staining properties—a point that has been extensively used by Meyer in his numerous studies on this question.

In *Eupomatus* a large part of the larval musculature has already been laid down before the formation of the bands has taken place, the greater part of this musculature persisting and ultimately forming a very considerable portion of the adult body.

Meyer (27) has criticised Hatschek's statement regarding the origin of the mesenchyme in *Eupomatus*, and has expressed himself as being very sceptical as to whether cells arise from the anterior ends of the bands. He is of opinion that, with more modern technique than that employed by Hatschek, whose observations were restricted to living material and optical sections, the facts of the case will prove different. He points out that while Hatschek describes the cœlomesoblast pole-cells as giving off cells into the blastocœl before they form the bands, he neither figures nor appears to have seen the division of these cells. Torrey (41), in speaking of the resemblance of the ectomesoblast in *Podarke* and *Thalassema*, says, "The striking similarity in the origin of the ectomesoblast in these two forms justifies us, I believe, in supposing that we may have the same condition of affairs in *Eupomatus* where the cleavage is also equal" (p. 226).

From Text-figs. 27, 28, which represent sections through the growing bands and head-kidneys of the four-day trochophore, it will be seen that there are numerous mesenchyme cells about the head-kidneys which could hardly have arisen from the cœlomesoblast cells (*cœ.m.*), which, moreover, show no evidence of having recently divided. I have examined a large number of such sections without observing in a single instance the division of these cells to form mesenchyme.

Treadwell (43) holds the view that this separation of the mesoderm in Annelids into apparently distinct portions is only a mechanical result of development, but the varied conditions under which a larval mesenchyme is present in Annelids seems to me to be against this view. Treadwell (43) has pointed out that we are forced to believe in two non-homologous sets of larval mesenchyme, the one arising from the ectoderm as in *Thalassema* and *Podarke*, and the other from the anterior ends of the germ-bands, as in *Nereis* and *Lumbricus*. These two sets do not, as a rule, exist together. "On the other hand," he says, "no one has proved, as far as I know, that no 'mesenchyme' arises from the germ-bands in cases where a larval mesenchyme exists." I have attempted to show that in *Eupomatus*, where a larval mesenchyme exists, no evidences of its origin from the bands can be observed, and the main result of my work has been to emphasise the distinction between ecto- and cœlomesoblast. I have already considered in the "Introduction" whether we are justified in laying any stress on this point. In Annelids we are at least certain that this separation seems general and definite.

SUMMARY.

Segmentation results in a round blastula with a very reduced blastocoel. Invagination produces at first an almost spherical gastrula. But this soon begins to assume the conical shape of the early trochophore. The blastopore, which is

small, closes from behind forwards, the anterior portion remaining as the mouth, while the posterior closes completely, the anus breaking through immediately at this point. The blastopore, which was originally ventral, becomes shifted to a ventro-lateral position. At a time when gastrulation is about half completed, some cells appear on either side of the endoderm and take up a bilateral position. They probably correspond to the lateral portions of the larval or ectomesoblast of *Thalassema*. They subsequently form the head-kidneys in *Eupomatus*. At the same time two conspicuous cells are usually distinguishable in the ventral lip of the blastopore. These are the cœlomesoblast cells. In the further progress of invagination, they are carried inwards in the wall of the archenteron, finally coming to lie in the anal end of the gut. Here at a considerably later stage they give rise to the mesoderm bands. There is a short stage in the early trochophore when the head-kidneys are already functional while the mesoderm bands are alone represented by these two cells in the gut-wall. With the formation of the bands the organisation of the trochophore is completed. The bands during their growth are never seen to bud off cells into the blastocœl. They remain from the first a compact mass of cells clearly distinguishable from the irregular cells of the ectomesoblast and the head-kidneys. The head-kidneys open into the proctodæum. They are formed from the ectomesoblast.

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EXPLANATION OF PLATES 21-23.

Illustrating Mr. Cresswell Shearer's paper "On the Development and Structure of the Trochophore of *Hydroides uncinatus* (Eupomatus)."

LETTERING.

œs. Œsophagus. *an.* Anus. *an. v.* Anal vesicle. *ap. s.* Apical muscle-strand. *bl.* Blastopore. *ca. m.* Cælomesoblast. *co.* Otocyst. *e.* Eye spot. *ect.* Ectoderm. *end.* Endoderm. *g.* Gut. *hk.* Head-kidney. *m.* Mouth. *mesc.* Mesenchyme or ectomesoblast. *oc.* Otocyst. *seg. c.* Segmentation cavity or blastocæl. *Stm.* Stomach.

PLATE 21.

Fig. 1.—Fully-grown free-swimming trochophore of *Eupomatus* three days old. The mesoderm bands are just commencing to appear. The head-kidney is shown opening into the proctodæum while the closed end is attached to the œsophagus. This and the subsequent figs. 3, 7 and 8 are drawn from living larvæ compressed slightly under a cover-glass.

Fig. 2.—Trochophore of *Hydroides norvegica*.

Fig. 3.—Trochophore of *Eupomatus* four days old, showing the otocyst and mesoderm bands well formed.

Fig. 4.—Head-kidney in a three-day old larva of *Eupomatus*.

Fig. 5.—Head-kidney in larva of *Hydroides norvegica*.

Fig. 6.—Trochophore of an unknown Annelid (probably *Hydroides pectinata*) from an outline drawing by Professor E. B. Wilson, showing the opening of the head-kidney into the proctodæum.

Fig. 7.—Trochophore of *Eupomatus* three days old. Seen from the ventral surface, showing the junction of the head-kidney on one side with the gut.

Fig. 8.—Trochophore of *Eupomatus* three days old seen from the oral side. The head-kidneys are shown on either side running down to open into the proctodæum.

PLATE 22.

Fig. 9.—Whole preparation of a larva of *Eupomatus* twenty-four hours old. In this and in the subsequent figures of this plate the cilia

on the external surface are not shown, for the sake of clearness. The head-kidney cell is seen on the ventral side of the œsophagus. In the apical region some ectomesoblast cells are shown.

Fig. 10.—Slightly older stage than that of the last figure. The head-kidney is represented by a string of three cells.

Fig. 11.—External view of a late gastrula of *Eupomatus* showing the portion of the blastopore that remains as part of the mouth.

Fig. 12.—Still later stage than that shown in fig. 10. This stage is about thirty-six hours old.

Fig. 13.—Still later stage than the last.

Fig. 14.—Anal end of a young trochophore of *Eupomatus* showing the double formation of the anal vesicle.

Fig. 15.—Early trochophore of *Eupomatus* older than that of fig. 13.

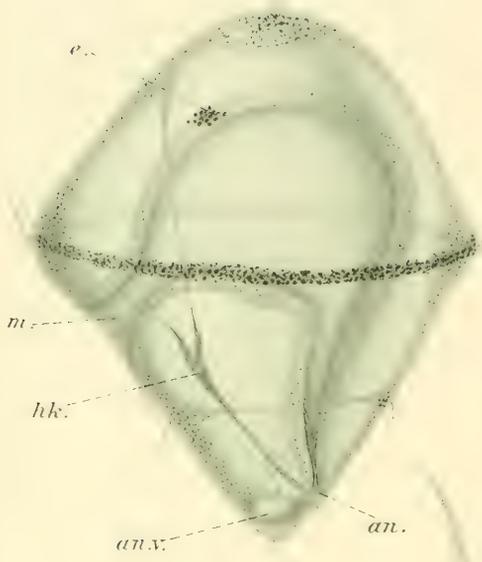
Fig. 16.—Early trochophore of *Eupomatus* forty-eight hours old. The cœlomesoblast cell is seen in the wall of the gut above the anal aperture.

PLATE 23.

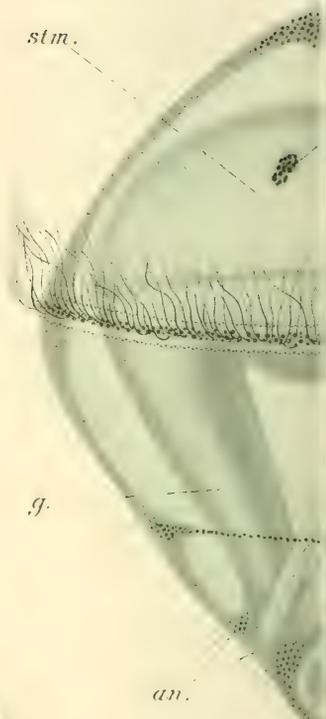
Fig. 17.—*Hydroides norvegica*. The trochophore in this figure is represented as tilted up and seen from the oral surface. The cœlomesoblast is seen arising from two cells in the gut-wall dorsal to the anal vesicle.

Fig. 18.—*Hydroides norvegica*. The lower portion of the trochophore is shown under high magnification and slightly compressed under the cover-glass. The opening of the head-kidneys into the proctodæum is shown, and the cœlomesoblast.

1.



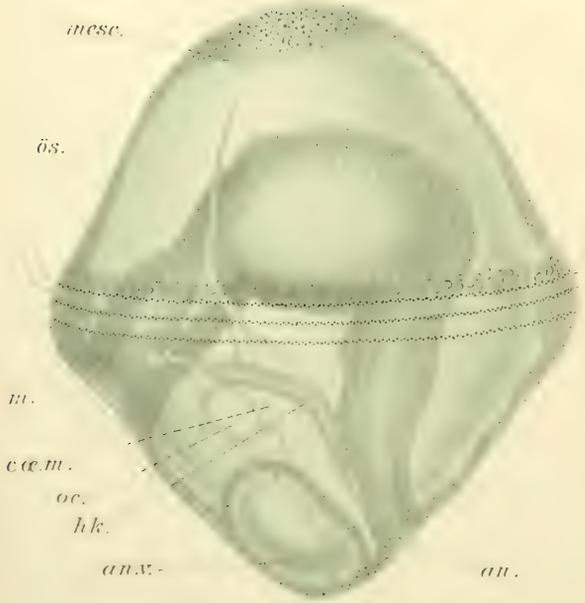
2.



4.

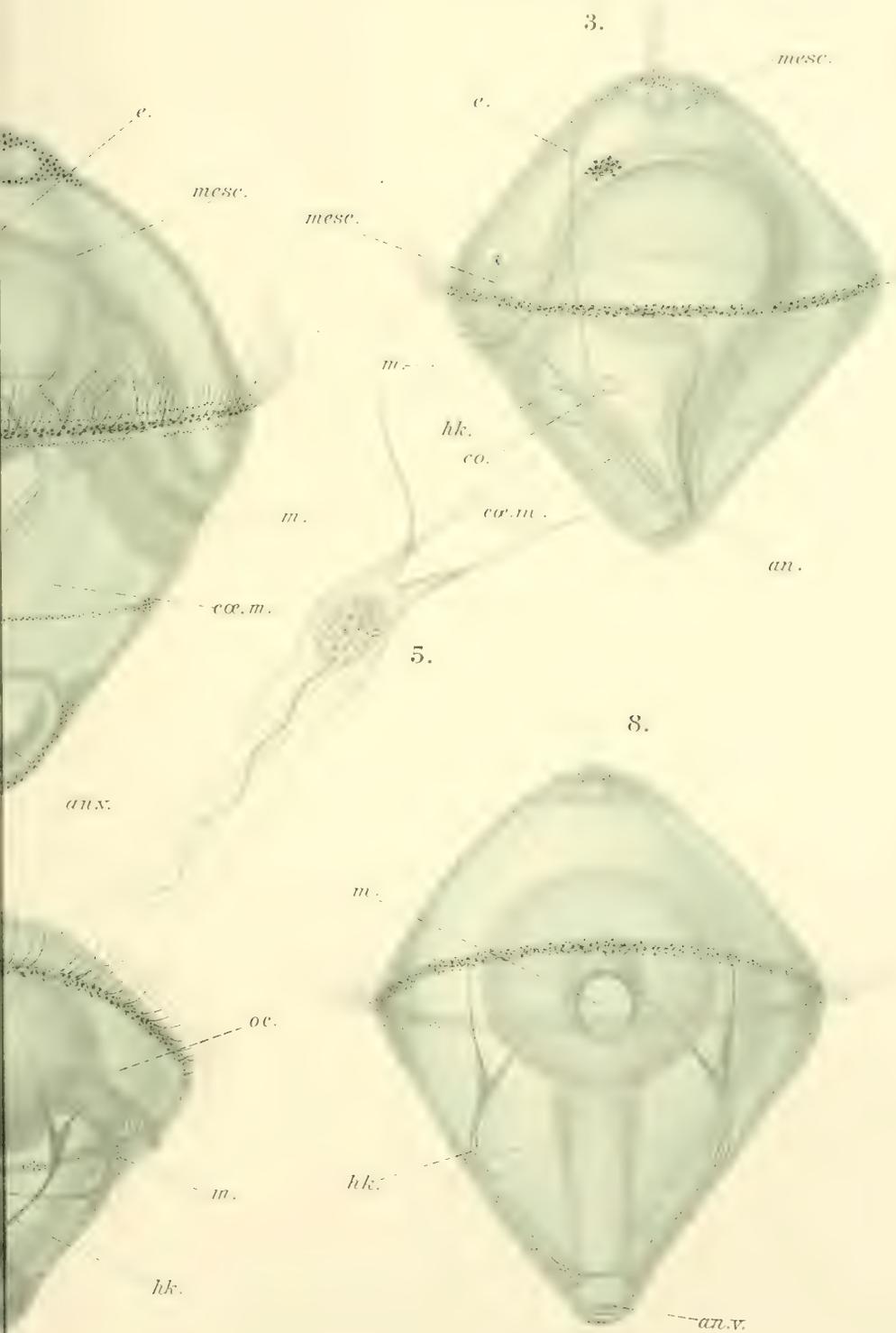


6.



7.





9.

mesc.



m.

12.

mesc.



m.

h.k.

15.

ca. m.

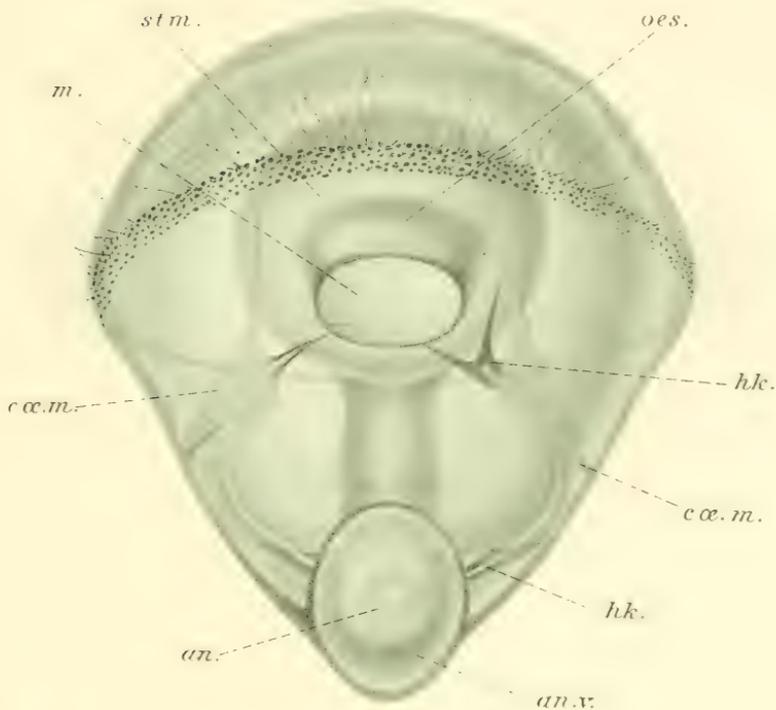


ca. m.

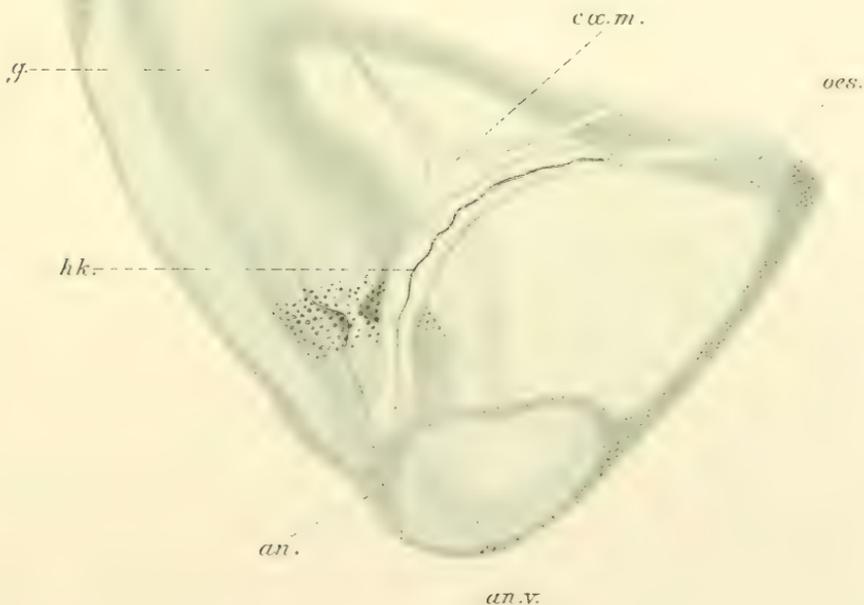
an.



17.



18.



Studies in the Experimental Analysis of Sex.

By

Geoffrey Smith, M.A.,

Fellow of New College, Oxford.

(From the Department of Pathology, University of Oxford.)

Part 5.—On the Effects of Testis-extract Injections upon Fowls.

IN the 'Proceedings of the Royal Society of Medicine,' vol. i, "Pathology," p. 153, 1907-8, Dr. C. E. Walker describes an experiment in which he injected two adult hens with extract of cock's testis for a certain period, with the apparent result that the combs grew very rapidly to quite twice their original area. On ceasing the injections they shrank gradually until they nearly reassumed their original size. A further experiment is alluded to in which several young hens of two months' age were treated in the same manner, and it is stated that, though the results differed somewhat from the first experiment, they were entirely satisfactory, but no further details, as far as I am aware, have been given.

On the strength of the above experiments Dr. Walker concludes, firstly, that the hen bird possesses the potentiality of developing the comb as in the male; secondly, that there is present some internal secretion in the testis which, when injected subcutaneously into the hen, calls forth the production of this and other secondary sexual characters proper to the male, e. g. the wattles and temperament.

This conclusion has been accepted, as proved by Dr. Walker's experiments, by a number of authorities on sex.

Since this conclusion, that the injection of testis extract into the female calls forth the production of certain secondary sexual characters of the male, is one of considerable theoretical importance, and since it rests at present entirely on the experimental evidence furnished by Dr. Walker, it appeared to me desirable to repeat the experiment on a rather larger scale, and to control the experiment with measurements on normal hens. It may be at once stated that the result of this inquiry has convinced me that the comb of the adult hen is usually in a state of fluctuating growth, varying between wide limits, and that this fluctuation is entirely uninfluenced by the injection of extracts of the cock's testis. Out of nine birds injected only one showed fluctuations in growth which fell outside the variability of the control birds, the other eight giving absolutely negative results. In the course of the experiments I also tested the influence of the injections upon the fertility of the eggs and upon the properties of the blood-serum of the injected birds.

The whole of the experiments have been done in the Department of Pathology, Oxford, under the supervision of Prof. Dreyer, to whom I tender my most hearty thanks for the help he has given me.

1. METHODS EMPLOYED.

As I was desirous of repeating Dr. Walker's experiments in the same manner as he performed them, the extract was made by crushing up the fresh testes of a cock with twice their weight of sterile saline and straining the emulsion through gauze. In this way everything except skin and connective tissue passes into the extract, which forms a fine emulsion. Dr. Walker injected the hens with .5 c.c. of such an emulsion every day. I have used various methods, in certain cases injecting the birds with greater amounts, up to as much as 10 c.c., at intervals of a few days, in other cases injecting

them every day with about 3 c.c. In all cases rather more extract was administered in my experiments in a given time than in Dr. Walker's. Since Dr. Walker obtained pronounced effects in three weeks, and very pronounced effects in less than two months, I have not continued the injections for more than a month except in a few cases. That this does not vitiate my results is, however, most clearly shown by the fact that in the two birds in which very marked variations in the comb were observed, comparable to Dr. Walker's, the full increase took place three weeks after the first injection, the injections being performed at intervals of two or three days. The injections were made with aseptic precautions in the pectoral muscles. In measuring the comb two methods have been used: firstly, by tracing an outline of the comb onto cardboard, and secondly, by measuring the two greatest dimensions of the comb directly with a pair of compasses. Both methods were used with the four birds in Experiment 1, but the numbers given in referring to this experiment in the schedule at the end were all taken from the tracings, as the direct measurements did not form a complete series for all the birds. As a consequence of this the numbers referring to comb measurement in these birds do not vary so smoothly as in the case of the later experiments. In Experiments 2 and 3, relating to fifteen birds, I relied entirely on direct measurements, which I consider liable to less experimental error. Measurements were not made of the wattles, as being too inconvenient. In calculating the percentage increase the following method is used. The height of the comb multiplied by the length, the same points being, of course, always taken, is considered as giving roughly the area of the comb. The increase of area observed is calculated as a percentage on the original area when the experiment began. Thus in Bird No. 1 the original area was 50×25 ; the area at the end of the injections was 72×37 , which gives, as the percentage increase—

$$\frac{(72 \times 37) - (50 \times 25)}{50 \times 25} \times 100 = 113 \text{ per cent.}$$

Besides measuring the combs the weights of the birds in grammes were taken at regular intervals, and as many observations as possible were made upon the fertility of the eggs by incubation. In certain cases samples of blood were drawn and their action upon suspensions of the testis extract was observed.

2. THE EFFECT ON THE GROWTH OF THE COMB.

In Experiment No. 1 four birds were used, three of them being white Leghorn hens of two years' age and one a buff Orpington of the same age. Two of the Leghorns were injected, namely, Nos. 1 and 2 in the schedule. No. 1 was injected with 35 c.c. extract in the course of twenty-four days. During this period the comb increased 113 per cent., the largest increase observed in any of the experiments. After the cessation of the injections the comb decreased a little and showed subsequent fluctuations of no very decided character, sometimes increasing considerably (see p. 11).

Bird No. 2 was injected with 102 c.c. in a period of seventy-five days. The comb fluctuated slightly in size, the greatest increase being 23 per cent.

In the two control birds, which were kept under the same conditions, but were not injected, one showed an increase of 24 per cent., the other remained constant.

The result of this experiment is that in one case the increase of comb in an injected bird was much greater than in the controls; the other injected bird, which was injected for a longer period, showed about the same increase as one of the control birds, and therefore gave a negative result. In the case of the injected bird which showed a large increase in the comb, there was no constant shrinking of the comb after the injections ceased.

The fluctuations in weight of all the birds did not show any correspondence with the fluctuations in the comb area.

In Experiment No. 2 six birds were used, all belonging to the same breed, viz. the Indian Jungle fowl, a small breed

a little larger than the ordinary Bantam. The combs in all these birds were similar single combs, but in Nos. 8, 9, and 10 the combs were larger, these birds being two years old and having a strain of Silky in them. The other three birds, Nos. 5, 6, and 7, were pure Jungle fowls of one year's age.

No. 5 was injected with 117 c.c. extract in sixty-two days. The increase of the comb was 76 per cent. After the cessation of the injections the comb fluctuated in size, but after having decreased a little it increased again, and five months after the injections had ceased it was rather larger in area than ever before. There was therefore no tendency to decrease after the cessation of the injections. Result doubtful, perhaps positive.

No. 6 was kept as a control bird from February 22nd to September 7th, during which period it showed an increase of comb of 29 per cent. It was then injected with 53 c.c. extract in twenty-one days, during which period the comb remained quite constant, showing no increase. Result entirely negative.

No. 7 was kept as a control from April 7th to June 14th, during which period the comb increased 16 per cent. It was then injected with 45 c.c. extract in fifteen days, during which it showed an increase of 35 per cent. The comb decreased again in August, but spontaneously increased 35 per cent. in September without any injections being administered, this increase being the same as that observed while the injections were going on. Subsequently, in November, the comb again increased, bringing up the percentage increase to 78. The result of injection was therefore entirely negative.

Bird No. 8 was injected with $71\frac{1}{2}$ c.c. extract in fifty-two days, during which period the comb increased 14 per cent. Result negative.

Nos. 9 and 10 were kept as controls during the whole period of the injections of the other birds, and they showed percentage increases of 33 and 31.

The result of this experiment, then, is that in no case was the percentage increase of the comb greater in the injected

bird during injection than in the control birds. The comb of the injected bird No. 5, which showed a large increase during injection, not only did not constantly shrink after the injections ceased, but actually, five months subsequently, attained its maximum size. Again, in these birds there was no correlation between growth of comb and general body-weight.

In Experiment No. 3 nine young birds, three months old, of the same parentage and brought up together, were used. They belonged to the Indian Jungle fowl breed, and all had similar combs. Three birds, Nos. 11, 12, and 13, were injected each with 39 c.c. extract in a period of twenty-one days, during which period their combs showed a percentage increase of 45, 62, and 30 respectively. The six control birds showed the following percentage increases during the same period: 53, 60, 62, 38, 14, and 30.

In case it might be argued that the effects of the injection might show themselves some time after the cessation of injection, measurements of the comb were continued for a month after the last injection. In that month the injected birds gave percentage increases of 26, 20, and 28, while the control birds in the same period gave 97, 9·8, 13, 35, 36, and 36.

The result of this experiment, therefore, conclusively showed that in young birds of three months old the injection of 39 c.c. of testis extract in a period of twenty-one days had absolutely no effect on the growth of the comb.

Summarising the results of the three experiments it will be seen that out of nine injected birds, eight gave absolutely negative results when compared with the controls. It cannot be objected to these negative results that the hens were not injected with enough extract or for a sufficiently long period, since all of them received as much, or in most cases more, and for an equal or longer period than the bird No. 1, which might be claimed as showing positive results. This bird showed an increase greater than in any of the controls (113 per cent.), but con-

sidering the wide limits of variation in uninjected birds, from 0 to 78 per cent., it is certain that no significance can be attached to this single case. Neither did the behaviour of this bird, subsequent to the cessation of the injections, lend any support to the idea that the injections were the cause of the increase. In Dr. Walker's two birds, after the injections ceased, the combs steadily shrank back nearly to their original dimensions. In my bird the comb shrank a little, but then afterwards increased again. Doubtless a larger series of measurements during the spring months on white Leghorn fowls would reveal as wide a range of fluctuation in untreated as in this injected bird. I have now under observation four Leghorn hens, whose combs have decreased to less than half their area during moulting, and doubtless they will again increase in the spring.¹ The measurements both on injected and control birds establish the fact that in adult as well as in young hens the comb is in a state of fluctuating growth, the fluctuations being often marked within a few days. If we attempt to correlate the variations in the comb with the variations of body-weight as given in the fourth column of the schedule, it will be seen that a simultaneous increase in the comb and in the body-weight is only to be observed in the young hens in Experiment 3, where such a correspondence would be naturally expected. It appears to me that an increase of comb is to be observed just before the hens begin laying.² Thus a reference to the fifth column in the schedule will show that the correspondence is marked, especially in Bird No. 7. It will be seen that the increase of comb is not confined to any particular period of the year, but may take place in autumn as well as in spring.

¹ This supposition has been confirmed, a normal Leghorn hen giving a percentage increase of 130 in twenty days. This is the greatest increase observed in any bird, normal or injected.

² In the next study evidence will be produced proving that the sudden increase of the comb is strictly correlated with egg-laying.

3. THE EFFECT ON THE BODY-WEIGHT, GENERAL HEALTH, ETC.

The series of weights of injected and non-injected hens shows that the injections do not have any constant effect on the weight of the body, even in the young animals used in Experiment 3. With regard to effect on general health it is true that one of the injected birds (No. 1) became unwell after the injections, appearing anæmic and with reduced temperature, and another (No. 8) died soon after the injections ceased, but seven of the injected birds showed no symptoms of any discomfort, laid well, and maintained their weight. It is therefore very probable that the two ill-haps were either purely coincidents or else due to accidental infection, and not to any poisonous action of the extract injected. Dr. Walker gives as the result of his injections that the hens became quarrelsome and attacked cocks that were put in with them. It may be mentioned that no such characteristics were developed by any out of my nine injected birds.

4. THE EFFECT ON THE FERTILITY OF THE EGGS.

Since the extract contains a large quantity of ripe and partially ripe spermatozoa, it was interesting to inquire if the injected hens might be rendered immune against the cock's spermatozoa, and if the presence of an immune substance in the body-fluids might render fertilisation impossible or abnormal. In order to test this, eggs laid by injected hens during the period of their injection were incubated to the third or fourth day together with eggs from control birds and the result noted. As will be seen from the schedule, four of the injected birds laid eggs during the full period of injection, and these eggs were in all cases fertile and normal in development. From some of these eggs healthy young were actually raised which showed no abnormalities. We may conclude, therefore, that the injection of the extract has no influence either on egg-laying or on the fertilisation

or development of the egg. It is a remarkable fact that one of the control birds (No. 3) laid a very large proportion of eggs which developed abnormally, abnormalities of a greater or less degree being observed in more than 50 per cent. of the eggs. Some of these abnormally developing eggs were simply abnormal in having the chick not sufficiently forward at the fourth day of incubation with the area vasculosa rather bloodless, but others showed actual structural abnormalities, in two cases double-headed or double-bodied monsters being produced, in others the back being twisted into a peculiar shape. I have recorded this fact, as it demonstrates very clearly that the production of these abnormalities must have been a fixed character in this hen and not an accidental occurrence.

5. THE EFFECT ON THE BLOOD.

The normal serum of the fowl has a very powerful agglutinating effect upon the live spermatozoa of the cock. If a small quantity of the spermatozoa from the vas deferens is mixed with a small quantity of normal blood-serum, it is observed that in a few moments the spermatozoa, instead of being dispersed through the fluid, are agglutinated in clumps and stringy masses. In the space of a quarter of an hour most of the spermatozoa will be found to be motionless, and on transferring them to saline they do not recover their mobility but are shown to be irrecoverably damaged. If we perform this experiment with a suspension of the testis extract used in our experiments, the same agglutinating effect is observed in test-tube experiments, but the agglutination does not proceed so rapidly even when the tubes are incubated at 37° C., because the suspension does not consist only of spermatozoa but very largely of fatty materials and cellular débris. The agglutinating effect of the serum obtained from an injected hen (No. 2) was tested twice against a suspension of spermatozoa and of testis extract and the result compared with samples of normal serum. No increased agglutinating power

was observed in the serum drawn from the injected bird, nor did this serum stop the motion of spermatozoa any quicker than in the case of normal birds. The various dilutions of the serums obtained from injected and non-injected birds also gave similar results.

It was therefore found in the limited experiments performed that no immunising process could be detected as the result of the injections, and this negative effect is in accordance with the observations on health, comb growth, and fertility, as affected by the injections.

SUMMARY OF RESULTS.

(1) The injection of testis extract into hens was found to have an entirely negative effect on the increase of the comb in eight out of nine adult and young hens when compared with the fluctuations in growth observed in control birds. In one case the injected hen showed an increase of comb slightly greater than any observed in the control birds,¹ but the comb in this bird did not show the constant shrinkage, after cessation of injections, observed in the cases cited by Dr. C. E. Walker, and regarded by him as an essential feature of the experiment.

(2) The injections had no observable effect on the health, body-weight, fertility, blood properties, or any other features, although very large quantities were administered over periods varying from fifteen to seventy-five days.

(3) The result of the experiments is to show that, although Dr. Walker's observations were doubtless correct, his conclusion that the increase of the comb was due to the testis extract injected was erroneous. There is, therefore, no evidence that the testis contains an internal secretion which, when injected into the female, can call forth the production of any of the male secondary sexual characters.

¹ Since the MS. was in proof, a control bird of two and half years age has given a percentage increase in comb of 130 in a period of twenty days, thus exceeding that of any of the injected birds (see note on p. 7).

EXPERIMENT No. 1.—No. of Bird, 1; White Leghorn,
2 years old.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
Feb. 1	Injected 2 c.c. extract	50 × 25	—	
" 3	" 2 " "	50 × 25	—	
" 7	" 4 " "	50 × 25	—	
" 10	" 4 " "	58 × 29	1520	
" 12	" 3 " "	57 × 28	1420	
" 15	" 5 " "	59 × 30	1380	
" 17	—	59 × 30	1510	
" 19	Injected 5 c.c. extract	67 × 34	1520	
" 22	" 5 " "	67 × 34	1650	
" 24	" 5 " "	72 × 37	1670	Increase of 113 %.
" 28	—	72 × 37	1500	From this date to March 7th the bird was very unwell, too weak to perch, and anæmic in appearance.
March 4	—	—	1320	Recovering.
" 7	—	67 × 34	1320	Recovered.
" 14	—	67 × 34	1380	Recovered.
" 18	—	67 × 34	1470	—
" 22	—	68 × 34	1510	—
" 24	—	—	—	Fertile normal egg.
" 25	—	70 × 36	1550	Increase of 10 % since March 7th.
" 30	—	70 × 36	1420	—
April 2	—	—	—	Fertile normal egg.
" 3	—	—	—	" "
" 4	—	—	—	" "
" 6	—	—	—	" "
" 7	—	70 × 34	1320	—
" 13	—	69 × 34	—	—
" 14	—	67 × 34	1080	—
" 19	—	67 × 34	1130	—
" 22	—	67 × 34	1200	—
" 26	—	66 × 33	1320	—
May 3	—	64 × 32	1410	—
" 14	—	59 × 30	1350	—
" 21	—	62 × 30	1240	—
June 1	—	61 × 28	1270	—
" 14	—	63 × 29	1290	—

No. of Bird, 2; White Leghorn, 2 years old.

Date.	Treatment.	(Comb) measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
Feb. 1	Injected 2 c.c. extract	95 × 50	—	—
.. 3	.. 2	—	—	—
.. 7	.. 4	—	—	—
.. 10	.. 3	95 × 50	2070	—
.. 12	.. 3	95 × 50	1900	—
.. 15	.. 5	97 × 52	1900	—
.. 17	—	97 × 52	1850	—
.. 19	Injected 5 c.c. extract	98 × 52	1970	—
.. 24	.. 5	101 × 54	1900	—
March 3	—	98 × 53	1820	—
.. 7	—	98 × 53	2050	—
.. 11	Injected 5 c.c. extract	99 × 53	2020	—
.. 14	.. 5	99 × 53	1870	Fertile normal egg.
.. 17	.. 10	98 × 53	1860	—
.. 22	.. 5	98 × 53	1700	—
.. 25	.. 10	98 × 53	1680	—
.. 30	.. 10	98 × 53	1620	Blood drawn for agglutination ex- periment.
April 6	.. 10	98 × 53	1650	Fertile normal egg.
.. 12	—	—	—	—
.. 14	Injected 10 c.c. extract	98 × 53	1620	Normal fertile egg.
.. 16	.. 8	98 × 53	1570	Increase of 23 %.
.. 18	—	—	—	Blood drawn for agglutination ex- periment.
.. 24	—	—	—	Normal fertile egg.
.. 25	—	—	—	—
.. 26	—	97 × 50	1790	—
May 1	—	—	—	Normal fertile egg.
.. 3	—	95 × 50	1670	—
.. 7	—	—	—	Normal fertile egg.
.. 14	—	94 × 50	1560	—
.. 21	—	94 × 50	1290	—
June 1	—	92 × 48	1320	—
.. 14	—	92 × 48	1300	—

STUDIES IN THE EXPERIMENTAL ANALYSIS OF SEX. 603

No. of Bird, 3; White Leghorn, 2 years old;
Control.

Date.	Treatment.	Comb measurement in millimetres.	Weight in grammes.	Remarks.
1910				
Feb. 22	—	85 × 46	2050	Several fertile eggs, many abnormal in development.
.. 28	—	87 × 50	1990	
March 1	—	87 × 52	1920	
.. 15	—	92 × 53	1940	
.. 18	—	90 × 54	1900	
.. 22	—	90 × 53	1900	
.. 25	—	91.5 × 54	1920	
.. 30	—	87.5 × 53.5	1790	
April 4	—	87 × 52	1720	
.. 14	—	89 × 52	1670	
.. 18	—	—	—	
.. 22	—	87 × 52	1750	
.. 26	—	89 × 52	1790	
May 3	—	89 × 51	1600	
.. 14	—	87 × 51	1560	
.. 21	—	85 × 50	1520	
June 14	—	86 × 53	1720	Increase of 24%.

No. of Bird, 4; Buff Orpington, 2 years old;
Control.

Date.	Treatment.	Comb measurement in millimetres.	Weight in grammes	Remarks.
1910				
Feb. 23	—	53 × 25	3270	Numerous fertile eggs, normal development.
March 2	—	53 × 25	3250	
.. 15	—	53 × 25	3300	
.. 18	—	53 × 25	3200	
.. 22	—	53 × 25	3160	
.. 25	—	53 × 25	3270	
.. 30	—	53 × 25	3070	
April 7	—	53 × 25	2920	
.. 14	—	53 × 25	2830	
.. 26	—	53 × 25	2840	
May 3	—	53 × 25	2820	No variations in comb.
June 24	—	53 × 25	2850	

EXPERIMENT No. 2.—No. of Bird, 5; Jungle Fowl, 1 year old.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes	Remarks.
1910				
Feb. 19	Injected 2 c.c. extract	23.5 × 10.25	720	—
" 22	" 3½ " "	23.5 × 10.25	720	—
" 24	" 4 " "	23.5 × 10.5	740	—
" 28	" 5 " "	24 × 10.5	750	—
March 3	" 5 " "	25 × 10.25	780	—
" 5	" 5 " "	26 × 10.5	760	—
" 8	" 3½ " "	27 × 11	800	—
" 11	" 5 " "	27.5 × 11.5	800	—
" 14	" 5 " "	28 × 11	820	—
" 17	" 9 " "	28.5 × 12	800	—
" 22	" 5 " "	29 × 12	800	—
" 25	—	29 × 12	760	—
" 28	Injected 10 c.c. extract	—	—	Normal fertile egg.
" 30	" 10 " "	29 × 12	720	" "
April 6	" 10 " "	29 × 12	740	—
" 7	—	—	—	Normal fertile egg.
" 8	—	30 × 11½	690	—
" 12	—	—	—	Normal fertile egg.
" 14	Injected 10 c.c. extract	30 × 12	740	—
" 16	" 10 " "	30 × 12	740	Normal fertile egg.
" 19	" 10 " "	30 × 12.75	710	—
" 22	" 5 " "	30.5 × 12.5	770	Increase of 76 %.
" 26	—	31.5 × 13.5	840	—
May 3	—	32 × 13	810	—
" 10	—	31.25 × 13	720	—
" 17	—	31 × 13	650	—
" 24	—	29 × 12	630	—
" 31	—	29 × 12	620	—
June 6	—	30 × 12.5	670	—
" 14	—	30 × 12.5	670	—
" 20	—	30 × 12	670	—
" 24	—	31 × 13	680	—
July 1	—	30.5 × 12.75	690	—
" 7	—	31 × 12.25	700	—
" 9	—	31 × 12	710	—
" 14	—	31 × 12	720	—
" 22	—	33 × 14.5	740	Largest dimen- sions of comb reached.
Aug. 16	—	30 × 12.5	770	—
Sept. 9	—	29 × 12	700	—
" 10	—	28 × 12	720	—
" 14	—	29 × 12	770	—
" 16	—	30 × 12.5	800	—
" 19	—	30 × 12	720	—
" 23	—	29 × 12	790	Egg laid.
" 28	—	30 × 12.5	820	—
" 30	—	31 × 12	840	Egg laid.
Oct. 3	—	32 × 13	840	—

No. of Bird, 6; Jungle Fowl, 1 year old.

Date.	Treatment.	Comb measure- in millimetres.	Weight in grammes	Remarks.
1910				
Feb. 22	—	22 × 7	670	—
March 1	—	22 × 7.5	660	—
" 8	—	22 × 7	720	—
" 18	—	23 × 7.5	770	—
" 22	—	23.5 × 7.25	690	—
" 25	—	23.5 × 7.5	720	—
" 30	—	23.5 × 7.5	700	—
April 6	—	22.5 × 7.25	700	Several normal fertile eggs.
" 14	—	23 × 7.5	640	
" 19	—	23.5 × 8	640	
" 22	—	23.5 × 8	690	
" 26	—	23.5 × 8	660	
May 3	—	23.5 × 8	720	
" 10	—	24 × 8	670	—
" 17	—	25 × 8	670	—
" 24	—	24.5 × 7.5	600	—
" 31	—	24.5 × 7.25	650	—
June 7	—	24.25 × 7	660	—
" 14	—	24 × 7	600	—
" 20	—	24 × 7	630	—
" 24	—	24 × 7	650	—
July 1	—	24.5 × 7	690	—
" 7	—	24.5 × 7.25	670	—
" 9	—	25 × 7.25	690	—
" 14	—	24.5 × 7	660	—
" 22	—	24.5 × 7	630	—
Aug. 16	—	24 × 7	700	—
Sept. 7	—	24 × 7	690	Greatest increase, 29 %.
" 10	Injected 4 c.c. extract	24 × 7	690	—
" 12	" 5 " "	24 × 7	670	—
" 14	" 4 " "	24 × 7	670	—
" 16	" 4 " "	24 × 7	670	—
" 17	" 4 " "	—	—	—
" 19	" 4 " "	24 × 7	670	—
" 21	" 4 " "	24 × 7	640	—
" 23	" 4 " "	24 × 7	650	—
" 24	" 4 " "	—	—	—
" 26	" 4 " "	24 × 7	670	—
" 28	" 4 " "	24 × 7	670	—
" 30	" 4 " "	23.75 × 6.5	670	—
Oct. 1	" 4 " "	—	670	—
" 3	—	24 × 6.5	690	No increase as result of injection, slight decrease.

No. of Bird, 7; Jungle Fowl, 1 year old.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes	Remarks.
1910				
April 7	—	26.25 × 9.25	840	
" 14	—	27 × 9.75	840	
" 19	—	27 × 10	820	
" 22	—	27 × 10	850	
" 26	—	26.5 × 10.25	870	
May 3	—	27 × 10.5	820	
" 10	—	26.5 × 10.25	820	
" 17	—	27 × 10.25	710	
" 24	—	26.5 × 10	670	Several normal fertile eggs.
" 31	—	26.5 × 10.25	670	
June 7	—	26 × 10	730	
" 14	—	27 × 10	770	Increase 16%.
" 15	Injected 3 c.c. extract	—	740	
" 16	" 3 " "	27 × 10	770	
" 17	" 3 " "	27.25 × 10	720	
" 18	" 3 " "	28 × 11	750	
" 19	" 3 " "	28 × 11	740	
" 20	" 3 " "	28.5 × 11.5	740	
" 21	" 3 " "	28.5 × 11	720	
" 22	" 3 " "	28 × 11.5	720	
" 23	" 3 " "	28.25 × 12	740	
" 24	" 3 " "	28.5 × 12	710	Egg laid, normal.
" 25	" 3 " "	28.5 × 12	700	
" 27	" 3 " "	28.5 × 12	700	Egg laid, normal.
" 28	" 3 " "	28.5 × 12	720	
" 29	" 3 " "	29 × 12	710	Egg laid, normal.
" 30	" 3 " "	29.5 × 12.5	750	Increase of 36%.
July 1	—	29.75 × 12	720	
" 7	—	29.25 × 11	670	
" 9	—	29 × 11	690	
" 14	—	28 × 10.5	660	
" 22	—	28 × 10.5	650	
Aug. 16	—	27.5 × 10	750	
Sept. 7	—	28 × 11	700	
" 10	—	28.5 × 10.75	750	
" 14	—	29.5 × 11.5	790	
" 16	—	30 × 12	800	Egg laid.
" 19	—	29.5 × 11.5	750	Increase of 35% since August 16th.
" 21	—	30.5 × 12.25	850	Egg laid.
" 23	—	29.5 × 11.5	820	
" 26	—	29.5 × 11.5	820	
" 28	—	29.5 × 11.5	820	
" 30	—	29.5 × 11	840	
Oct. 3	—	29.5 × 11.25	840	
" 18	—	31.75 × 12	890	Eggs laid.
" 25	—	34 × 13	900	
Nov. 1	—	35 × 14	850	Increase of 78% since August 16th.
" 8	—	31.5 × 13	800	

STUDIES IN THE EXPERIMENTAL ANALYSIS OF SEX. 607

No. of Bird, 8; Jungle Fowl, 2 years old.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
March 1	Injected $3\frac{1}{2}$ c.c. extract	26.5 × 12	800	—
" 11	" 5 " "	27 × 12	790	—
" 14	" 5 " "	27 × 12	740	—
" 17	" 5 " "	27 × 12	730	—
" 22	" 5 " "	26.5 × 12	730	—
" 25	" 8 " "	26.5 × 12	750	—
" 30	" 8 " "	27.5 × 12.25	740	Normal fertile egg.
April 6	" 8 " "	27 × 12	770	Normal fertile egg.
" 8	—	27.25 × 12	750	—
" 14	Injected 5 c.c. extract	27 × 12.25	790	—
" 19	" 9 " "	27.5 × 12.5	820	—
" 22	" 10 " "	28 × 13	820	Increase of 14%.
" 26	—	27.5 × 12.75	800	—
" 30	—	—	—	Found dead on nest. Peritoneal cavity full of clear yellow pus. Slight infection of tuberculosis in liver, hardly sufficient as cause of death.

No. of Bird, 9; Jungle Fowl, 2 years old.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
March 1	—	37.5 × 15	900	—
" 11	—	36 × 14	900	—
" 18	—	35.5 × 14	870	—
" 22	—	34.5 × 13.5	870	—
" 25	—	35 × 14	890	—
" 30	—	35 × 13.5	920	—
April 7	—	35 × 13.5	920	—
" 14	—	35 × 14	950	—
" 19	—	35 × 13.75	950	—
" 22	—	36 × 14	970	Several normal fertile eggs.
" 26	—	36 × 14	950	—
May 5	—	37.15 × 14	990	—
" 10	—	37.5 × 15	940	—
" 17	—	38 × 15	970	—
" 24	—	39 × 15.25	990	—
" 31	—	39 × 16	990	—
June 5	—	39 × 16	1000	Increase of 33%.
" 14	—	38 × 15	950	Suffering from hard tumour-like swelling in abdomen. Killed, and on dissection oviduct found occluded with large masses of yolk; ovary also full of large-yolked eggs.

No. of Bird, 10; Jungle Fowl, 2 years old.

Date.	Treatment.	Comb measurement in millimetres.	Weight.	Remarks.
1910				
Feb. 23	—	20.5 × 12.75	—	—
March 3	—	20.5 × 12.75	—	Numerous fertile normal eggs laid.
.. 30	—	21 × 13.5	—	
April 25	—	23 × 15	—	
June 2	—	22 × 13	—	
.. 24	—	20 × 13	—	
Sept. 26	—	23.5 × 13.5	—	

EXPERIMENT No. 3.—No. of Bird, 11; Jungle Fowl, 3 months old.

Date.	Treatment.	Comb measurement in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	20 × 6.5	350	
.. 9	Injected 3 c.c. extract	20 × 6.75	350	
.. 12	.. 3	20.25 × 6.75	410	
.. 14	.. 3	20 × 6.75	420	
.. 16	.. 3	20 × 6.75	450	
.. 17	.. 3	—	—	
.. 19	.. 3	20 × 7.25	460	
.. 21	.. 3	20.25 × 7.25	500	
.. 23	.. 3	20.5 × 7.5	490	
.. 24	.. 3	—	—	
.. 26	.. 3	21 × 8	490	
.. 28	.. 3	21.25 × 8	500	
.. 30	.. 3	21.25 × 8	500	
Oct. 1	.. 3	—	500	
.. 3	—	21.75 × 9	510	Increase of 45 %.
.. 18	—	23.25 × 10	570	
.. 25	—	23.75 × 10	600	
Nov. 1	—	24.5 × 10	600	
.. 8	—	24.75 × 10	600	Increase of 26 % since October 3rd.

No. of Bird, 12; Jungle Fowl, 3 months old.

Date.	Treatment.	Comb measurement in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	18 × 5	350	—
" 9	Injected 3 c.c. extract	18.5 × 5.25	350	—
" 12	" 3 " "	19 × 5.75	420	—
" 14	" 3 " "	19 × 6	410	—
" 16	" 3 " "	19 × 6	450	—
" 17	" 3 " "	—	—	—
" 19	" 3 " "	19.5 × 6	470	—
" 21	" 3 " "	19.5 × 6	500	—
" 23	" 3 " "	19.75 × 6.5	500	—
" 24	" 3 " "	—	—	—
" 26	" 3 " "	20 × 6.5	500	—
" 28	" 3 " "	20 × 7	500	—
" 30	" 3 " "	20.5 × 7.25	520	—
Oct. 1	" 3 " "	—	520	—
" 3	—	21 × 7.5	520	Increase of 62 %
" 18	—	22.5 × 8	640	—
" 25	—	23 × 7.5	620	—
Nov. 1	—	23.25 × 8	600	—
" 8	—	23.75 × 8	600	Increase of 20 % since October 3rd.

No. of Bird, 13; Jungle Fowl, 3 months old.

Date.	Treatment.	Comb measurement in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	16.5 × 4.75	270	—
" 9	Injected 3 c.c. extract	16 × 4.75	270	—
" 12	" 3 " "	16.25 × 4.75	340	—
" 14	" 3 " "	16.5 × 4.75	340	—
" 16	" 3 " "	16.75 × 5	380	—
" 17	" 3 " "	—	—	—
" 19	" 3 " "	16.75 × 5	400	—
" 21	" 3 " "	17 × 5	440	—
" 23	" 3 " "	16.75 × 5	440	—
" 24	" 3 " "	—	—	—
" 26	" 3 " "	17.25 × 5	430	—
" 28	" 3 " "	17.25 × 5.25	420	—
" 30	" 3 " "	17.5 × 5.5	440	—
Oct. 1	" 3 " "	—	440	—
" 3	—	18 × 5.5	450	Increase of 30 %
" 18	—	19 × 6	550	—
" 25	—	19 × 6	570	—
Nov. 1	—	19.5 × 6.75	620	—
" 8	—	19.5 × 6.5	600	Increase of 28 % since October 3rd.

No. of Bird, 14; Jungle Fowl, 3 months old;
Control.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	18.5 × 6	410	—
" 9	—	18.5 × 6	420	—
" 12	—	19 × 6	500	—
" 14	—	18.75 × 6	490	—
" 16	—	19 × 6.5	500	—
" 19	—	19.25 × 6.75	530	—
" 21	—	19.5 × 7	570	—
" 23	—	19.75 × 7	570	—
" 26	—	20 × 7	550	—
" 28	—	20.5 × 7	540	—
" 30	—	21 × 7.5	550	—
Oct. 3	—	21.25 × 8	540	Increase of 53%.
" 18	—	23 × 8.5	600	—
" 25	—	25.5 × 10	630	—
Nov. 1	—	27 × 11.25	650	—
" 8	—	28 × 12	650	Increase of 97% since October 3rd.

No. of Bird, 15; Jungle Fowl, 3 months old;
Control.

Date.	Treatment.	Comb measure- ment in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	17 × 4	300	—
" 9	—	17 × 4	310	—
" 12	—	17.5 × 4.25	320	—
" 14	—	17 × 4.25	320	—
" 16	—	17.25 × 5	350	—
" 19	—	17.25 × 5	370	—
" 21	—	17.25 × 4.75	350	—
" 23	—	17.25 × 5	370	—
" 26	—	18 × 5	380	—
" 28	—	18.25 × 5.25	400	—
" 30	—	18.25 × 5.25	400	—
Oct. 3	—	19 × 5.75	410	Increase of 60%.
" 18	—	20 × 6.5	480	—
" 25	—	20 × 6.5	500	—
Nov. 1	—	21 × 7	500	—
" 8	—	20 × 6	570	Increase of 9.8% since October 3rd.

STUDIES IN THE EXPERIMENTAL ANALYSIS OF SEX. 611

No. of Bird, 16; Jungle Fowl, 3 months old;
Control.

Date.	Treatment.	Comb measurement in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	15.5 × 3	250	—
" 9	—	15.75 × 3.5	260	—
" 12	—	15.75 × 3.5	300	—
" 14	—	16 × 3.5	300	—
" 16	—	15.75 × 3.5	320	—
" 19	—	15.5 × 3.75	320	—
" 21	—	15.5 × 4	350	—
" 23	—	16 × 4	350	—
" 28	—	16.25 × 4.25	370	—
" 30	—	16.5 × 4.25	370	—
Oct. 3	—	16.75 × 4.5	390	Increase of 62%.
" 18	—	17.5 × 5	490	—
" 25	—	17.75 × 4.75	500	—
Nov. 1	—	18 × 4.75	500	—
" 8	—	18 × 4.75	500	Increase of 13% since October 3rd.

No. of Bird, 17; Jungle Fowl, 3 months old;
Control.

Date.	Treatment.	Comb measurement in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	16 × 4	250	—
" 9	—	16.5 × 4.25	270	—
" 12	—	16.25 × 4.25	310	—
" 14	—	16 × 4.5	320	—
" 16	—	16.5 × 4.75	340	—
" 19	—	16 × 4.75	380	—
" 21	—	16.5 × 4.75	370	—
" 23	—	16.75 × 4.75	380	—
" 26	—	17.25 × 5	380	—
" 28	—	17 × 5	390	—
" 30	—	17 × 5	400	—
Oct. 3	—	17.75 × 5	410	Increase of 38%.
" 18	—	19 × 5.75	500	—
" 25	—	19.25 × 5.75	510	—
Nov. 1	—	20 × 5.25	500	—
" 8	—	20 × 6	500	Increase of 35% since October 3rd.

No. of Bird, 18; Jungle Fowl, 3 months old;
Control.

Date.	Treatment.	Comb measurement in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	17 × 4.5	300	—
" 9	—	17.5 × 4.5	350	—
" 12	—	17.5 × 4.5	370	—
" 14	—	18 × 4.25	370	—
" 16	—	18 × 4.25	390	—
" 19	—	17.5 × 4.25	420	—
" 21	—	17.5 × 4	430	—
" 23	—	17.75 × 4.5	450	—
" 26	—	17.75 × 4.5	460	—
" 28	—	18 × 4.75	470	—
" 30	—	18.25 × 4.75	470	—
Oct. 3	—	18.5 × 4.75	470	Increase of 14%.
" 18	—	19 × 5.75	580	—
" 25	—	20 × 5.75	580	—
Nov. 1	—	20 × 6	570	—
" 8	—	20 × 6	570	Increase of 36% since October 3rd.

No. of Bird, 19; Jungle Fowl, 3 months old;
Control.

Date.	Treatment.	Comb measurement in millimetres.	Weight in grammes.	Remarks.
1910				
Sept. 7	—	17 × 5	280	—
" 9	—	17.25 × 5	280	—
" 12	—	17.5 × 5	320	—
" 14	—	17.25 × 5.25	320	—
" 16	—	17.25 × 5	340	—
" 19	—	17.5 × 5.5	370	—
" 21	—	17.5 × 5.5	380	—
" 23	—	18 × 5	390	—
" 26	—	18 × 5.75	400	—
" 28	—	18 × 6	400	—
" 30	—	18 × 6	410	—
Oct. 3	—	18.5 × 6	410	Increase of 30%.
" 18	—	19.5 × 6.25	510	—
" 25	—	19.5 × 6.75	550	—
Nov. 1	—	20 × 6.75	550	—
" 8	—	21 × 7.25	550	Increase of 36% since October 3rd.

**Cortical Cell Lamination of the Hemispheres of
Papio Hamadryas.**

By

E. H. J. Schuster, M.A., D.Sc.,
Fellow of New College, Oxford.

With Plates 24-30.

PREFACE.

THE localisation of function in the cerebral cortex is a problem on the importance of which it is hardly necessary to dwell. Its practical value to the surgeon and its theoretical interest to the physiologist and psychologist are obvious and have been sufficiently recognised. It is a problem which concerns many of the biological sciences, since it can be approached in many different ways. The physiologist has, by the electric stimulation of different parts of the surface of the brain in various animals, obtained responses from different parts of the body, and has thus been enabled to say what areas control the movements of the various groups of muscles. Occasional opportunities are afforded to the surgeon for making similar experiments on the human subject. The ablation of different parts of the cerebral cortex in the living animal is another physiological method from which valuable conclusions have been drawn. Observations on the alterations in function associated with local cortical lesions due to accident or to disease form a parallel method of research in the domains of pathology.

The contribution called for from the anatomist and histo-
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logist is the mapping out of the cortex into areas differing from one another in structure. In doing this he may take into account the form and arrangement either of the nerve-cells or of the nerve-fibres, or he may divide the surface into regions distinguished by the period at which they arrive at maturity. In an exhaustive treatment of the subject evidence obtained in all these ways must be considered and co-ordinated—a difficult task, since the interpretation of the observations is beset with pitfalls, and in consequence a number of apparent inconsistencies have to be explained away or harmonised.

On some points already a considerable degree of certainty has been arrived at, namely the extent of the area concerned directly with the movement of the voluntary muscles, and of that region in which the visual impulses set up in the retina reach the cortex. With regard to these points the evidence from all sources is in agreement, and the definite function is in each case correlated with a perfectly definite structure, which, particularly in the case of the visual area, sharply delimits it. A perfectly definite structure also marks the olfactory cortex, while to many other structurally distinct areas a special function may be assigned with some degree of confidence.

That a better appreciation of a physiological and anatomical problem may be obtained by extending it into the realms of comparative physiology and comparative anatomy is generally accepted. Both the points of agreement and the points of divergence in structure between animals with different habits living in different environments may elucidate the relation between structure and function. The present paper aims at making a small but definite contribution to the subject, in that it consists of a description of a special aspect of the histology of the cortex in an animal not hitherto treated in this way. The histological study of the cortex may be pursued in at least three different ways. Firstly, the nerve-fibres may be stained by some method like that of Weigert; secondly, the nerve-cells may be stained either by the Golgi or Cajal process. These are uncertain in action and only

select a few cells for staining, but show them complete with dendrites and axon, and may enable one to trace for a considerable distance the path of the latter, in some cases even as far as its terminal arborisation. These methods are of great importance, and throw light on the relations of one cell to another within a particular cortical area, and thus on the individual functions of the different types of cell. They are, however, unsuitable for the survey of the arrangement of the cells in their several layers, which is necessary in order to divide the cortex into regions according to the type of cell lamination present in each. For this purpose it is necessary to employ a method of a third type, such as that of Nissl, in which every cell is rendered visible by the stain. Nissl's method shows the general shape of each cell and its principal dendrites, also the structure of the nucleus and the arrangement of the extra-nuclear chromatin; it does not show the axon nor the nerve-fibrils running through the body of the cell.

It is a variation of Nissl's methylene-blue which has been used for the purposes of the present paper, namely Unna's polychrome methylene-blue, as the particular point dealt with is the arrangement of the layers. Much work of the kind has been done on the human brain, without, however, exhausting the subject, but the other Primates have been somewhat neglected. Of the few papers dealing with them the earliest is that of Schlapp ('Archiv f. Psychiat.,' Bd. xxx), "Der Lebenbau der Gross linn rinde des Affen *Macacus cynomolgus*." Schlapp does not go into any great detail: three types only of cortex are described and somewhat roughly figured, their extent being shown by a sketch of the lateral surface of the hemisphere.

Campbell ('Histological Studies on the Localisation of Cerebral Function,' Cambridge, 1905) describes the brains of the chimpanzee and orang-outang. This work, which fills a volume of considerable size, deals principally with the human brain, and treats the subject with praiseworthy thoroughness. Not only is the arrangement of the cells

described, but that of the fibres also. The human brain is compared with that of the two apes, and the clinical, pathological, and experimental evidence is fully discussed in its relation with the histological results.

An excellent series of papers on the cell lamination have been published by Brodmann in the 'Journal f. Psychologie und Neurology,' 1903 and onward, and summarised in his book, 'Vergleichende Lokalisations lehre der Grosshirnrinde,' Leipsig, 1909. The human brain is described in great detail, a very large number of separate areas (more than fifty) being distinguished histologically. Similar detailed descriptions of the brains of Cercopithecus and of the lemurs are given, while the marmoset (*Hapale*) is dealt with more shortly. The homologies between different regions in the human and other Primate brains are dealt with as far as possible.

Mott and Kelley publish a "Complete Survey of the Cell Lamination of the Cerebral Cortex of the Lemur" ('Proceedings of the Royal Society,' B., vol. lxxx, 1908), in which the histological results are compared with the results of stimulation experiments performed on the lemur's brain by Prof. Halliburton, and a paper of a similar nature, but dealing with the brain of the marmoset, has been produced by Mott, Halliburton, and the present author (Mott, Schuster, and Halliburton, "Cortical Lamination and Localisation in the Brain of the Marmoset," 'Proceedings of the Royal Society,' B., vol. lxxxii, 1910).

MATERIAL AND METHODS.

The material used in the present paper consisted of the brain of a specimen of *Papio hamadryas* which had died in the gardens of the Zoological Society.

Each hemisphere was cut into a number of blocks (seventy or more), arranged in such a way as best to secure that as much as possible of the cortex was cut in planes at right angles to its surface, since in oblique sections the nature of the cell lamination is almost impossible to determine. The

outlines of the blocks were carefully plotted in drawings of the brain. From each block sections 10μ thick were taken and stained with polychrome methylene-blue. As no satisfactory sections of the insula were obtained, the cortex in this part of the brain has not been described.

In the investigation of these sections the admirable works of Brodmann¹ on *Cercopithecus* and Campbell² on the chimpanzee and orang-outang were of great assistance to me, and as far as possible my observations have been brought into line with theirs.

My observations differ from Brodmann's in that I have not described so many types of cortex. This is due to a difference in judgment and not to antagonism with regard to matters of fact. Since in the area covered by each main type local differences are present, and since the main types in many cases shade into one another, it would be possible to describe many more varieties of structure than are mentioned by Brodmann. One must arbitrarily fix a limit to the number of types distinguished, and I have fixed mine at a smaller number than he.

For illustrating the structures described in the text, outline drawings of the cells were made under the Abbé Zeichenapparat, and these were afterwards blacked in with ink so that the cells are represented in silhouette.

It was thought advisable to prefix a short description of the fissures to the principal part of the paper.

FISSURES IN THE BRAIN OF PAPIO HAMADRYAS.

The Sylvian fissure (*FS.*) and sulcus centralis (*c.*) call for no special comment. Anterior to the latter the following fissures are present:

¹ K. Brodmann, "Beitrage zur histologischen Lokalisation der Grosshirnrinde Dritte Mittheilung; Die Rindenfelder der Niederen Affen," 'Journ. für psych. u. Neurol.,' Bd. iv, Heft 5/6, 1905.

² A. W. Campbell, 'Histological Studies in the Localisation of Cerebral Function,' Cambridge, 1905.

Sulcus arcuatus (arc.).—From the most posterior portion of the backwardly directed convexity of this fissure a straight branch runs upwards and backwards. The upper limb in the right hemisphere is united at its extremity with one of the sulci of the frontalis superior series. The lower limb in both hemispheres runs straight downwards and forwards, and ends about a centimetre above the anterior end of the Sylvian fissure.

Sulcus inferior transversus (it.) runs obliquely upwards and forwards between the lower ends of the sulcus centralis and sulcus arcuatus; in the right hemisphere it is connected with the Sylvian fissure.

Sulcus rectus (rect.) runs horizontally forwards from a position about 5 mm. in front of the sulcus arcuatus, to end about the same distance from the frontal pole. Between its posterior end and the lower portion of the sulcus arcuatus lies the sulcus diagonalis (*d.*), a shallow oblique fissure better marked in the right hemisphere than in the left.

The sulcus præcentralis superior and sulcus frontalis superior (*fs.* 1, 2, 3, 4) are together represented by a series of shallow fissures, of which the posterior (*fs.*₁) is the best developed. This lies in a more or less sagittal direction, while the other members of the series are more transversely placed.

On the orbital surface is found a sulcus orbitalis (*orb.*) lying near the mesial border; this is triradiate in the right hemisphere, but a simple, more or less sagittal, fissure in the left. On the lateral side of this is the obliquely placed sulcus fronto-orbitalis (*fo.*), the anterior outer end of which just reaches the lateral surface of the hemisphere. This fissure is quite well defined, though not more than three or four millimetres deep. Its shape is complicated in the left hemisphere by the presence of a shallow forwardly directed bifurcated branch.

The sulcus temporalis superior (*ts.*) is a long, well-developed fissure extending both in front of and behind the Sylvian fissure. Its posterior end is bifurcated in the right

hemisphere but not in the left. Below it, in the temporal lobe, lie one or two shallow grooves ($Tm_{1,2}$, etc.), which may be taken to represent the sulcus temporalis medius.

The sulcus post-centralis superior ($pcs.$) is a shallow groove lying almost horizontally opposite the upturned posterior end of the sulcus cinguli.

The sulcus intra-parietalis ($ip.$) starts anteriorly in the space which is bounded above and in front by the sulcus centralis, and below and behind by the Sylvian fissure. It runs at first upwards and backwards, then turning backwards, disappears underneath the occipital operculum. Here it bifurcates. Its outer branch runs outwards on the anterior wall of the sulcus lunatus, and then divides into two rami, of which one runs to the bottom of that fissure while the other runs upwards. The inner branch, by far the larger of the two, is called the ramus parieto-occipitalis ($rpo.$) of the sulcus intra-parietalis. It cuts very deeply through the supero-mesial border of the hemisphere, then runs straight downwards to end in a bifurcation, the branches of which lie more or less parallel to the sulcus calcarinus.

Owing to the extent of the occipital operculum, this ramus parieto-occipitalis appears to be a mesial continuation of the sulcus lunatus; in reality the two fissures are partially separated by a submerged gyrus lying near the supero-mesial border.

The fissura parieto-occipitalis ($fpo.$) is a triradiate fissure, the two posterior rays of which arch round the anterior limb of the terminal bifurcation of the parieto-occipital branch of the sulcus intra-parietalis. These two rays are better developed in the left hemisphere than in the right, and there the lower one forms a superficial connection with the posterior limb of $rpo.$ The anterior ray is poorly developed.

The sulcus subparietalis ($sp.$) (posterior limbic sulcus) lies between the fissura parieto-occipitalis ($fpo.$) and the sulcus cinguli. The main portion of this fissure runs upwards and slightly backwards; its lower end is divided into two

branches, which are better marked in the left hemisphere than in the right.

The sulcus cinguli (*Sc.*) (intercalary or calloso-marginal fissure) is quite typical in its arrangement. At its anterior end lie two shallow sulci rostrales (*ro.*), with the upper of which it is, in the right hemisphere, in connection.

The sulcus collateralis (*Col.*) is in the right hemisphere divided into three segments; the most anterior of these (*col.*₁) lies obliquely between the sulcus rhinalis and the anterior end of the sulcus temporalis superior. The middle segment (*col.*₂) is the longest; it runs sagittally backward. Between its posterior portion and the sulcus calcarinus lies the third segment (*col.*₃), the main portion of which runs parallel to that fissure, to end posteriorly in a transverse member. In the left hemisphere only two segments are present, the anterior (*col.*₁) corresponding exactly to the anterior segment in the right hemisphere, while the posterior segment of the left hemisphere corresponds to the two posterior segments of the right; at its hinder end it runs upwards and ends very close to the sulcus calcarinus.

In the left hemisphere a shallow T-shaped sulcus (*T.*) is present lying between the posterior part of the sulcus collateralis and the sulcus calcarinus.

The sulcus rhinalis (*rh.*) is quite typical.

The sulcus lunatus (*lun.*) ("Affenspalte") is well developed in both hemispheres; its posterior lip is produced forwards to form an operculum. Curving round its lower end is the deep sulcus occipitalis inferior.

The sulcus occipitalis inferior (*Oi.*), the upper lip of which is produced downwards to form a well-marked operculum. Behind this fissure, curving round the tentorio-lateral margin, is another sulcus, which is independent in the right hemisphere, but connected with it in the left.

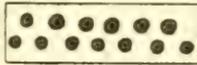
The sulcus occipitalis lateralis (*Ol.*) lies horizontally between the sulcus lunatus and the occipital pole. It is a very deep fissure, which cuts obliquely downwards into the hemisphere so that its lower lip forms an operculum. Its

anterior end is bifurcated in the right hemisphere. One or two shallow grooves lie between this and the sulcus occipitalis inferior.

The sulcus calcarinus (*cal.*) at its anterior end joins the fissura hippocampi; it runs a sigmoid course backwards, to end in a long bifurcation near the occipital pole. The upper branch is by far the longest, and its upper end just reaches the lateral surface of the hemisphere. In the right hemisphere it gives off a short downward branch.

FRONTAL LOBE.

Precentral (Motor) Type (fig. 1).

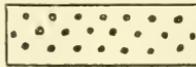


This type of cortex is characterised by its great depth, which reaches to over 2·7 mm., the presence of the Betz cells, and the absence of a distinct layer of "granules." The drawing (fig. 1) is taken from the lateral surface of the hemisphere a short way in front of the upper end of the sulcus centralis. Here the breadth of the cortex is about 2·7 mm., but only the upper 2·4 mm. are drawn. The Betz cells occupy a broad zone, otherwise rather poor in cells, which stretches from 1·3–1·9 mm. from the surface. They appear to be larger here than in other parts of the precentral area. The cell bodies of the largest are about 70 μ in length by 50 μ in breadth, but the massive processes given off from the apex and base render accurate measurements impossible, since it is difficult to say where the cell-body ends and the bases of the apical process or other dendrites begin. These cells are slightly smaller on the mesial surface, and gradually diminish in size as one passes downwards over the lateral surface towards the Sylvian fissure. About half-way down they measure only 50 \times 30 μ .

The other cell layers are as follows :

- (i) The lamina zonalis is about 1 mm. broad.
- (ii) Lamina granularis externa reaches to a depth of about .3 mm. Its constituent cells are small pyramids and granules ; these vary in size in different regions, and appear to be rather larger on the mesial than on the lateral surface. The largest of the small pyramids are about $15 \times 12 \mu$.
- (iii) Lamina pyramidalis is about 1 mm. broad, extending from a depth of .3 to a depth of 1.3 mm. ; as is usual, the cells increase in size from above downwards. The largest cells in the area figured are about 35μ in length \times 20μ in breadth ; but this particular strip of cortex, though exceptionally rich in large Betz cells, is rather poorer than others in large pyramids of layer iii.
- (iv) Lamina granularis interna is represented by a few scattered granules in the lower part of layer iii and upper part of layer v.
- (v) Lamina ganglionaris. The Betz cells have already been described ; the other cells contained in the layer resemble either those of layer iii or of layer vi.
- (vi) Lamina multiformis is rather poor in cells. These are arranged in regular radial rows, and many of them have strong, downwardly directed processes.

Anterior Precentral Type (fig. 2).



The whole depth of the cortex is about 2.2 mm. ; it is built up as follows :

- (i) Lamina zonalis, breadth just over .1 mm.
- (ii) Lamina granularis externa, breadth about .2 mm. This is composed of small pyramids and granule cells, the former measuring $10-15 \mu$ in length by $9-12 \mu$ in breadth, and the latter being some $9-12 \mu$ in diameter.
- (iii) (iv) and (v) Lamina pyramidalis, lamina granularis interna, lamina ganglionaris are not very clearly separated ; a

narrow strip, such as is figured, hardly shows any lamination, but if a wide area of cortex is examined under the low power of the microscope one can distinguish (1) an upper zone (iii *a*) consisting mostly of medium-sized pyramids, which stretches downwards to a depth of about .8 mm. (2) A tendency to form a definite layer of large pyramids (iii *b*) at a depth of .9–1.2 mm. from the surface. The size of these cells may be 30–50 μ in length by 20–25 μ in breadth. (3) A stratum, rather poor in cells, extending from 1.2–1.4 mm. (4) Another, but less well-defined, layer of large pyramidal cells (v) at 1.4–1.6 mm. These cells are in some places rather larger than those at iii *b*, in other places about the same size or rather smaller; they are in all places much less numerous. A few granule cells and small triangular and irregular-shaped cells are present between, and interspersed with, both the upper and lower layers of large pyramids.

(vi) Lamina multiformis occupies the rest of the cortex; it contains cells of varied shapes, the most prevalent being roughly triangular cells with long processes extending both upwards and downwards.

Extent and Boundaries.—The precentral cortex on the mesial surface is bounded below by the sulcus cinguli; on the lateral surface it extends posteriorly to the bottom of the sulcus centralis; below it falls considerably short of the Sylvian fissure. Along its free anterior and lower border it passes gradually into the anterior precentral type.

The anterior precentral type is also bounded below on the mesial surface by the sulcus cinguli; along the supero-mesial border it extends to more than half the distance between the upper end of the sulcus centralis and the frontal pole. Its upper anterior extension forms a broad band on the lateral surface, the posterior part of which is bounded below by the horizontal limb of the sulcus arcuatus. The vertical portion of that fissure serves as the anterior boundary of the narrow strip of this cortex, which lies in front of the lower portion of the precentral area. Approaching the Sylvian fissure the anterior precentral type alters considerably in character; the large

pyramids, both supra- and sub-granular, become smaller and more densely packed together, and the lamina granularis interna is more easily recognisable. In this condition the cortex is prolonged forward beyond the lower end of the sulcus arcuatus, where it passes gradually, on the one hand, into the frontal, and, on the other, into the posterior orbital type. In structure this lower forward extension of the anterior precentral bears a marked resemblance to the anterior part of the posterior orbital cortex. In position it has some points in common with Brodmann's type 10, which it resembles in having a layer of granules distinguishable but not well developed.

Posterior orbital type (fig. 5).



The position of the particular strip of cortex shown in fig. 5 was near to the posterior border of the orbital surface just lateral to the sulcus orbitalis. The cortex is here 1.8 mm. in depth. The lamina zonalis extends to a depth of .25 mm., and is succeeded by a very poorly developed lamina granularis externa; this in turn gives place to the lamina pyramidalis. The lower and larger pyramids of the latter form together with the cells of the lamina ganglionaris a zone rich in cells of moderate size, which extends from a depth of .7 mm. to a depth of 1.3 mm. A faint indication of the lamina granularis interna lying at a distance of about .9 mm. from the surface suggests that only the upper border of this zone should be reckoned to the lamina pyramidalis, and that the greater part of it is derived from the lamina ganglionaris. The lower half millimetre of the cortex is occupied by the lamina multiformis, the greater proportion of the cells of which are of a broad pyramidal form with basal processes extending horizontally. These cells tend to be arranged in horizontal rows, separated by tangentially running bundles

of fibres, a type of arrangement which, though obvious even in a narrow strip like that figured, becomes very conspicuous when a considerable width of cortex is examined under a low power.

The posterior orbital type is of practically identical structure with Brodmann's type 11, but it shows some differences in its extent and boundaries.

According to Brodmann, this type covers the greater part of the orbital surface in the hemisphere of *Cercopithecus*, being absent only from its anterior portion and the gyrus rectus. In *Papio* a type of cell-lamination exactly answering to the description given above is found only in the posterior quarter of this part of the hemisphere, and there it extends across the gyrus rectus, and is in continuity with the anterior limbic type over the orbito-mesial border. In front it extends further forward along the lateral than along the mesial border, but it gradually changes its character; the internal layer of granules becomes more marked, and the tangential arrangement of the lamina multiformis less marked, till finally, at about the position indicated in the figure, it is definitely replaced by frontal or prefrontal cortex, though where the exact boundary may be it is impossible to say owing to the gradual nature of the transition.

The intermediate precentral area of Campbell is of approximately the same structure and extent both in man and in the higher apes (orang and chimpanzee). In structure it resembles the precentral (motor) cortex in almost all points, except that no giant Betz cells are present in the internal layer of large pyramidal cells. The elements of this layer are smaller and less numerous than those of the outer layer of large pyramids. Between the two layers is a stratum poor in cells, which occupies the position of the layer of stellate (granule) cells present in most other regions. This type of cortex extends from the mesial surface, over the supero-mesial border, then downwards and outwards over the lateral surface, and ends by occupying a considerable area of the orbital face of the hemisphere. On the mesial surface it

is bounded behind by the precentral area, below by the calloso-marginal fissure, and extends forward for a considerable distance. Its anterior boundary crosses the superomesial border about 2 cm. in front of the precentral area in the chimpanzee, and somewhat further forward in the orang. On the lateral surface it forms a zone lying in front of the precentral area, which is broad above, but becomes narrower lower down, being bounded in front for a short distance by the sulcus arcuatus.

As it nears the Sylvian fissure it extends forwards over the lower portion of the sulcus arcuatus and occupies a more or less rectangular area bounded above by the sulcus rectus and below by the superior limiting sulcus of the island of Reil—the part of the hemisphere which, according to Campbell, corresponds to the pars basilaris of the human brain. In front it invests the upper end of the sulcus fronto-orbitalis, “then turning abruptly downwards, and still following the fronto-orbital sulcus, it coats the convolution forming its anterior wall and is finally arrested well down on the inferior (orbital) surface by the sulcus orbitalis.”

In Brodmann's description of the brain of *Cercopithecus* the area which corresponds to Campbell's intermediate precentral is occupied by three types of cortex. Type 6, which agrees in structure with the intermediate precentral, has approximately the same extent on the mesial surface and on the upper and middle portions of the lateral surface, but it never extends far beyond the sulcus arcuatus, which lies along its whole length just behind the anterior boundary. The region roughly corresponding to the pars basilaris is occupied by type 10, which in the orbital surface gives place to type 11.

Type 11 resembles type 6 in the absence of a layer of granules but differs from it in the following points: (1) The external and internal layers of large pyramids (Brodmann's layers iii *b* and v) are fused to form a clearly delimited band, the cortex is narrower, and to judge from the photographs the cells are considerably smaller.

In Type 10 the granule layer is present but poorly developed. Types 10 and 11 agree in a point which Brodmann regards as important, namely, that owing to the tangential arrangement of the fibres in the lower part of the cortex the cells of the innermost layer tend to lie in tangential rows and not in radiating rows more or less vertical to the surface.

The Frontal and Prefrontal Types (figs. 3 and 4).



The drawing of the frontal cortex (fig. 3) is taken from the lateral surface of the hemisphere on the upper side of the sulcus rectus, about two thirds of its length from its posterior end. Here the depth of the cortex is approximately 2 mm. The lamina zonalis (i) occupies the upper .2 mm. of this. The lamina granularis externa (ii) extends to a depth of rather over .3 mm., while the lamina pyramidalis (iii) forms a zone of which the lower border lies .9 mm. from the surface.

There is nothing remarkable about any of these layers; as is usually the case the pyramids of layer iii increases in size from above downwards, but the large cells near the lower border do not form at all a well-marked layer. The lamina granularis interna (iv) extends from a depth of .9 to a depth of 1.2 mm. or lower, but it is overlapped by the lamina ganglionaris (v), the large cells of which spread upwards among the granules and downward to a depth of about 1.4 mm. The rest of the cortex is occupied by the lamina multiformis (vi), which fades away into the white matter. The cells of this layer are more concentrated near its upper border, thus forming a distinct band between which and (v) is a slightly clearer stratum. This type of cortex corresponds exactly with Brodmann's type 9 or Campbell's "frontal."

The prefrontal type is illustrated in fig. 4—a drawing of a strip of cortex taken from the gyrus rectus about half-way along it.

The prefrontal type differs from the frontal in its comparative poverty in cells and in the smaller size of the cells of layers iii *b* and v, particularly the former. Its depth is also less. Measurements of the cells in the two layers mentioned were made near the posterior boundary of the frontal region and in the prefrontal. It was found that in the former position the largest cells at iii *b* were about 40μ in length \times 30μ in breadth, while in the latter $30\mu \times 23\mu$ represents approximately their dimensions. The general average is, however, in each case much less. The corresponding figures for layer v are: in posterior part of the frontal area about the same as for layer iii *b*, while in the prefrontal area, $35\mu \times 27\mu$ roughly indicates their size. In fig. 3, which is taken fairly far forward in the frontal region, it will be noticed that the subgranular pyramids are rather larger than those situated above the granules, and that the cells of each layer are slightly larger than the corresponding cells in fig. 4.

The extent of the lamina multiformis and the shape of its cells depend to a great extent to the prevalent direction of the fibres of the underlying white matter; where this is tangential the layer is shallow and its cells tend to be drawn out tangentially; where the fibres radiate upwards towards the surface the cells are elongated along an axis vertical to the surface, and the layer tends to be deep. This effect of the direction of the fibres is not always confined to the lamina multiformis, but the layers lying above it may all be involved. It is not confined to one part of the hemisphere, but may be noted in all. It is mentioned here because, in the part of the prefrontal region figured, the fibres are arranged tangentially, while in fig. 3 they are radial in direction. It seemed desirable to explain that this difference is not an essential difference between the two types. In the region of the frontal pole the direction of the fibres of the prefrontal cortex is radial, and the result is a marked difference of appearance in the shape and arrangement of the cells, particularly of the lower layers. The condition described by Brodmann as type 12 may thus be distinguished: "slender pyramids," "layer vi much

extended and passing gradually into the white matter through isolated, scattered spindle-cells."

Extent of Frontal and Prefrontal Types.—The extent and boundaries of the frontal and prefrontal types are shown in the figures, and hardly call for verbal description. The limits are in most places vague and indefinite, but an exception to this statement must be made in the case of the sulcus arcuatus, which forms the posterior, and, to a certain extent the upper boundary of the frontal area on the lateral surface. On the anterior wall of that fissure the cortex is intermediate in character between the frontal and anterior precentral types.

The granule layer is slightly less accentuated than in the former, while a few very well developed pyramidal cells, lying some above it and some below it, are distinctly suggestive of the latter type. This intermediate strip corresponds to Brodmann's type 8. A difference will be noted in the region between Papiro and Cercopithecus, namely, that in the latter the anterior precentral cortex extends in front of the sulcus arcuatus and the intermediate type 8 lies well in the surface; in the former the anterior precentral type stops at the bottom of the fissure.

The whole of the frontal lobe in the chimpanzee and the orang lying in front of the intermediate precentral and limbic areas is covered by the frontal and prefrontal types of cortex. These agree in the possession of a distinct layer of granules, which is broad and well-defined, but not so densely crowded with cells as the corresponding stratum of the parietal or temporal areas. Above and below there is in each case a layer of large pyramids. The chief point of difference lies in the smaller size, slenderer forms, and reduced numbers of the constituent elements of these two layers in the prefrontal area. There is no well-marked line of demarcation, but the large pyramids, which are best developed near the posterior border of the frontal area, gradually undergo reduction in size and number.

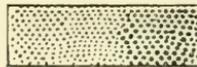
Brodmann's description of the corresponding region in the

hemisphere of *Cercopithecus* agrees in the main very well with Campbell's account summarised above. The slight differences to be noted may be due to the gradual nature of the transition between one type of cortex and its neighbours. Thus Brodmann recognises four different types against Campbell's two, namely, Nos. 8, 9, 12, and 25. Of these, No. 9 clearly corresponds with Campbell's frontal type, and No. 12 with the prefrontal, though the extent of the area occupied by No. 12 is relatively considerably less.

No. 8 lies immediately in front of the sulcus arcuatus; it occupies a narrow strip of the lateral surface of the hemisphere, bounded behind by type No. 6 and in front by type No. 9. In structure it resembles the posterior portion of Campbell's frontal area, being intermediate between the two types, which it separates. No. 25 lies completely on the mesial surface, where it occupies a comma-shaped area between No. 9 (frontal area) and No. 24 (the anterior limbic area). Its structure is intermediate between these.

PARIETAL OCCIPITAL AND TEMPORAL LOBES.

Post-central Type (fig. 6).



The post-central type of cortex is illustrated in fig. 6, which is taken from the crown of the post-central gyrus a little way below the anterior end of the sulcus post-centralis superior (*pcs.*). The cortex is here 1.7 mm. in depth, of which the lamina zonalis (i) occupies the upper .1 mm. The lamina granularis externa (ii), composed as usual of small pyramids and granules, is fairly well developed, but not so closely crowded with cells as to be sharply marked off from the underlying lamina pyramidalis; it reaches to a depth of about .25 mm. The lamina pyramidalis (iii) is very distinctly subdivided into an upper stratum (iii *a*) containing medium-sized

pyramids in no wise remarkable, and a lower stratum (*iii b*), which is very conspicuous, and constitutes the most characteristic feature of the post-central cortex. *iii b* extends from a depth of .5 mm. to a depth of .85 mm., and its principal constituents are large cells closely crowded together, and lying about three deep. Their shape and approximate size may be gathered from the drawing; they are elongated pear-shaped or pyramidal cells drawn out above into a broad process which takes the stain rather faintly. It is difficult to give a numerical indication of their size, but this may be as much as 60 or 70 μ \times 30 μ . The lamina granularis interna is a dense layer of granules intermixed with small pyramids; its lower limit is .1 mm. below that of *iii b*, but above it is extended by columns of small cells which lie between the large elements of that lamina.

Closely underlying *iv* is the lamina ganglionaris *v*. This consists of a number of triangular quadrilateral and other elements of comparatively small size, among which are scattered occasional pyramids of notably superior size; these cells, which lie near the lower border of the layer, at a depth of 1.2 mm., are inferior in size to the supra-granular pyramids, and differ from them also in shape; their cell-bodies are more slender, and their apical processes narrower and more darkly staining. The rest of the cortex is occupied by the lamina multiformis (*vi*), which is separated from *v* by a zone comparatively poor in cells. It cannot be subdivided into a lamina triangularis and a lamina fusiformis.

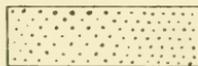
The cortex described above corresponds to Brodmann's types Nos. 1 and 2. As it dips over in front into the sulcus centralis it alters somewhat in character; the whole cortex becomes narrower; the large cells at *iii b* become reduced in size and number; the layer of granules (*iv*) grows less distinct, and the lamina ganglionaris suffers the same changes as *iii b*.

According to Brodmann, the large cells at *iii b* and *v*, which form the most characteristic feature of the post-central cortex, are larger and more numerous near the posterior lip

of the gyrus centralis posterior than at the summit of the gyrus. He thus distinguishes two types—No. 1 clothing the summit of the gyrus, and No. 2 its posterior lip. I do not find that this is the case in Papio. Here the post-central type is at its best in the middle portion of the gyrus, becomes less well-developed near the posterior border, and as it passes downwards into the intra-parietal fissure it loses its distinctive features as it changes into the neighbouring type. A gradual reduction of the size and numbers of these large cells, particularly those of the lamina ganglionaris, can also be observed as one passes downwards over the lateral surface; they are largest and numerous in the upper half of the gyrus, and become very much less conspicuous near the lower end of the sulcus centralis. On the mesial surface the large cells of the lamina ganglionaris are particularly well represented, while those of the lamina pyramidalis inferior are neither so large nor so numerous.

Extent of the Post-central Type.—The post-central cortex, modified locally as described above, occupies on the mesial surface the posterior portion of the para-central lobule. On the lateral surface it extends anteriorly to the bottom of the sulcus centralis, and occupies the greater part of the gyrus centralis posterior. Above, where the sulcus intra-parietalis bends backwards, it passes into the superior parietal type, while below it extends in front of the lower end of the sulcus centralis, and becomes continuous anteriorly with the lower portion of the anterior precentral, while posteriorly it changes into the inferior parietal cortex.

Superior Parietal Type (fig. 7).



The superior parietal type is illustrated in fig. 7. The general plan of its layers and their relative extent is very much the same as that of the post-central type. The most

striking difference is to be found in the lamina pyramidalis inferior (iii *b*). Here, though numerous large pyramids are present forming a fairly definite layer, they are notably inferior in size to those of the post-central type, while in number also they are conspicuously less. They are confined to a narrow zone lying immediately above the granules, while in the post-central cortex they form a broad stratum which occupies more than half of the whole lamina pyramidalis.

Extent and Boundaries.—The superior parietal cortex occupies on the lateral surface the greater part of the area bounded laterally by the sulcus intra-parietalis, mesially by the border of the hemisphere, posteriorly by the upper part of the sulcus lunatus, and anteriorly by the post-central cortex. It extends also on to the mesial surface, where it lies between the sulcus cinguli and the ascending limb of the sulcus subparietalis. Below it passes into the posterior limbic type, and behind into the inferior parietal.

Inferior Parietal Type (fig. 8).



The inferior parietal cortex is illustrated in fig. 8, which is taken from the lateral surface of the hemisphere just posterior to the sulcus intra-parietalis at a level a little below the posterior end of the sulcus temporalis superior. The cortex is here 1.9 mm. deep. The lamina granularis interna (iv) is very well marked; it is about .2 mm. in breadth, its upper margin lying at a depth of .8 mm. Above it lies a zone of large pyramids (iii *b*) of approximately the same breadth. The cells contained in it are smaller than their homologues in the superior parietal cortex. In shape they are rather slender: their length may be as much as 40 μ , but their breadth is never much over 20 μ .

Below the granules is a fairly well-developed lamina ganglionaris (v). The majority of the cells in this layer are

much smaller than those at iii *b*, but scattered among them are a few of outstanding size, yet not so large as the corresponding cells of the post-central or superior parietal regions. The lamina multiformis may be divided into an upper lamina triangularis and a lower lamina fusiformis. The cells of the former are large and numerous with broad triangular or quadrilateral forms predominating, while those of the latter are smaller and slenderer, and many of them approach the fusiform in shape. On the mesial surface the breadth of the cortex is about $\frac{1}{5}$ mm. less. In this reduction, which is associated with the more tangential arrangement of the fibres in the underlying white matter, the lamina multiformis is alone much affected. Not only is this layer reduced in breadth, but its constituent cells are reduced in size and numbers.

This type of cortex bears a considerable resemblance to the temporal and some to the frontal cortex. It differs from the latter, however, in many important points. It is of about the same breadth but much richer in cells. The lamina zonalis is narrower, the lamina granularis externa more clearly defined. The large pyramids at iii *b* are much more numerous and form a more distinct layer. The lamina granularis interna is also much richer in cells, and more obviously separated from the adjacent strata. The lamina ganglionaris is not so well developed, but the lamina multiformis is broader and richer in cells, and does not show the gradual transition to the white matter which Brodmann observes is a characteristic of the frontal lobe.

Extent and Boundaries.—The inferior parietal cortex lies behind and below the superior parietal both on the lateral and on the mesial surface. On the mesial surface it covers a broad area bounded in front by the sulcus subparietalis and behind by the ramus parieto-occipitalis of the intra-parietal fissure. Below it passes gradually into the posterior limbic and occipital types. Above it passes over the supero-mesial border and forms a narrow strip between the superior parietal area and the upper end of the sulcus lunatus, then crosses the sulcus

intra-parietalis, and invests the triangular area lying between that fissure and the upper end of the sulcus temporalis superior; below this it extends forwards and downwards on the upper side of the Sylvian fissure; it gradually becomes narrower, and finally dies away a little in front of the lower end of the sulcus centralis.

The inferior parietal cortex corresponds in position and structure to Brodmann's type 7. That part of it which lies in the mesial surface and on the lateral surface above the intra-parietal corresponds with Campbell's parietal area, but is notably less in extent than in the higher apes or in man. The remaining area is according to Campbell clothed with temporal cortex, but there is so great a similarity between the two types that there is no great antagonism in the two descriptions.

Calcarine Type (fig. 9).



The calcarine type of cortex is illustrated in fig. 9, a drawing taken from the wall of the lower branch of the posterior bifurcation of the calcarine fissure. Here the cortex is just over 1.5 mm. in breadth.

The lamina zonalis occupies rather more than the upper .1 mm. of this, and the lamina granularis externa forms a narrow border of small pyramids and granules below it. The lamina pyramidalis, consisting of small- and medium-sized pyramids, extends to a depth of .4 mm. It is densely crowded with cells, which follow the usual arrangement in being larger near the lower border. The largest of them do not appear to exceed a length of 25 μ . What is considered by Brodmann to be homologous with the lamina granularis interna occupies about half the total breadth of the cortex, extending, as it does, from a depth of .4 to a depth of 1.15 mm. It can be divided into three strata: (1) An upper layer of granules

interspersed with a few small pyramidal cells; this extends from .4–.55 mm. (2) A middle layer, .25 mm. wide, corresponding to Campbell's layer of large stellate cells. This is rather poorer in cells than the rest of the cortex, being the region occupied by the stria of Gennari. A considerable number of cells are, however, present, and these seem to be concentrated rather nearer to the upper than to the lower border. The most conspicuous are the large stellate cells themselves, the length and breadth of which may be about 25 μ . The majority of these seem to be in the form of rather broad pyramids, sending out an apical process and horizontal basal processes. Scattered among them are granules and smaller pyramids. (3) The lower layer of granules extends from a depth of .8 to a depth of 1.15 mm. This can be clearly subdivided again into an upper layer of large pyramidal and irregular-shaped cells and a lower layer consisting almost entirely of small granules. The lamina ganglionaris is comparatively poor in cells, and the cells themselves are small. The solitary cells of Meynert, which are reckoned to belong to this layer, are actually sunk within the upper part of the underlying lamina multiformis; they are not very numerous, and only one of them is shown in the drawing; in size they may be about 30 μ in length by about the same breadth. The lamina multiformis is very narrow, but richer in cells than the layer above it; the cells themselves are small.

Extent and Boundaries.—The calcarine cortex is on the mesial surface largely confined to the calcarine fissure; at the posterior bifurcation of the latter it emerges on to the surface and covers the whole area lying behind it. Spreading on to the lateral face of the hemisphere it occupies the greater part of the large area, bounded in front by the sulcus lunatus, to the lip of which it almost extends. It covers the walls of the lateral occipital fissure. Near the occipital pole it does not extend much below that sulcus, but in front it spreads downwards much farther, yet always falls far short of the sulcus occipitalis inferior.

Occipital Type (fig. 10).



The occipital type is illustrated in fig. 10, which is drawn from the lateral surface of the hemisphere between the sulcus lunatus and the upturned end of the sulcus temporalis superior. The cortex is here 1.6 mm. deep, of which the lamina zonalis occupies rather over .1 mm. The lamina granularis externa is not clearly separated from the subjacent lamina pyramidalis. The latter extends downwards to a depth of .75 mm., and its lower half is characterised by the presence of a number of elongated pyramids, which reach a length of 50 μ and more. The lamina granularis interna is narrow but very well marked; the granules are arranged closely together, and very few cells of other forms are present among them except in so far as they themselves extend upwards among the bases of the large pyramids. The lower border of this layer lies at a depth of .9 mm. The lamina ganglionaris is composed of rather small pyramids; it is poor in cells and appears as a clear band under the low power of the microscope. It is the conspicuous presence of a layer of large pyramids above the granules, coupled with their conspicuous absence below, which gives the distinctive character to this type of cortex. The lamina multiformis is well developed and about .4 mm. broad. Along its upper border the elements are larger and more triangular, while near its lower edge they are smaller and more fusiform.

The occipital type, which corresponds to Campbell's visuo-psychoic and Brodmann's types 18 and 19, is not of uniform structure over the whole area which it invests. Immediately adjacent to the calcarine area, which it completely surrounds, it is much narrower than in the position selected for illustration, fewer cells are present, and the lamina ganglionaris is relatively clearer. As it approaches the temporal or inferior parietal areas which form the greater part of its anterior boundary it assumes more the character of those

types. It is this intermediate structure which forms Brodmann's type 19.

Extent and Boundaries.—The occipital type completely surrounds the calcarine, the boundary between the one type and the other being quite abrupt and definite. Very different is the transition between the occipital and the inferior parietal temporal and posterior limbic types, into which it passes anteriorly. This is so gradual that it is almost impossible to say where the one ends and the other begins, and the boundary is in consequence very difficult to describe. Under these circumstances I do not attempt a verbal description, but merely refer to the figures A, B, C, D.

Temporal Type (fig. 11).



The essential features of the cortex covering the greater part of the temporal lobe are shown in fig. 11, which is taken from the middle temporal gyrus in its posterior half. Its depth is here about 2 mm.

The lamina zonalis extends to a depth of .2 mm. The lamina granularis externa is closely packed with cells and fairly well separated from the underlying lamina pyramidalis. The latter extends to a depth of rather more than half the whole thickness of the cortex. The large pyramids (iii*b*), which reach a length of 30 μ or more, form a broad zone near the inner border of the layer.

The lamina granularis interna is exceptionally well developed, and stretches from 1.1 mm. to 1.3 mm. from the surface.

Beneath this layer the development of the cortex is poor in comparison with the parts above, but the lamina ganglionaris is fairly well developed, though its cells are slightly smaller than those at iii*b*. A slightly clearer space separates it from the lamina multiformis.

The description given above applies in its details only to the middle temporal gyrus. The cortex of the superior temporal gyrus differs in many respects. It is broader and poorer in cells. The lamina granularis interna is not so well developed. The lamina ganglionaris is, on the whole, not so clear, but contains a few large cells scattered through it.

A change in structure is also apparent as one moves round the temporal lobe in the other direction. As one approaches the hippocampal gyrus the cortex becomes narrower, and the layer of granules less well developed.

The extent of the olfactory cortex is shown in fig. c, but it has not been thought necessary to give a special description or drawing of its structure.

Anterior Limbic Cortex (fig. 12).



The anterior limbic type is illustrated in fig. 12, which is taken from the mesial surface of the hemisphere just above the anterior portion of the corpus callosum. The cortex here reaches a depth of 1.9 mm. Its chief characteristic is the absence of any very definite system of stratification. The lamina granularis externa is very poorly developed; it passes gradually into the lamina zonalis above it and is hardly possible to separate from the lamina pyramidalis below. The latter is somewhat sparsely populated with small and medium-sized pyramids until a depth of .6 or .7 mm. is reached; below this, extending for about half a millimetre, is a zone of cells considerably larger in size and more closely arranged, which probably represents the lower part of the lamina pyramidalis (iii *b*) and the lamina ganglionaris (v). There is no trace of the lamina granularis interna. The lamina multiformis is also poor in cells, of which the upper ones are of fair size and triangular form, while the lower ones are smaller and more spindle-shaped.

Posterior Limbic Cortex (fig. 13).



The posterior limbic cortex is illustrated in fig. 13, which is taken from the mesial surface of the hemisphere just above the posterior end of the corpus callosum. It differs from the anterior limbic type in (1) a greater richness of cells, (2) the better development of the lamina granularis externa, (3) the presence of a lamina granularis interna. The latter, though clearly defined, is not so well developed as in the inferior parietal or occipital types, which it resembles in some respects. The lamina ganglionaris follows closely below the internal layer of granules. It is well developed, the cells on the whole being larger and more numerous than in the strip actually drawn.

Extent and Boundaries.—The anterior limbic cortex lies between the sulcus cinguli and the corpus callosum; it is continued forward as a broad band round the anterior end of the latter. This anterior portion is separated from the frontal cortex by an area intermediate in character. Posteriorly it extends a little way behind the region of the mesial surface, in which the anterior precentral changes into the precentral type. At this point, by the gradual acquisition of an internal layer of granules, it becomes transformed into the posterior limbic type. The latter extends round the posterior end of the corpus callosum, changing gradually above and behind into the types of cortex on which it abuts.

The posterior limbic cortex corresponds to Brodmann's type 23, the anterior to his type 24, while the area intermediate in character between the limbic and the frontal is equivalent to his type 25.

EXPLANATION OF PLATES 24-30,

Illustrating Mr. E. H. J. Schuster's paper, "Cortical Cell Lamination of the Hemispheres of Papio Hamadryas."

PLATE 24.

The two hemispheres seen from different points of view to show fissures and distribution of various types of cortex.

Fig. A.—Dorsal view of both hemispheres.

Fig. B.—Ventral view of both hemispheres.

Fig. C.—Mesial view of left hemisphere.

Fig. D.—Lateral view of left hemisphere.

Fig. E.—Mesial view of right hemisphere.

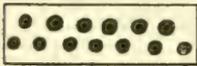
Fig. F.—Lateral view of right hemisphere.

The left hemisphere in figs. A, B, C, D is shaded to show the superficial extent of the different types of cortex described. The lettering on the right hemisphere in figs. A, B, E, and F refers to the fissures, while the numbers show approximately the position from which the strips of cortex drawn in figs. 1-13 are taken. These drawings were, many of them, taken from the left hemisphere, but their position has for the sake of simplicity been transferred to the right hemisphere in the diagrams.

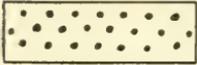
FS. Sylvian fissure. *c.* Sulcus centralis. *arc.* Sulcus arcuatus. *it.* Sulcus inferior transversus. *rect.* Sulcus rectus. *fs. 1, 2, 3, 4.* Sulcus precentralis superior and sulcus frontalis superior. *orb.* Sulcus orbitalis. *fo.* Sulcus fronto-orbitalis. *Ts.* Sulcus temporalis superior. *Tm.* Sulcus temporalis medius. *pes.* Sulcus post-centralis superior. *ip.* Sulcus intra-parietalis. *ppo.* Ramus parieto-occipitalis of sulcus intra-parietalis. *fpo.* Fissura parieto-occipitalis. *sp.* Sulcus subparietalis. *Sc.* Sulcus cinguli. *ro.* Sulcus rostralis. *Col.* Sulcus collateralis. *rh.* Sulcus rhinalis. *lun.* Sulcus lunatus. *oi.* Sulcus occipitalis inferior. *ol.* Sulcus occipitalis lateralis. *Cal.* Sulcus calcarinus.

Magnification in figures 1-13 = 130.

EXPLANATION OF SHADING IN FIGURES A, B, C, D, PLATE 24.



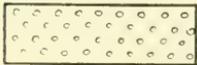
Precentral cortex (Pl. 25, fig. 1).



Anterior precentral cortex (Pl. 25, fig. 2).



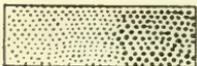
Frontal cortex (Pl. 26, fig. 3).



Prefrontal cortex (Pl. 26, fig. 4).



Posterior orbital cortex (Pl. 27, fig. 5).



Post-central cortex (Pl. 27, fig. 6).



Superior parietal cortex (Pl. 28, fig. 7).



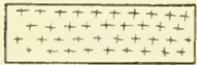
Inferior parietal cortex (Pl. 28, fig. 8).



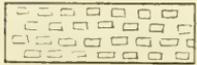
Calcarine (visual) cortex (Pl. 29, fig. 9).



Occipital cortex (Pl. 29, fig. 10).



Temporal cortex (Pl. 30, fig. 11).



Anterior limbic cortex (Pl. 30, fig. 12).



Posterior limbic cortex (Pl. 29, fig. 13).



Olfactory cortex (Pl. 24, fig. E, *rh*, and fig. C).

PLATE 25.

The shading on figs. A, B, C, D in Pl. 24, as shown in the diagram on the opposite page, corresponds in position to the sections of cerebral cortex shown in the several figures in Pls. 25 to 30.

Fig. 1.—Precentral motor cortex.

Fig. 2.—Anterior precentral cortex.

PLATE 26.

Fig. 3.—Frontal cortex.

Fig. 4.—Prefrontal cortex.

PLATE 27.

Fig. 5.—Posterior orbital cortex.

Fig. 6.—Posterior central cortex.

PLATE 28.

Fig. 7.—Superior parietal cortex.

Fig. 8.—Inferior parietal cortex.

PLATE 29.

Fig. 9.—Calcarine (visual) cortex.

Fig. 10.—Occipital cortex.

Fig. 13.—Posterior limbic cortex.

PLATE 30.

Fig. 11.—Temporal cortex.

Fig. 12.—Anterior limbic cortex.

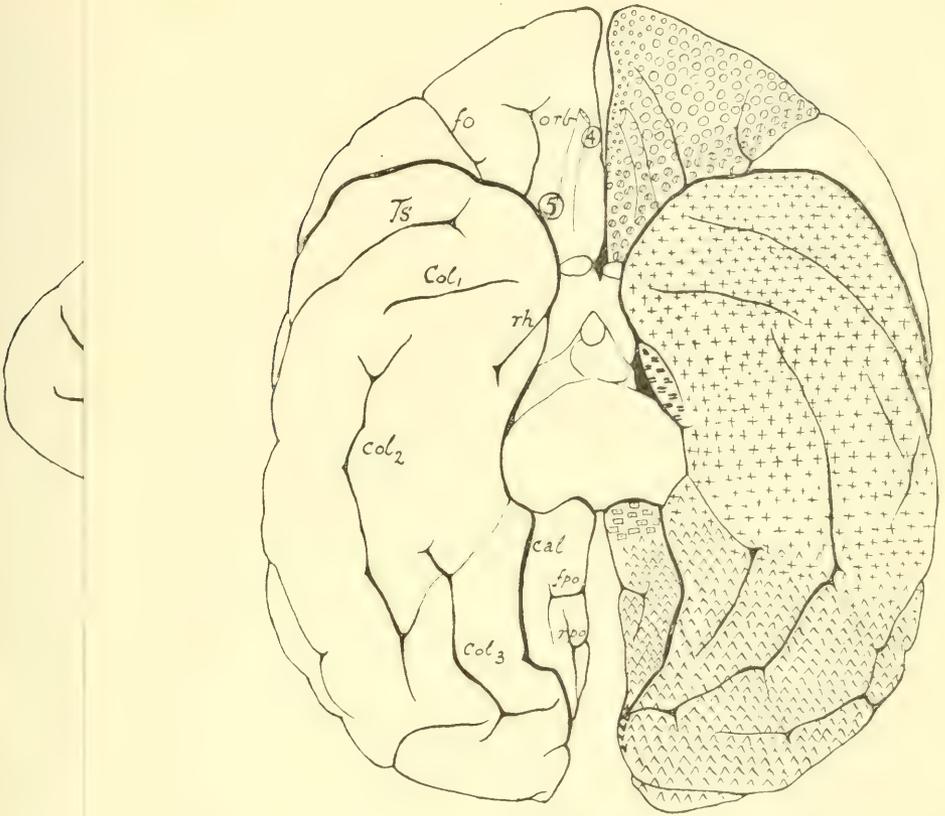


FIG. B.

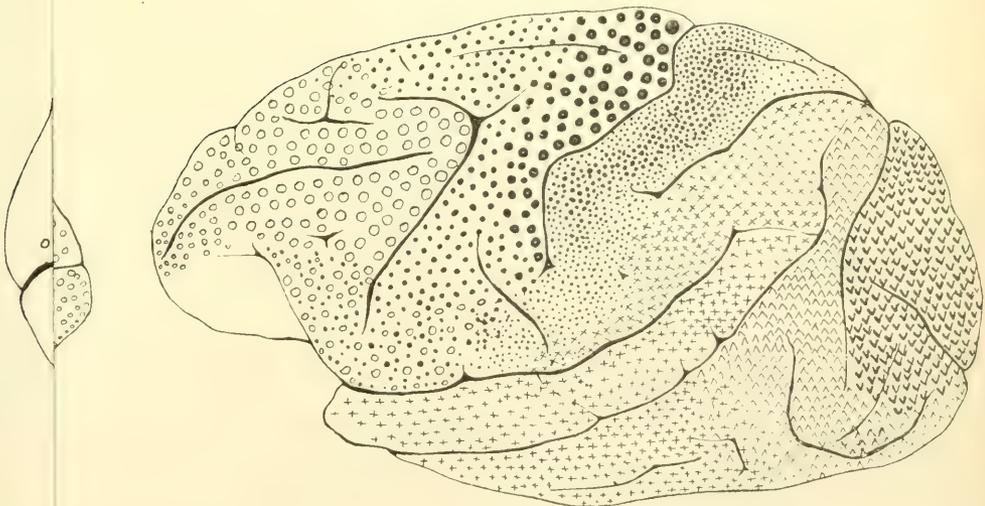


FIG. D.

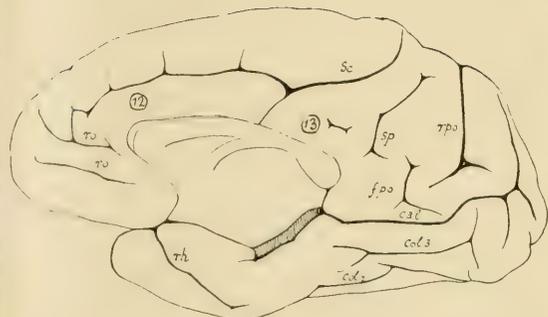


FIG. E.

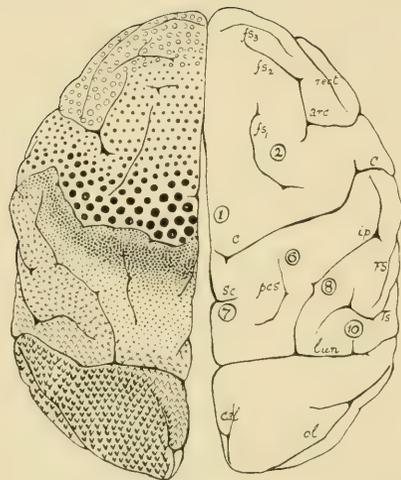


FIG. A.

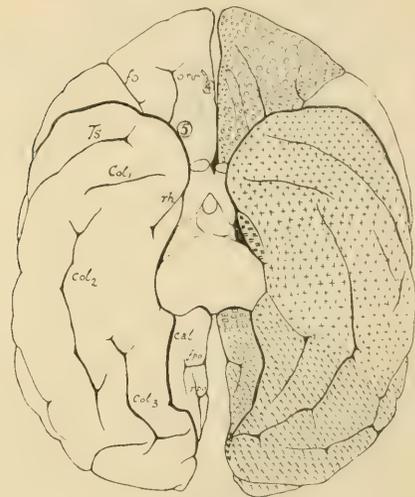


FIG. B.

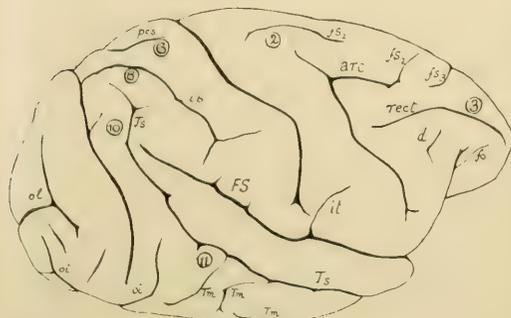


FIG. F.

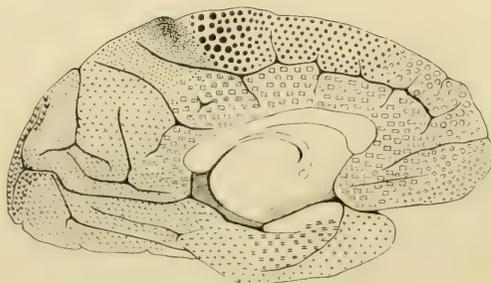


FIG. C.

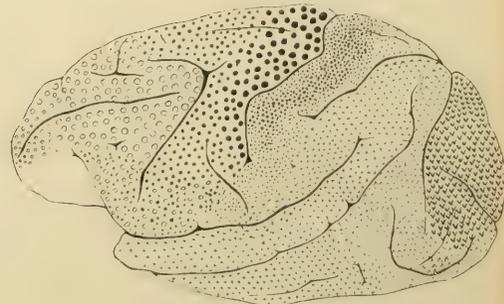


FIG. D.



ANTERIOR PRAECENTRAL CORTEX.

FIG. 2.



PRAECENTRAL (MOTOR) CORTEX.

FIG. 1.



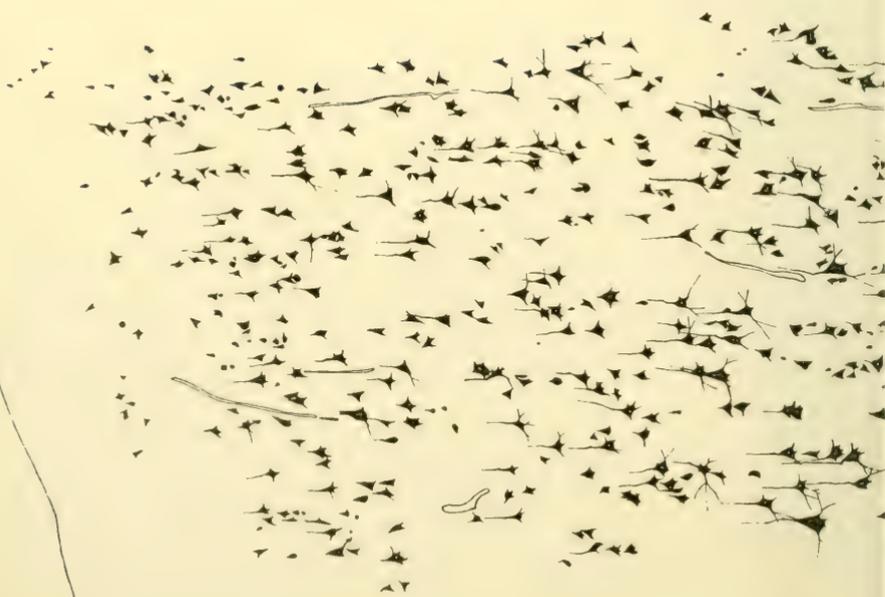
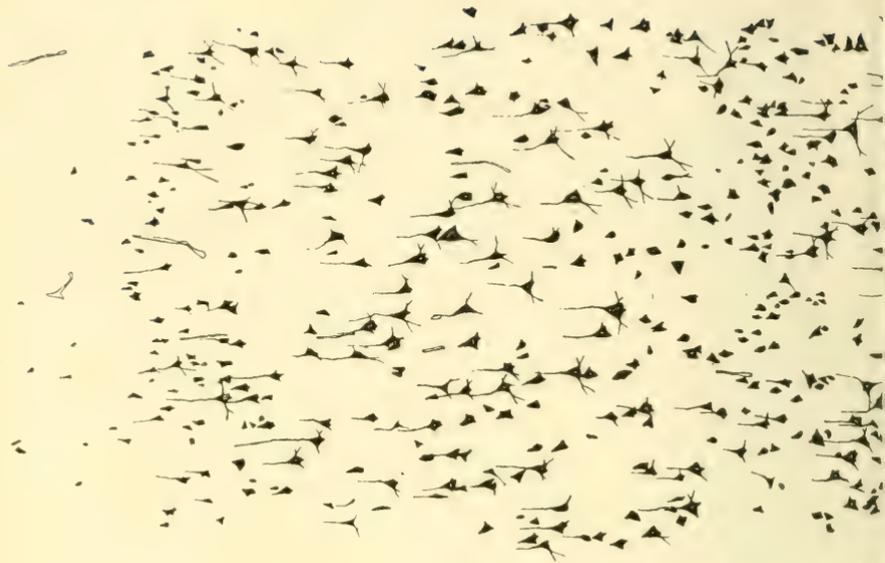
ANTERIOR PARACENTRAL CORTEX

FIG. 2.



PARACENTRAL (MOTOR) CORTEX.

FIG. 1.





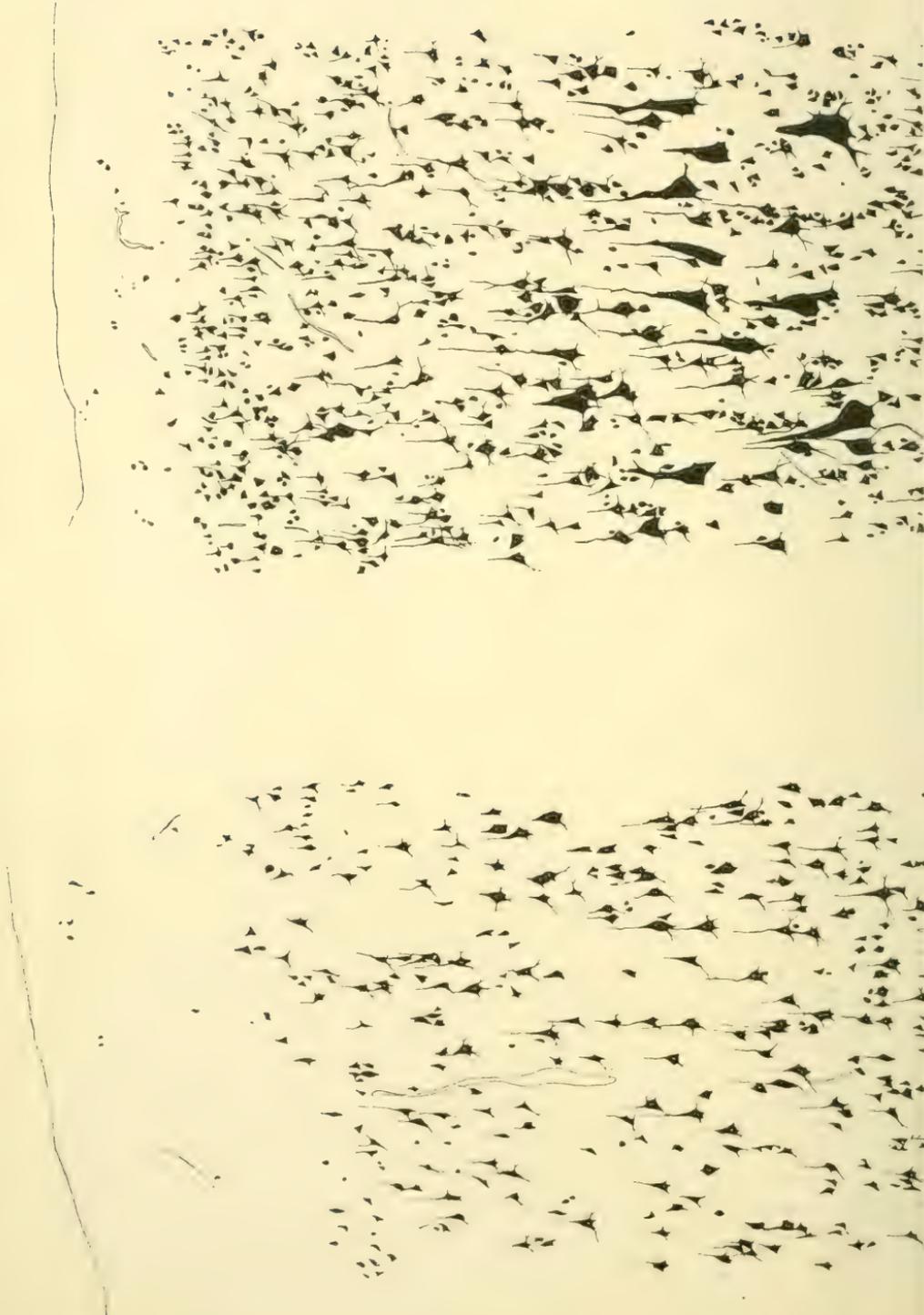
PREFRONTAL CORTEX.

FIG. 4.



FRONTAL CORTEX.

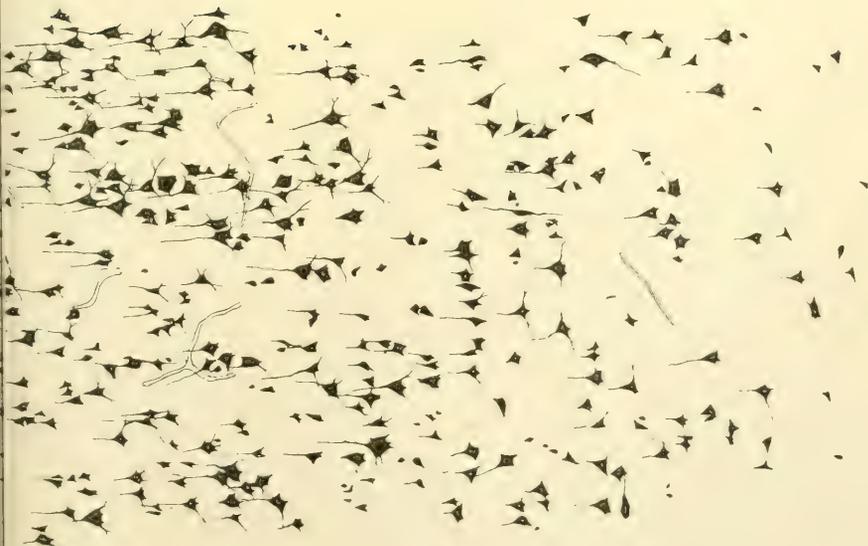
FIG. 3.





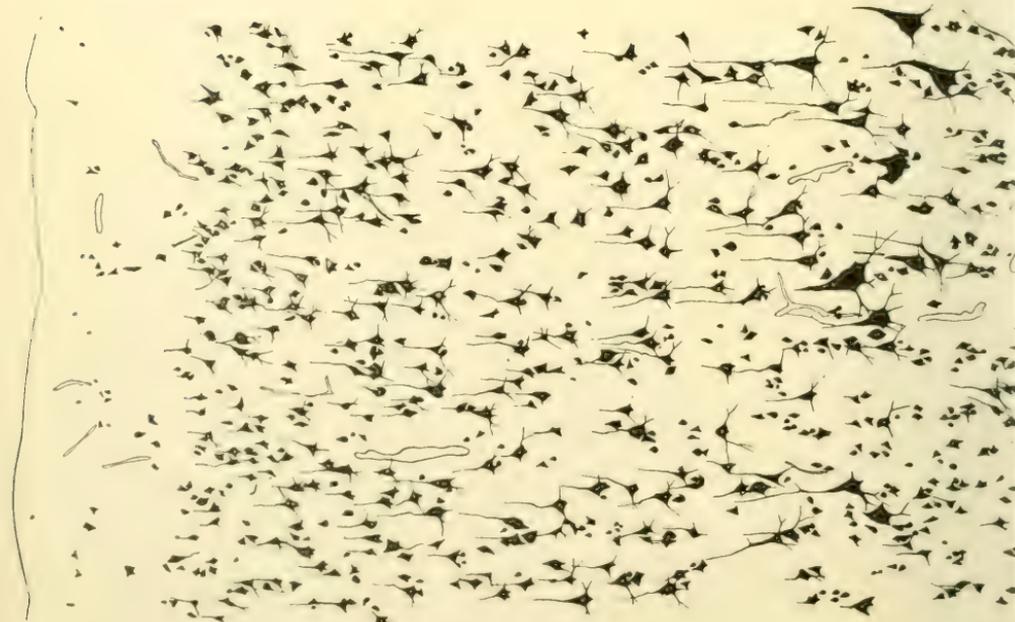
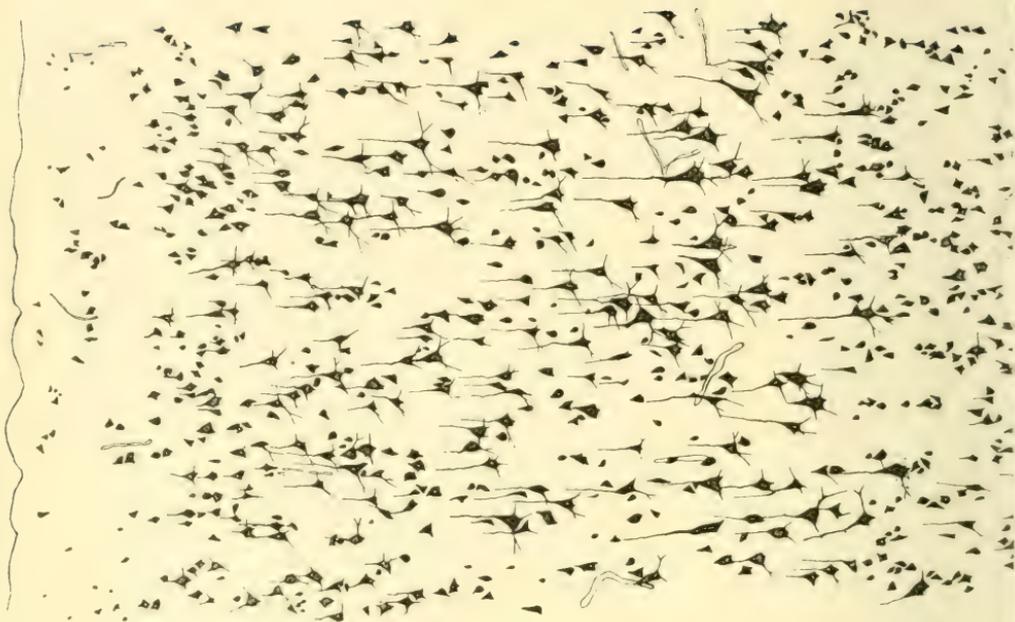
POSTERIOR CENTRAL CORTEX.

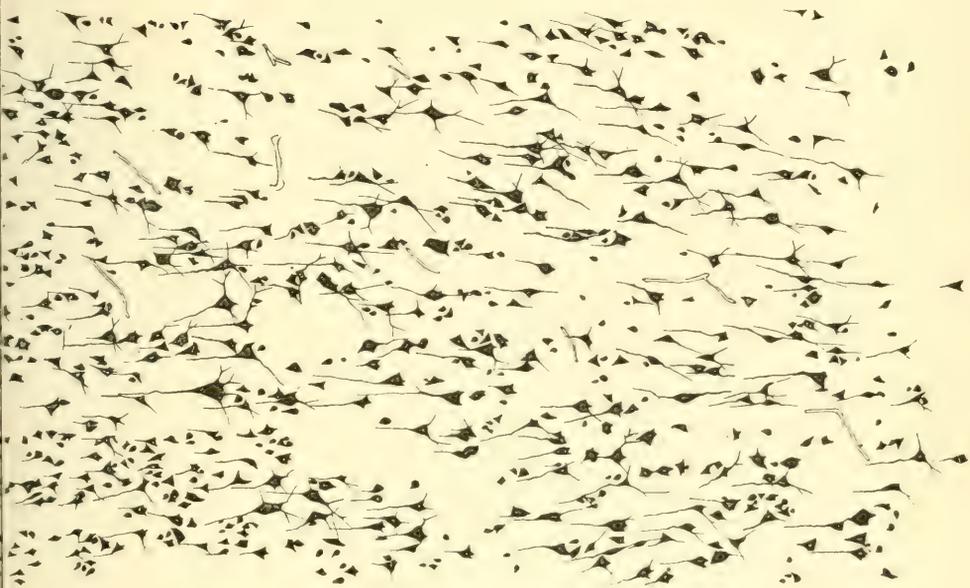
FIG. 6.



POSTERIOR ORBITAL CORTEX.

FIG. 5.





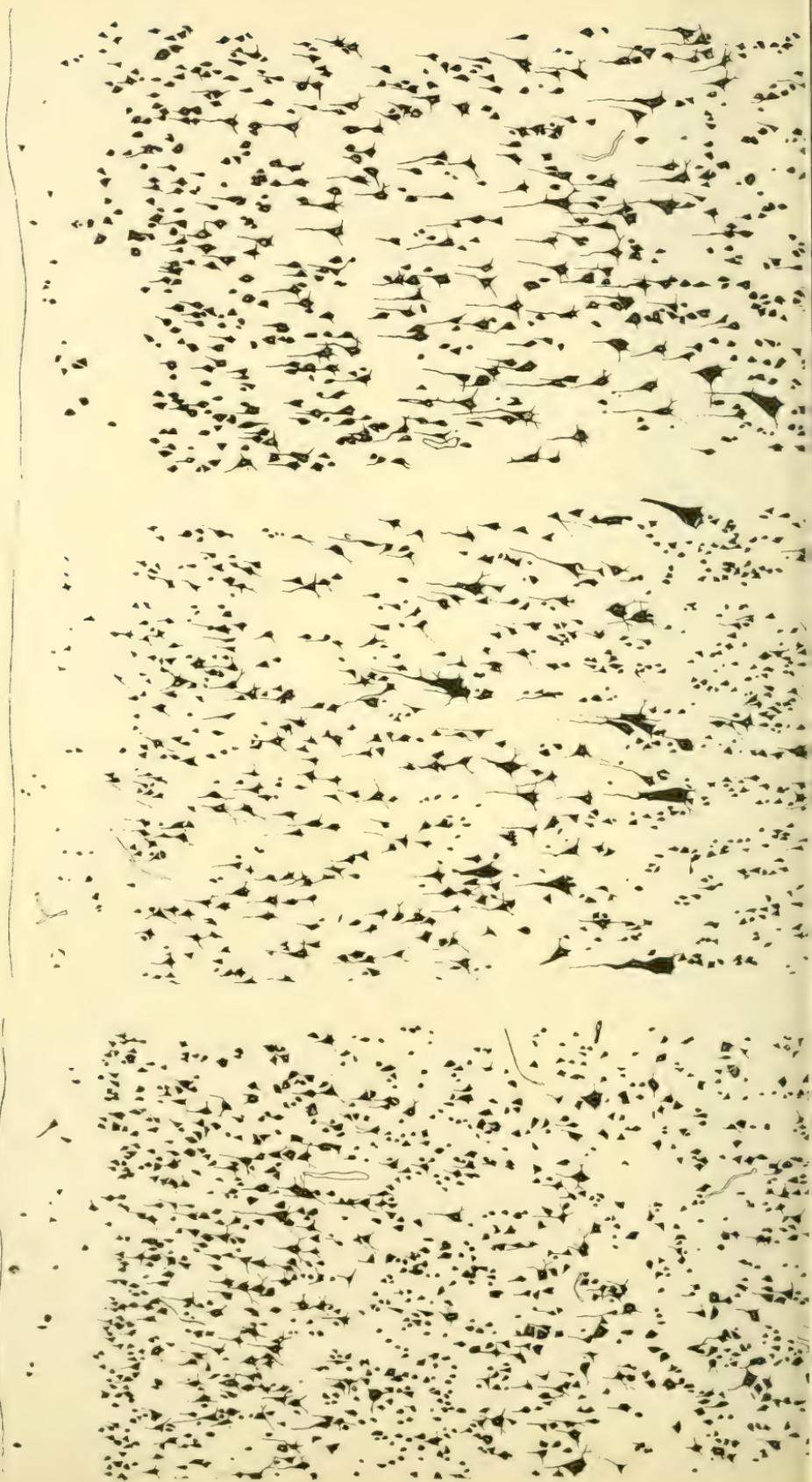
INFERIOR PARIETAL CORTEX.

FIG. 8.



SUPERIOR PARIETAL CORTEX.

FIG. 7.

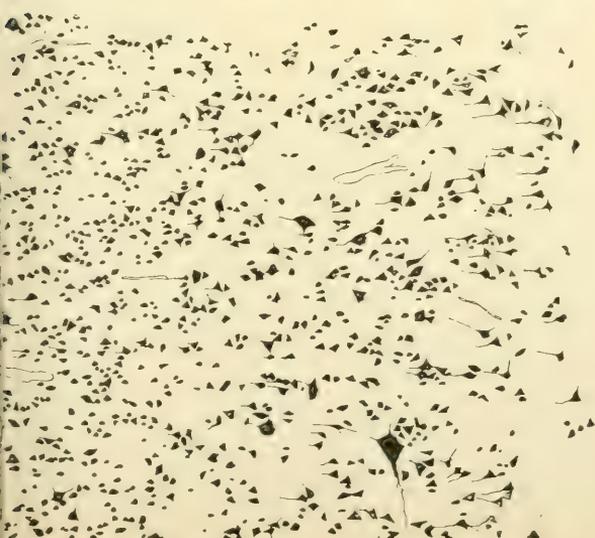




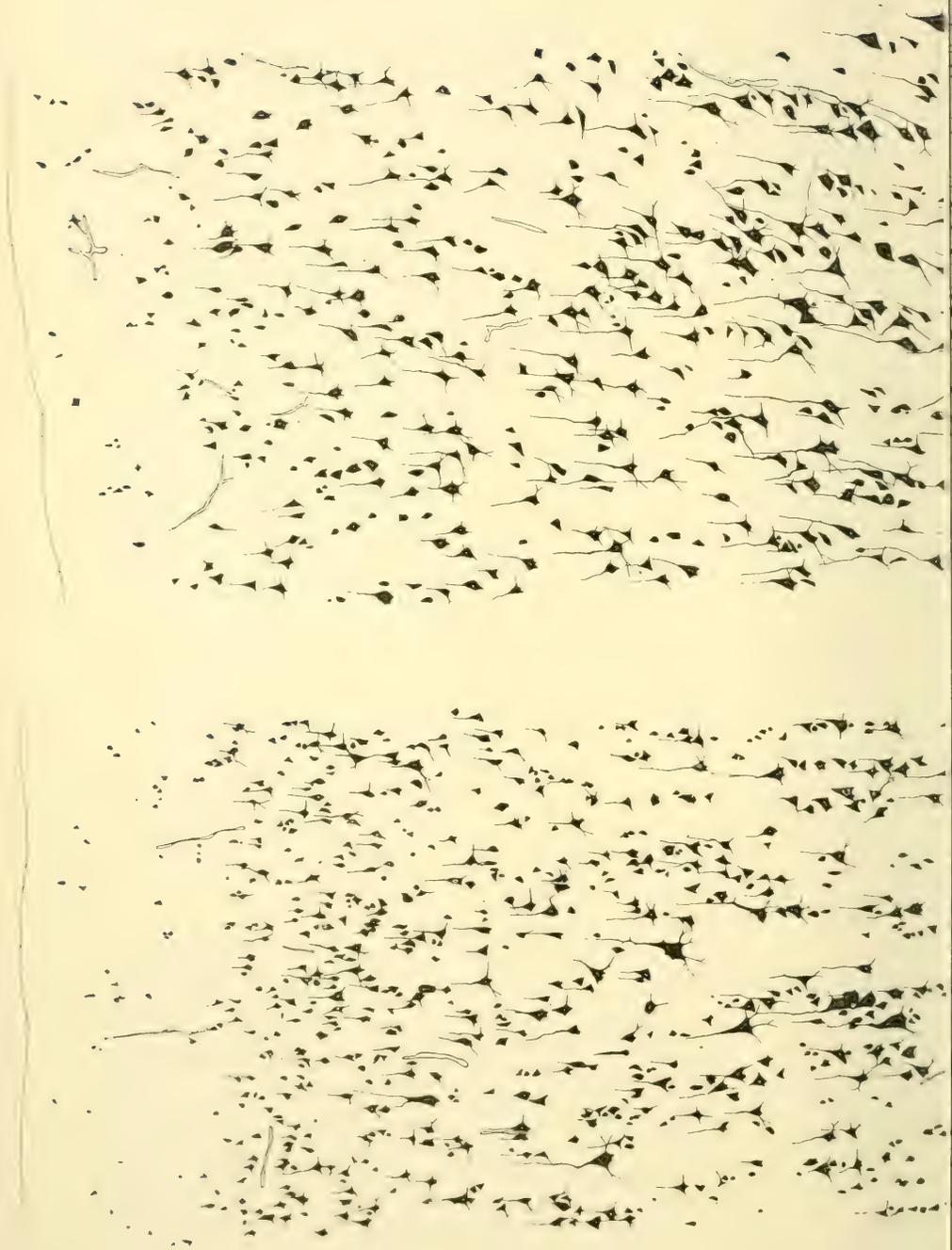
POSTERIOR LIMBIC CORTEX.
FIG. 13.



OCCIPITAL CORTEX.
FIG. 10.



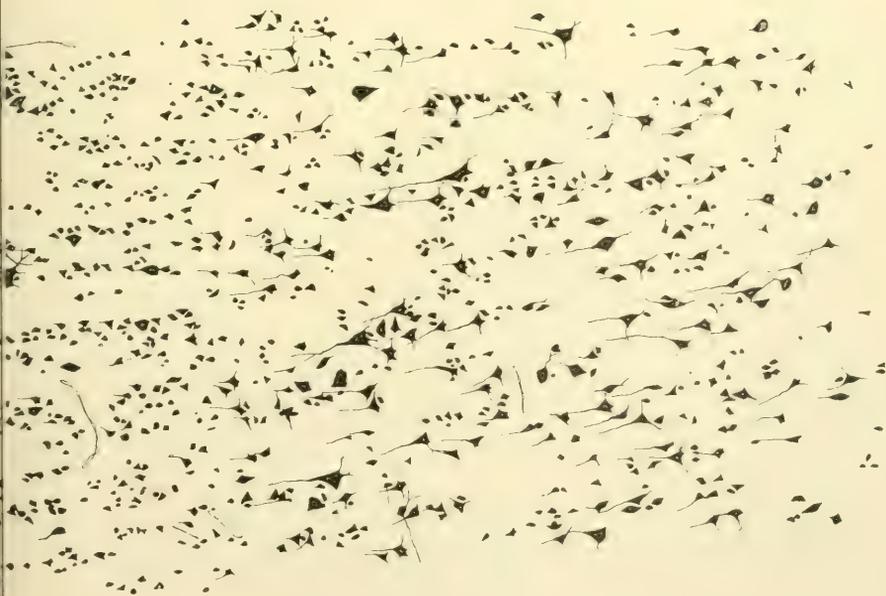
CALCARINE (VISUAL) CORTEX.
FIG. 9.





ANTERIOR LIMBIC CORTEX.

FIG. 12



TEMPORAL CORTEX.

FIG. 11

On Some Stages in the Life-History of *Leptomonas muscæ domesticæ*, with some remarks on the Relationships of the Flagellate Parasites of Insects.

By

J. S. Dunkerly.

With Plate 31.

IN an endeavour to examine the biflagellate character of *Herpetomonas* as described by Prowazek (20), I have dissected and examined a number of house-flies. At first *Musca domestica* was investigated, as I had supposed that this was the animal indicated by the word "Stubenfliege." In this country, however, *M. domestica* does not seem to be commonly infected, as I was unable to find the parasite in it, and Hewitt (6), who examined a good number of these flies, was similarly unsuccessful. In the smaller house-fly, *Homalomyia canicularis*, flagellate parasites were found to be present, but Hewitt had confined himself to *M. domestica*. Still, the infections were very rare. I examined these flies taken in three distinct localities: Chelsea and Wandsworth in London, and Benfleet in Essex. Parasites were found in flies from each place, but always in a low percentage, about 4 per cent., of the flies examined. Other species of flies¹ have been examined, but not in large numbers, so that it is not surprising that no *Herpetomonads* have been met with in them as yet.

¹ I take this opportunity of expressing my thanks to Mr. Austen, who kindly assisted me in the identification of these flies.

I will first describe the forms met with by me in *Homalomyia canicularis*, and afterwards discuss their significance. (1) In the midgut (ventriculus) of two flies were seen some large typical *Herpetomonas* forms (Pl. 31, fig. 1). This form had a body $25\ \mu$ to $30\ \mu$ long, and the flagellum was $30\ \mu$ long. Its movements were characteristic, the body being clumsily swung from side to side by the lashing of the long thick flagellum. With Giemsa's stain the double character of the flagellum described by Prowazek is evident in most cases. If, as Patton (17) states, this is merely a stage in division, then it is an unusual type of division, since the kinetonucleus is not even transversely elongated when the flagellum has divided along its whole length, this being very unlike the state of affairs found by me in dividing forms (Pl. 31, fig. 9). My material for the study of these forms has been very scanty, and I can only say that they are very different, both in appearance and size, from the other flagellates met with in the fly.

(2) A commoner form (Pl. 31, figs. 2-14) was found in the intestine, and once in the Malpighian tubules. These infections were always heavy ones, the parasites occurring in dense clusters, either on the intestinal wall or free in the lumen of the intestine. On slides the clusters were seen to be formed by the typical rosettes, or more correctly, agglomerations, with the flagella pointing to the centre, as described by Woodcock (26) for cultural forms of trypanosomes. The body of this form was $15\ \mu$ to $18\ \mu$ long, and its movement was rapid and graceful, the anterior part of the body often undulating. A large number of dividing forms were usually present (Pl. 31, figs. 6-10). In one case, in which the forms were particularly elongated (Pl. 31, fig. 14), cysts were also found, and doubtful intermediate stumpy forms. The characters to which I wish to draw particular attention are the varying position of the kinetonucleus and the presence of an undulating membrane (Pl. 31, figs. 3, 4, and 11-14). (The forms shown in figs. 3, 4, and 11 are from the same fly). All intermediate stages between the short form, with its

anterior end truncated, and the elongated one with anterior extremity drawn out into a membrane attached to the flagellum, are found (Pl. 31, figs. 3, 4, and 11). A form resembling that in fig. 11 was found by Chatton and Alilaire (2) in *Drosophila confusa*, and described under the name *Trypanosoma drosophilæ*, although the authors recognised the possibility of its being a stage in the life-history of a *Leptomonas* found by them in the same fly. Werner (24) also described the same form from "Stubenfliegen," and named it *Crithidia muscæ domesticæ* to distinguish it from the biflagellate *Herpetomonas muscæ domesticæ* of Prowazek. Miss Mackinnon (14) also, whose paper appeared while this work was in progress, in describing what she regards as a *Herpetomonas* from *Homalomyia corvina* (?) pointed out the similarity between some of the forms found by her and the *Crithidia* of Werner, which would not be surprising if both are stages in the life-history of organisms belonging to the same genus, possibly to the same species. The forms shown in Pl. 31, figs. 12, 13, and 14 possess, undoubtedly, an undulating membrane, though the flagellum is not produced beyond it, and these resemble in a striking manner some stages of *Trypanosoma cazalbouri* in cultures, described by Roubaud (22, pl. viii, figs. 2 and 6), thus indicating a close relationship between the parasite of a non-blood-sucking fly like *Homalomyia* and the trypanosomes of vertebrates. Patton (18, and 17, p. 142, note), in objecting to Prowazek's account of *Herpetomonas* (which, however, has been supported by other observers, Lingard and Jennings [12], Roubaud [22]) decided that all uni-flagellate parasites of insects with the kinetonucleus anterior to the trophonucleus and without undulating membrane are to be called *Herpetomonas*, and that those having the kinetonucleus posterior to the trophonucleus, and possessing an undulating membrane, should receive the generic name of *Crithidia*. Lühe (13) and Hartmann and Jollos (5) have pointed out that Patton's failure to see the characters observed by Prowazek and others does not prove their non-existence; and as to his

use of the name *Crithidia*, this is certainly a misuse of Leger's term, which he applied (8) to a short rounded form, "en form de grain d'orge légèrement aplati et tronqué à l'extrémité antérieure . . . ," and usually without an undulating membrane. However, it seems from the evidence of the forms found in *Homalomyia* that the same organism may be without an undulating membrane at one stage of its life-history, while possessing one at another stage. I shall return later in this paper to this question of nomenclature.

(3) In the rectum, near the rectal glands, were found masses of small oval bodies (Pl. 31, fig. 15) attached to the rectal epithelium. On examining these in water I was able to observe the mass apparently swell, as though the walls of the oval bodies were gelatinous, and after a short time some of the bodies were seen to become actively motile, with a small anterior flagellum (Pl. 31, figs. 16 and 17). The flies containing these cysts had no other flagellate stages in them, but came from the same locality as those that had. Similar cysts have been described by Minchin (15) for *T. grayi*, by Prowazek (20), Rosenbusch (21), and Mackinnon (14) for *Herpetomonas*, the latter having observed them giving rise to flagellates. The cysts stained with Giemsa (Pl. 31, fig. 15) show a faint trophonucleus and a distinct kintonucleus, with a large number of scattered granules stained a deep purple colour, and have a definite wall surrounded by a remarkable substance which stains deeply, and may be gelatinous in nature (vide supra). But iron-hæmatoxylin shows little of these peculiar effects (Pl. 31, fig. 17A). The commencement of development of the flagellum is indicated by a clear area in Giemsa preparations (Pl. 31, fig. 15B), the borders of which appear to stain with iron-hæmatoxylin, showing a triangular area with the kintonucleus as base (Pl. 31, fig. 17A and D), and the same appearance has been seen in non-flagellate forms of *T. lewisi* by Prof. Minchin, who kindly showed me his original drawings.

In the life-cycle of *Leptomonas*, as far as I have investigated it, we find the following forms: (1) A typical *Lepto-*

monas (fig. 2), which actively divides in the intestine or in the Malpighian tubules of the fly (figs. 6–10), producing (2) very active, slender forms, often with undulating membrane (figs. 11–14). These probably encyst while attached in large numbers to the rectal wall, and the cysts (figs. 15 and 17A) may be passed out with the fæces to give rise to flagellate forms in another fly, as described by Patton (19) for the *Herpetomonas* (? *Leptomonas*) of *Musca nebulosa*, the Madras bazaar fly. But whether the large *Herpetomonas* form (fig. 1) should have a place in this life-history I am at present unable to decide. Almost certainly the above is but a part of the whole life-cycle, and the low percentage of infections have prevented the completion of it up to the present. It might be thought improbable on *à priori* grounds that flies in England and in India should be infected by the same pair of parasites, yet in smears of house-flies' guts which Dr. Row brought from India and kindly left at the Lister Institute, there are large *Herpetomonads* and small *Leptomonads* just as in *H. canicularis* in England. If these should prove to be different forms of the same organism, and at the same time have a trypanosome-stage in their life-history, considerable changes in our nomenclature of flagellate parasites will be necessitated.

As to Prowazek's description of elaborate autogamy and hereditary infection in *Herpetomonas*, one is tempted to interpret some of his figures (which hardly bear out his account), as being those of a Sporozoan infection, and I hope to publish shortly an account of a Microsporidian which I have found in *Homalomyia*.

The nomenclature of these forms, interesting on account of their probable relationship with the trypanosomes, is in a very confused state, and it is with a view to the clearing up of at least one part of the vexed question that I wish to re-state the following facts in their history.

Saville Kent in 1881 (23) established the genera *Leptomonas* and *Herpetomonas* for unflagellate parasites found in a Nematode, *Trilobus*; and in *Musca domestica* respectively. The only points of distinction mentioned by him

which are of any service are that *Leptomonas* was $\frac{1}{2300}$ in. long, and formed rosettes, while *Herpetomonas* was $\frac{1}{680}$ to $\frac{1}{430}$ in. long, and had, at any rate, not been seen in rosettes or agglomerations. In 1902 Leger (9) found flagellate parasites in *Homalomyia* and other Diptera, and named an elongated form *Herpetomonas* (sp. var.), while a short rounded form, "en form de grain d'orge," he called *Crithidia* (sp. var.). Later (10, A and B), he described *H. subulata* from *Tabanus* as possessing an undulating membrane, still retaining the name *Crithidia* for short pyriform forms. Prowazek (20) in 1904 had investigated the parasite of the house-fly, and described it as possessing two flagella united by a membrane and arising from an anterior double basal-granule or diplosome. Novy, MacNeal, and Torrey, in 1907 (16) followed Leger's nomenclature for types found in mosquitoes, their *Herpetomonas* in cultures showing an undulating membrane. They described a diplosome, not where Prowazek had placed it, but at the posterior end of the body, and bearing, as they themselves point out, a considerable resemblance to a *Diplococcus*, which was generally adherent to the body of *Herpetomonas* in the cultures. Lingard and Jennings (12) in 1906 found in a Muscid fly forms showing the typical diplosome described by Prowazek, but most of their figures are not clear, and they claim to have seen the actual folding of the flagellate to form the biflagellate condition according to the Prowazek-Schaudinn theory respecting the origin of the double flagellum.

The history of *Herpetomonas* up to this point has been related in greater detail by Woodcock (25). His conclusions are—(1) That some of these parasites of mosquitoes are probably connected with *Trypanosomes* of vertebrates; (2) some of the typical *Herpetomonads* found may be simply and primarily parasites of the insects; (3) that forms adapted for life in sanguivorous insects, by which are meant "*Crithidia*" forms with an undulating membrane, following Patton's nomenclature, may be unrelated to any trypanosome

of a vertebrate. But no forms were then known with an undulating membrane in a truly non-sanguivorous insect. In 1908, however, Chatton and Alilaire (2) described flagellates found in *Drosophila confusa*—a *Leptomonas* (as distinct from Prowazek's *Herpetomonas*) and a *Trypanosoma* without a clear undulating membrane, but with the blepharoplast at the posterior end of the body. They named these forms *L. drosophilæ* and *T. drosophilæ*, but at the same time put forward the suggestion that they are really two stages of the same life-cycle. Werner (24) in 1909, and Rosenbusch (21) in 1910, have stated that there are two distinct parasites of the house-fly, a *Herpetomonas* of Prowazek and a *Crithidia* with posterior kinetonucleus, of which Rosenbusch describes the encystation. Roubaud, in an interesting article in 1909 (22), has used an old generic term, "*Leptomonas*," for the uniflagellate parasite of the fly *Pycnogonum*, excluding *Herpetomonas* of Prowazek, which he also found in the same fly. He regards, then, *Herpetomonas* of Prowazek as biflagellate, and *Leptomonas* as uniflagellate, with kinetonucleus usually anterior, but with a so-called trypanosome stage in its life-history. The evidence of Rosenbusch (21), Chatton and Alilaire (2), and Mackinnon (14), and that given by my figures, all goes to show that a form resembling *Leptomonas* of Saville Kent is found in non-sanguivorous flies (in three cases, house-flies), developing in the course of its life-history a form resembling a cultural trypanosome, and having an encysted stage. The fact that many observers have seen a large form (shown in Pl. 31, fig. 1), which differs very much in appearance from *Leptomonas*, renders it possible that the other observers who fail to see the two flagella are dealing with a different organism.

This much, however, seems certain: (1) That Leger's original pear-shaped *Crithidia* is only a stage of the *Leptomonas* life-history; also (2) that the "*Crithidia*" of later authors—Patton (18), Woodcock (25)—found in blood-sucking flies, or in cultures, are in some cases developmental stages of a *Trypanosoma*. The evidence of the forms found by me

(Pl. 31, figs. 11-14) in the house-fly, *Homalomyia canicularis*, shows that Rosenbusch's *Crithidia muscæ domesticæ*, and therefore probably *Trypanosoma drosophilæ* of Chatton and Alilaire, are merely forms assumed by a *Leptomonas*.

Should *Leptomonas* or *Herpetomonas* be the name given to these parasites of the Insecta? The *Leptomonas* of Saville Kent was described as being of a size comparable with that of the small *Leptomonas*, of, e. g., *Homalomyia*, whereas *Herpetomonas* was evidently a huge form. Again, *Leptomonas* was said to form rosettes. A diagnosis based on morphological grounds is of more value than one depending upon habitat. At present, therefore, *Leptomonas* would appear to be a correct name for the uniflagellate parasites found in the gut of non-sanguivorous insects, including house-flies, *Pycnogonum* (22), *Bombyx* (11), and in some plants (7), while *Herpetomonas* may be retained as a provisional name for a large form with peculiar flagellar apparatus and a complicated life-history, as described by Prowazek. Should the latter prove to be but a stage in the *Leptomonas*' life-history, then *Herpetomonas* should be merged in *Leptomonas*, since the latter would then have been the first which was accurately described. *Crithidia* cannot be applied as a generic name to any form, as it has simply been the name given to two stages in the life-history of *Leptomonas*, or in other cases to what are probably stages of *Trypanosoma*. That *Leptomonas* had priority over *Crithidia* was pointed out by Hartmann and Jollos (5), but it was not clear then that "*Crithidia*" was a form in the *Leptomonas*' life-history.

A paper by Flu on parasites of the house-fly, *Musca domestica*, appeared ('Centralblatt f. Bakt., etc.,' Bd. lvii, 1911, p. 522) after this paper had been sent to press, and is in the main confirmatory of the chief points emphasised above.

PROTOZOOLOGICAL LABORATORY,
LISTER INSTITUTE,
LONDON.

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EXPLANATION OF PLATE 31,

Illustrating Mr. J. S. Dunkerly’s paper “On Some Stages in the Life-history of *Leptomonas muscæ domesticæ*, with Some Remarks on the Relationships of the Flagellate Parasites of Insects.”

[All figures are outlined with the aid of Zeiss-Abbé drawing apparatus, and are drawn at a magnification of 2400.]

Fig. 1.—Large *Herpetomonas* from stomach of *Homalomyia canicularis*. Osmic vapour, Giemsa.

Fig. 2.—*Leptomonas* from intestine of *H. canicularis*, showing distinct blepharoplast. Flemm.-Fe. hæm.

Figs. 3 and 4.—*Leptomonas* from intestine of *H. canicularis*, showing varying positions of the kintonucleus. Schaud-Fe. hæm.

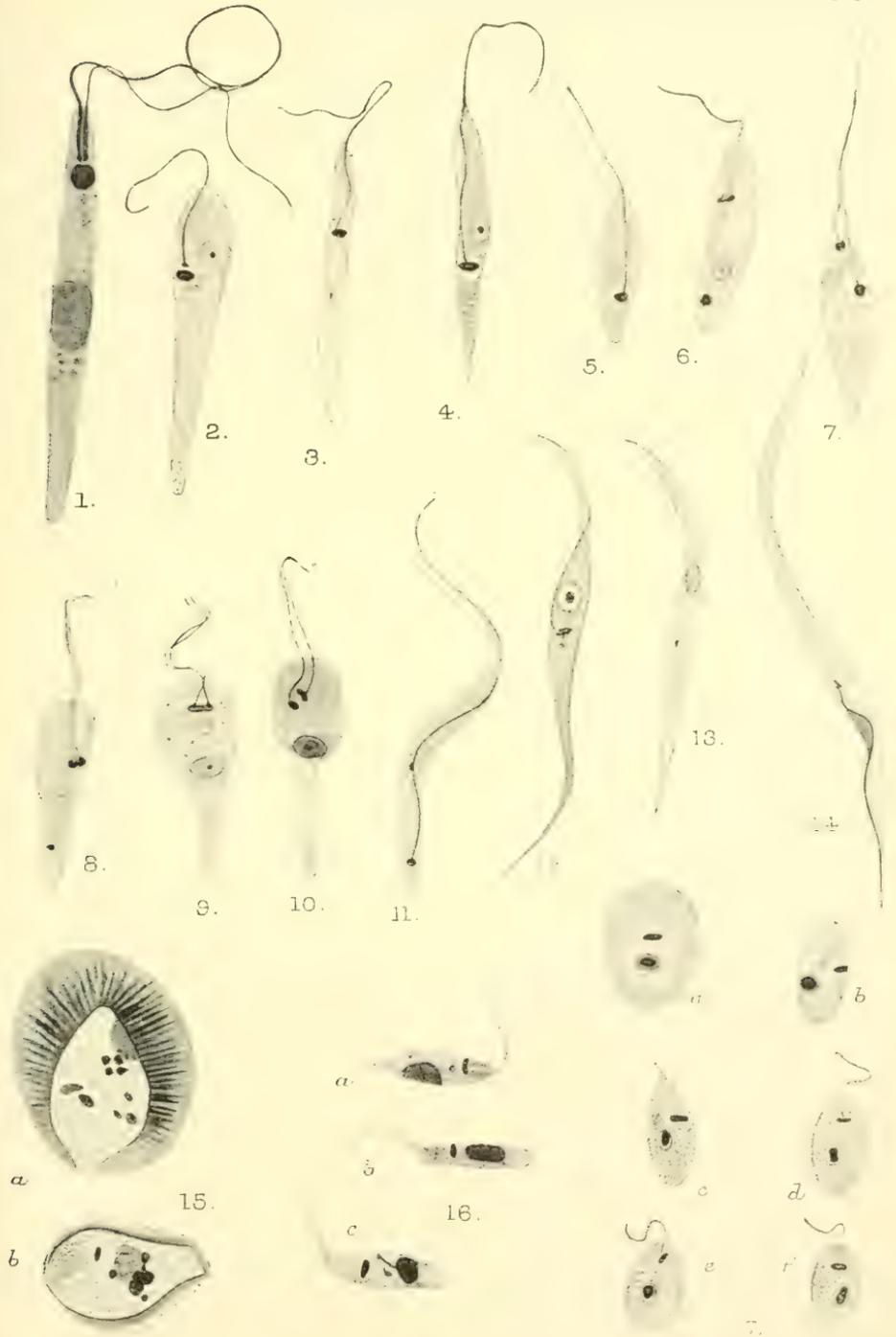
Figs. 5-10.—*Leptomonas* from intestine of *H. canicularis*; various stages in division. Flemm.-Fe. hæm.

Figs. 11-14.—*Leptomonas* from intestine of *H. canicularis*; trypaniform individuals, Schaud.-Fe. hæm.

Fig. 15.—Cysts of *Leptomonas muscæ domesticæ* from rectum of *H. canicularis*, showing scattered nuclear material. Osmic vapour, Giemsa.

Fig. 16.—Small flagellate forms a few minutes after leaving cyst. Osmic vapour, Giemsa.

Fig. 17.—Small flagellate forms a few minutes after leaving cyst showing development of the flagellum. Flemm.-Fe. hæm.



LEPTOMONAS MUSCAE DOMESTICAE.

On *Merlia normani*, a Sponge with a Siliceous and Calcareous Skeleton.

By

R. Kirkpatrick.

With Plates 32-38 and 5 Text-figures.

A GOOD deal of the work in connection with the following investigation was done at the Lister Institute, in the laboratory of Professor E. A. Minchin, and I take this opportunity of thanking him for the continual help and instruction which he very kindly gave me in methods of technique.

Also I would express my sincere thanks to Senhor A. C. Noronha, who accompanied me to Porto Santo to help with dredging for *Merlia*.

Most of the drawings have been done by Mr. P. Highley, who has put them on stone. The drawings of young stages of *Merlia* on Pl. 38 and figs. 1-4 on Pl. 36 were done by Miss Rhodes.

In 1908 Canon Norman, who had been working out the Polyzoa of Madeira, sent to the Natural History Museum four small, dried, incrusting calcareous organisms resembling Polyzoa. The crusts were about a square centimetre in area, and a millimetre or less in thickness. They had been detached from a small mass of calcareous conglomerate hooked up by a fisherman from sixty fathoms off Porto Santo island, about twenty miles N.E. of Madeira.

The specimens were covered with a thin yellow pellicle stretching across a white calcareous network, with very minute polygonal meshes, and with small rough tubercles rising from the nodes and pushing up the pellicle. A vertical

section showed a series of vertical tubes divided up by horizontal partitions or tabulæ. The vertical walls were imperforate, but marked with longitudinal vertical sutures extending from the upper surface to the base, one suture being between any two tubercles. The horizontal tabulæ usually had a central hole or slit, but were sometimes imperforate. In the uppermost spaces of this honeycomb-like framework were bundles of slender pin-shaped spicules. In the small fragment that could be spared for the making of preparations I found a "tuning-fork" spicule, seemingly imbedded in one of the tabulæ (Pl. 38, fig. 6).

Apparently the pin-shaped spicules were not present in the particle of *Merlia* used for decalcification, and I wrongly concluded that these spicules were calcareous and that they had been dissolved in the acid.

I named the incrusting organism *Merlia normani*, and regarded it as a Pharetronid sponge.¹ (1) Even if I

¹ If it had been lawful to base any opinion at all on the investigation of such scanty material, then the conclusion arrived at was, I think, a legitimate one. "Tuning-fork" spicules with thick, parallel, wide-apart prongs have been found only in Pharetronid sponges. Finding this rare and peculiar form of spicule seemingly imbedded in one of the tabulæ of a mysterious calcareous skeleton unlike that of any known recent organism, it seemed justifiable to conclude that the spicule belonged to the framework, and that therefore the latter had been made by a calcareous sponge. The upper surface of the skeleton of *Merlia* shows, too, certain resemblances to that of the Pharetronid sponges, *Porosphæra* and *Plectronia*. Further, a Pharetronid—*Murrayona phanolepis*, Kirkp. ('Proc. Roy. Soc.,' 1910)—has now been found, in which solid skeleton fibres are devoid of an axial core of spicules, and it was on these characters that I founded the sub-family *Merlinæ*. The spicule, which sent me on the wrong track, was a genuine "tuning-fork" and not a simulacrum made by some boring fungus or Alga, for when I crushed under the cover-slip the fragment of sponge containing the spicule, the latter floated out solid and free into the balsam. At present only three Pharetronid Lithonine sponges are known with a similar kind of tuning-fork, and these have been recorded from the Indian Ocean and Pacific. Off Porto Santo Island, in submarine holes or caves, possibly almost inaccessible to dredges, there must be a Pharetronid sponge. Unfortunately I failed to secure examples, in spite of twelve days' dredging.

had known that the pin-shaped spicules were siliceous—as, indeed, they were—I would have considered them as part of a siliceous sponge growing over a Pharetronid. In January, 1909, mainly with the object of procuring living specimens of *Merlia*, I spent a winter holiday at Madeira and Porto Santo. After dredging for nine days off the latter island I found the sponge in sixty fathoms off a little rocky islet called Cima, at the S.E. corner of Porto Santo. The living specimens were in the form of little bright vermilion crusts, with a smooth surface. At first, when removed from the water, nothing else was seen excepting the bright smooth patch of colour, but soon the surface sank a little, and the porcelain-white skeleton network with its nodal tubercles became visible, thus enabling *Merlia* to be distinguished from certain other small red incrusting organisms brought up in the dredge, viz. a red Ectyonine sponge, a polyzoan, a compound ascidian and a coralline alga. It is true these latter all had slightly different shades of red, but *Merlia* itself varied slightly in this respect. A crust of *Merlia* broken in half showed the cavities of a calcareous framework filled with orange-coloured jelly.

Specimens were fixed in .5 per cent. osmic acid in seawater, washed, stained immediately in Weigert's picrocarmine, and either graded into alcohol or put into glycerine. Others were fixed in Flemming mixture, and others again in absolute alcohol.

When I came to examine the first decalcified sections of the fresh material I was expecting to find a Pharetronid sponge, and great was my surprise on seeing at the surface a siliceous sponge, and below the latter and in continuity with it a series of separate but closely packed parallel moniliform cylinders chiefly made up of large granular cells imbedded in a tough transparent maltha. In specimens decalcified whole the cylinders hang down from the thin surface lamina, the various segments of each cylinder being united by narrow central isthmuses of tissue which had passed through the holes in the centre of the tabulæ. After a long search

among many specimens I failed to find any calcareous spicules, "tuning-fork" or otherwise.

At first sight it seemed that *Merlia* must be some unknown calcareous organism, viz. Foraminiferan, Pharetronid sponge, Coral or Polyzoan, infested by a remarkable siliceous sponge. Weltner, (2) who had seen some of the fresh specimens and sections, published a short paper entitled "*Ist Merlia Normani* Kirkp. ein Schwamm?" in which he stated his belief that *Merlia* was an unknown calcareous organism, and that the siliceous sponge associated with it was merely a "raumparasit" which would probably be discovered sheltering itself in other suitable situations. Later, in a paper on *Astrosclera willeyana*, Weltner (3) again expressed his opinion that the siliceous sponge had simply incrustated and grown into the calcareous organism. There was, however, an important objection to this theory. When shells on which the *Merlia* was growing were ground down till the base of the *Merlia* appeared, the cavities or "crypts"¹ of the calcareous framework were found crammed with the large granular "crypt" cells even five stories down below the surface, and frequently such crypts were roofed over by tabulæ in which the central hole has become reduced to an almost imperceptible slit from 1 to 3 μ in diameter. À propos of the crypts and the cells inside them, to the question "Whose grave is this?" the answer seemed to be, "'Tis indeed thine, since thou liest in it." I concluded that the cells had been formed in situ and had not grown down from the surface. I named the siliceous sponge *Noronha scalariformis*, but failed to arrive at any definite opinion concerning the calcareous structure, pointing out, however, its resemblance to certain Palæozoic fossils (*Monticulipora sensu lato*, *Rhaphidopora*) and to *Heliopora*. (4)

Further examination showed that the masses of crypt-tissue were in vital continuity with the sponge at the surface. In some instances bundles of siliceous spicules were found imbedded amongst them. The existence of a sponge with

¹ A name suggested by Prof. E. A. Minchin.

siliceous and calcareous skeleton had seemed to me as improbable as that of a centaur, for no distinction between siliceous and calcareous sponges has been considered so profound as that of the chemical constitution of the skeleton.

After examining about 1000 specimens, and tracing the development of the soft tissues and skeleton at the growing edges, I became convinced that *Merlia* was, beyond doubt, a sponge with a siliceous and calcareous skeleton (5). The grounds on which this conviction is based are so firm that the final proof, such as would be afforded by seeing an embryo of *Merlia* settle down and develop the siliceous and calcareous elements, is, in my opinion, no longer necessary. I am having, however, relays of specimens sent from Porto Santo every fortnight, in the hope of discovering when *Merlia* forms its reproductive cells and embryos, and of thereby being enabled to work out the development.

DESCRIPTION OF MERLIA NORMANI.¹

The great majority of the specimens, of which there are about 1000, were obtained from sixty fathoms off Porto Santo Island. All the specimens from this depth are in the form of small incrustations, on an average about a square centimetre in area and a millimeter or less in thickness. The crusts grow on shells, branches of corallines, Foraminifera, worm-tubes, etc., are bright vermilion in colour, and have a smooth surface. They conform closely to the surface of the objects upon which they grow, creeping round the edges of shells and encircling the branches of corallines (Pl. 32, figs. 1-3). They are flat and thin when spread over the smooth inner surface of a shell, but in other situations they may form slightly thicker convex bosses. *Merlia* always grows on hard unyielding surfaces, and never on soft objects such as sponges or Ascidians. The *Merlia* crust is always so intimately united to the surface of foreign objects that it is almost impossible to flake it off without removing some of the

¹ A certain amount of repetition of details given in the introductory account seems unavoidable.

foundation on which it is growing. This close union is one of the characters that has made the problem of *Merlia* difficult to solve, and has led to false inferences being drawn, as I shall endeavour to explain later.

In addition to the above-mentioned specimens there are two much larger ones requiring special notice. One of these, a fine example forming a large patch over 30 square centimetres in area on a small block of volcanic rock, was hooked up by a fisherman from ninety fathoms off Porto Santo Island (Pl. 32, fig. 4). The specimen was nearly dry when brought to me, but it still retained its red colour. Lastly, in the Seminario Museum at Madeira there is a large fragment of a dead *Dendrophyllia* covered over a considerable area with a thin crust of *Merlia*. The coral was hooked up from ninety fathoms off Cape Garajau, Madeira. Pl. 32, figs. 5, 5*a* shows a small piece of incrustated coral, which the authorities of the Seminario Museum kindly allowed me to break off. The *Merlia* incrusts both the sides and free end of the coral branch. Evidently the sponge flourishes better in ninety fathoms than in sixty, and it may be inferred that the habit of *Merlia* is always incrusting.

When living specimens are removed from the sea the soft, smooth surface sinks down a little, thus allowing the porcelain-like network, with its minute circular or polygonal meshes and little nodal tubercles, to be seen imbedded in the red flesh. Usually no openings of any kind are to be seen, but in the case of two specimens killed suddenly by dropping alive into Flemming's mixture, several very small circular or oval holes, from 20 to 60 μ in diameter, were visible (Pl. 32, figs. 7, 8). These two specimens were the best preserved of all, for they were in the expanded condition, and the fixing fluid had penetrated well into the interior.

The thin growing edge of the sponge extends well beyond the edge of the calcareous skeleton in examples in which the crust spreads freely over a surface, but often the growing or creeping edge is dammed up, so to speak, and the sponge and its calcareous skeleton increase in depth at such places.

A living sponge broken in half—i. e. in vertical section—shows (Pl. 32, fig. 10), beneath the soft surface-layer, a more or less regular white calcareous honeycomb, with blocks of reddish orange-coloured jelly filling in the spaces, the appearance being that of pots of jelly superposed one on another in from one to three or four or, rarely, five storeys from edge to centre of the sponge. The blocks often form fairly regular horizontal and vertical rows. Occasionally the uniformity of a vertical row is interrupted by a block of double breadth, and very frequently the regularity of the horizontal rows is broken owing to the blocks of "jelly" being longer or shorter than the average. Pl. 32, fig. 9, shows variation both in the length and (once) in the breadth of the blocks of soft tissue filling in the spaces or crypts in the calcareous skeleton. Pl. 35, fig. 17, shows a section of the skeleton with nearly equal and uniformly arranged compartments. In the youngest specimens, which form little red spots only 2 or 3 mm. in diameter, there are no crypts at all, the calcareous skeleton consisting merely of slender bars of a wide-meshed polygonal network. Also, in the case of specimens which can extend freely over the flat or curved, smooth inner surfaces of old bi-valve shells, there may be only a few crypts in perhaps one or two storeys at the centre of the crust, the rest of the skeleton being composed of deeper or shallower pits, with the floor formed by the surface of the shell.

Not infrequently the sponge grows in depth rather than in extent, and the crypts may then become four, or even five, storeys deep, or rather, it should be said, high, for the growth is from below upwards. In such specimens the storeys may diminish gradually to four, three, two, one, and finally to none at the growing edge.

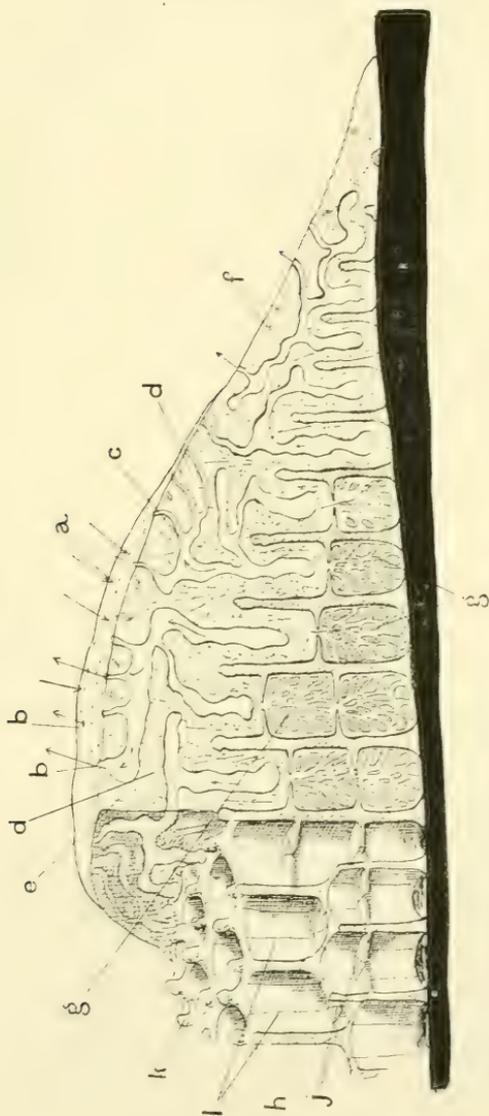
On viewing by powerful reflected light under a low magnification a stained specimen in glycerine, it is possible to see, below the transparent surface, circular masses of flagellated chambers filling in the uppermost spaces of the calcareous framework, and joined to each other by radiating spokes, also composed of masses of flagellated chambers, crossing

over the upper edges of the calcareous walls and between any two tubercles (Pl. 33, fig. 1). The circular masses show a lighter central area, or occasionally two such areas. Further, from one to three nearly vertical bundles of fine needles are present in or above each circular mass. Scattered mostly in or upon the surface of the sponge are very minute, oval siliceous rings. The siliceous spicules are not visible in glycerine preparations, but clearly so in balsam ones. Thin specimens decalcified whole show very clearly the darker node-like circular areas of masses of flagellated chambers joined to each other by five to seven radiating spokes, the blank spaces between the spokes having been occupied by the calcareous tubercles. The under-surface of fairly thick decalcified specimens presents an extraordinary appearance, such as has never been observed before in any sponge. Hanging down from the thin surface-layer of the sponge are closely packed but separate parallel moniliform cylinders. The segments of each cylinder vary in breadth from 75 to 100 μ (or rarely 150 μ), and in length from 75 to 250 μ . The segments are joined to those above and below by narrow central strands, which had passed through the holes or slits in the tabulæ.

Fig. 9 on Pl. 32 shows a vertical section of a decalcified specimen. The spaces between the cylinders extend from the base of the sponge to the apex of the gaps left by the dissolved tubercles, not taking into account the fine organic basis of the skeleton remaining after decalcification. The upper part of the section, including the ectosome and part of the choanosome, is extremely contracted, but below this the flagellated chambers, bundles of spicules, and the cylindrical masses of large granular cells are to be seen.

Fig. 3 on Pl. 33 shows a decalcified vertical section of one of the two expanded specimens; in the part of the section shown in the figure there happens to be only one mass of crypt cells that had been covered by a tabula. All the other basal extensions of soft tissue had filled in calcareous pits, the floors of which had been formed by the surface of the shell on which the sponge grew (see below, Section 3).

TEXT-FIG. I.



Diagrammatic plan of *Merlia normani*. Soft tissues removed on the left side of figure. *a*, Incurrent pores. *b*, Oscules. *c*, Excurrent canal. *d*, Flagellated chambers. *e*, Long siliceous spicules (tylostyles). *f*, Clavidses (siliceous microsclepes). *g*, Crypt cells (calceocytes) in crypts. *h*, Calcareous skeleton. *j*, Tabulae (in section). *k*, Nodal tubercles. *l*, Longitudinal sutures.

By macerating specimens in Eau de Javelle the calcareous framework stands out clear (Pl. 32, fig. 6, and Text-fig. 3, see Section 2 *b*).

Having given a general account of the external appearance and coarse anatomy of *Merlia*, I will now describe the sponge more in detail under the following headings:

- (1) The canal system.
- (2) The skeleton; (*a*) siliceous, (*b*) calcareous.
- (3) The soft tissues and cells.
- (4) Young stages of *Merlia*.
- (5) Theory of construction of the calcareous skeleton.
- (6) Systematic position of *Merlia*.
- (7) On some resemblances between the calcareous skeleton and certain Palæozoic fossils.
- (8) Summary.

(1) THE CANAL SYSTEM: A NEW TYPE.

It would have been very difficult to have learned the structure of the canal system from contracted specimens. Under such conditions no surface openings are to be seen, and the upper part of the sponge is squeezed down—like a closed concertina—into the open crypts.¹ Some examples of this very contractile and sensitive sponge, dragged up with a mass of shells and *débris* from sixty fathoms, were resuscitated by trailing them in a bottle in the sea, and then suddenly dropping them into Flemming. In these specimens not only are some of the oscules and pores open, but the whole sponge is expanded and shows wide ectosomal spaces. In contracted specimens the sponge surface almost rests on the calcareous tubercles, but in expanded examples the latter are a considerable distance below.

The larger openings or oscules measure about 60μ in

¹ Open crypts—the uppermost spaces in the calcareous skeleton. In the macerated skeleton these spaces are open and not roofed over by tabulæ.

diameter, and the smaller ones or pores about 20-30 μ . Both kinds are oval or circular, and quite flush with the surface.

The pores may form a circle round an oscule (Pl. 32, fig. 7), but sometimes no regular arrangement is perceptible (fig. 8). The regular plan of a central oscule and a ring of pores would seem to conform to the shape of the upper crypts and tubercles, but the reverse is probably the case, the crypts conforming to the arrangement of the canal system, as I hope to make clear later.

Both oscules and pores are provided with a very well-developed sphincter apparatus, consisting of concentric and radial contractile cells for respectively closing and opening the orifices. Some of these cells are remarkable in shape, viz. with long processes which curve round the orifices and which may actually anastomose, thereby becoming porocytes. These cells seem to indicate the mode in which porocytes may have arisen, viz. by the fusion of processes curving round an orifice, and not by the appearance of a hole in the solid body of the cell (Pl. 37, fig. 3).

The incurrent canals pass down and bifurcate, passing right and left (as seen in sections). Some of them present a puzzling appearance, for they are surrounded by flagellated chambers. Sometimes a string or tube of flagellated chambers is seen traversing an open space. The lumen of the tube is the incurrent canal and the open space is an excurrent one, into which the apopyles on the outer surface of the tube open.

Fig. 13, on Pl. 33, which depicts the growing edge of a very young sponge, will show how this arrangement has probably come about. In the youngest stage the canal structure is that of a simple rhagon, i. e. of a gastral cavity with much folded walls, the folds branching out to the periphery. The spaces between and outside the folds constitute the incurrent, and those in the lumen of the folds, channels or canals, the excurrent system.

The incurrent canals pass between the little clusters of flagellated chambers, which clusters at this stage have a

narrow lumen (or excurrent canal). In the course of growth the clusters expand at the same time that their lumen increases, and they encroach on the incurrent spaces, which may now consist of narrow channels or canals completely surrounded by flagellated chambers.

A tube of flagellated chambers may be compared to a hollow central column (say with a "pore" at the summit opening on the roof) supporting a groyned vault, the concave surfaces of which face the interior of the building which leads to a door (oscule).

The excurrent spaces or channels in the upper part of the sponge often form wide pouch-like out-foldings, but those in the deeper parts which are crowded into the uppermost open crypts of the hard skeleton are in the form of narrow canals.

In a section the in- and excurrent canals are easily distinguished by the direction of the apopyles and the collar-cells. A terminal excurrent canal or space opens into the floor of one of the large ectosomal spaces, and the latter open to the exterior by an oscule.

The flagellated chambers possess a remarkable structure not known to occur elsewhere. They are semi-oval or hemispherical, and about 33μ in diameter. They are usually closely approximated to each other, being joined—or separated—only by a few connective-tissue cells.

Stretched across the excurrent aspect of each chamber is a membrane with a thin-edged circular apopyle in the centre. Nearly surrounding each apopyle is a single contractile cell which acts as a sphincter muscle. The apopyles vary in diameter from a point up to nearly the width of the flagellated chamber.

The prosopyles consist simply of spaces between the fused rays of the stellate bases of the collar-cells (Pl. 33, fig. 6)—a character recalling the membrana reticularis of Hexactinellida. This stellate arrangement of the cell-plasma is not easy to see, and I am thereby led to believe that this mode of union may possibly occur among other tetraxonid

sponges, especially among those with a eurypylous type of canal system.¹

The collar-cells vary greatly in appearance according to their state of contraction. Figs. 8-12 on Pl. 33 are drawings of collar-cells on one and the same slide. Some are relatively short and thick, with the collar contracted down and joined to the collars of neighbouring cells, and with a wide opening with polygonal outline. Others are elongated and with separate cylindrical collars. The collar-cells vary in height from 4.5μ to 8.5μ . The usually spheroidal but sometimes hemispherical body is about 3.3μ in diameter. The spherical nucleus is at or near the base of the cell. The flagellum passes down through the cell to the nucleus (Pl. 33, fig. 12). The name "hymenopylous"² is proposed for the new type of canal system, which seems to be a modification of the eurypylous type.

(2A) THE SILICEOUS SKELETON.

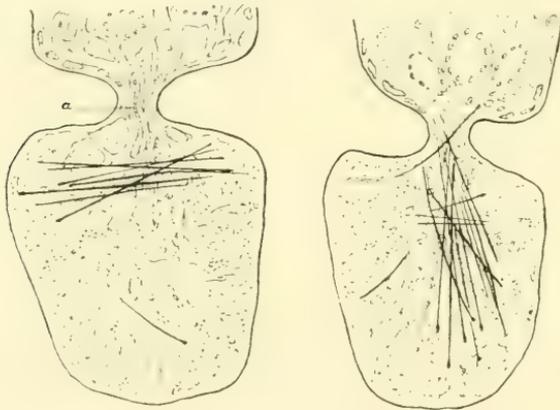
The siliceous skeleton consists mainly of upright bundles of

¹ Fig. 6, Pl. 33, is, I find, slightly diagrammatic in places, but it is sufficiently accurate in places to justify the statement that the collar-cells are joined to each other by stellate basal processes. Further investigations of new, well-fixed material, prepared with a good plasma stain, are desirable. It is not surprising that we have so little knowledge concerning the inter-relations of collar-cells in siliceous sponges. The difference between the tissues of healthy living sponges and dead ones fixed under the best possible conditions must be very great, especially in the matter of contraction. Minchin and Reid have shown ('Proc. Zool. Soc., London,' 1908, p. 674, pl. xxxvii, fig. 24) that in *Clathrina contorta* the bodies of the collar-cells are separated by delicate extensions of the gelatinous ground-substance of the sponge. Muriel Robertson and Minchin ('Quart. Journ. Micr. Sci.,' November, 1910, p. 621) write that probably the collar-cells are connected across this intervening substance by protoplasmic fibrils, though they have not yet seen such connections. Certainly the manner in which a contracted Acon with its collar-cells squeezed out among the porocytes expands again with all its collar-cells "dressed" and in line would lead one to suspect that here also there may be a kind of collar-cell membrane, as in Hexactinellids.

² ὑμῆν, ἔνος, membrane; πύλη, gate.

slender tylostyles¹ with pointed ends outwards, along with a few slender raphides, which form more or less vertical pillars of support round the large ectosomal spaces and incurrent canals. In contracted sponges one to four of these vertical wisps are drawn down into the upper crypts, but in expanded specimens the wisps are outside the crypts and form supporting pillars to the ectosome and canals (Pl. 32, fig. 10, and Pl. 33, fig. 3). Sometimes a bundle of spicules lies transversely on the floor of an upper open crypt. Only very rarely are spicules of any kind found in the lower crypts, but neverthe-

TEXT-FIG. 2.



Sections of masses of crypt-tissue showing siliceous spicules.
a. An elongated calcocyte in neck of crypt. Soft crypt-tissue mostly disintegrated owing to insufficient fixation. $\times 225$.

less they do occur there. (The probable reason for this rarity is explained in Section 5).

Numerous microscleres in the form of oval rings—for which I propose the name “clavidiscs”²—are scattered about on the surface, and also, but much less abundantly, deeper down.

¹ In the report on the “Discovery” Tetraxonida I have used the term “tyle” in place of “tylostyle,” because it was short, and by way of antithesis to “amphityle,” but I now return to the commonly used designation “tylostyle.”

² Clavis, key, referring to the key-hole notches; discus, quoit.

A second kind of microsclere, viz. a very minute, slender, simple sigma is found in fair abundance in the choanosome, especially in the immediate neighbourhood of the flagellated chambers. At one time I thought these spicules were the broken curved ends of rhabdites, but latterly I have seen the little spicules in their scleroblasts.

Rhabdites and trichodragmata constitute a third and fourth kind of microsclere.

The Spicules.—The slender tylostyles (Pl. 35, fig. 1), which are commonly curved at the distal end, though sometimes nearly straight, are about 140μ long, 1.8μ thick, and with oval heads 5 by 2.2μ in length and breadth. The rhabdites (Pl. 35, fig. 2a) are about 80μ long. They are found separately or mixed in with tylostyles. Trichodragmata (Pl. 35, fig. 2) occur, but are rather rare. In one specimen there are toxa-like spicula with a central kink or bend (Pl. 35, fig. 13), but this is an exceptional feature. The clavidiscs (Pl. 35, figs. 3–9) are about 45μ long, 30μ broad, and with the rim, which is bevelled inwards to a thin edge, 3μ broad. A key-hole shaped sinus or notch is present on the inner margin at each end of the long axis. The axial canal is in the centre of the thickness of the outer edge of the rim. Numerous variations and sports occur, which are interesting because they show the mode of origin of these spicules, viz. from deeply curved rods which have bent round till the ends met and joined. Sometimes the ends cross or do not meet at all, or a transverse bar may cross from side to side (Pl. 35, figs. 7, 8). Fig. 14 shows clavidiscs with a disc-like plate in place of the key-hole sinus. Fig. 7 shows a sigma-shaped spicule which is probably merely a deviation from the ring shape. Again, the key-hole sinuses may be absent from one or both ends. Lastly, the clavidisc may sometimes be in the form, not of a ring, but of a solid disc (not figured).

I had formerly supposed (6) that the clavidiscs were related to chelate spicules of Desmacidonidæ, but I now consider their affinities to be with the diancistra of Hamacantha (see Section 6 on the affinities of Merlia). These spicules

are mostly scattered at the surface, in which they lie horizontally.

The oval rings found deeper down in the sponge usually have thinner rims. In one instance six rings followed at equal intervals on one side and five on the other side of the mass of sponge filling an open crypt. Hence I called the upper part of the sponge *Noronha scalariformis*.

The very fine primitive simple sigmata are commonly found in the neighbourhood of the flagellated chambers.

There seems to be no transition between the sigmata and the clavidiscs. At the same time the clavidiscs probably developed from some such form. In one or two of the myocytes acting as sphincters round the apopyles there seemed to be an appearance of a slender curved axial rod of silex. Possibly the slender sigmata may originally have come into existence owing to the presence of sphincters, which surround not only the pores and oscules, but also the apopyles of the flagellated chambers.

To sum up, normally there are five kinds of spicules in *Merlia*, viz. tylostyles, long rhabdides, trichodragmata, clavidiscs, and slender sigmata. Rarely thicker sigmata and toxa occur.

Pl. 35, figs. 11-15 show abnormal forms of spicules, all found in one specimen.

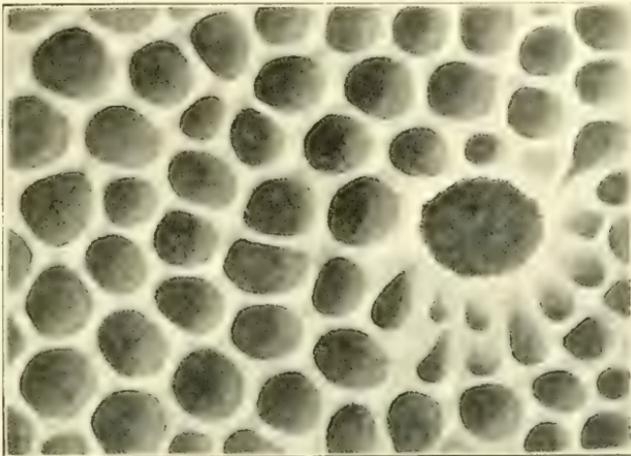
(2B) THE CALCAREOUS SKELETON.

When a living sponge is taken from the water presently the semi-transparent, red, fleshy surface sinks a little, and the porcelain-white calcareous skeleton becomes visible. Under a lens it is possible to see the very minute circular or polygonal meshes of a fine network, and the still more minute tubercles rising from the nodes. In dried specimens the flesh forms merely a thin yellowish pellicle, covering the surface, which has a uniformly granular aspect due to the tubercles below, with here and there a polygonal pattern where the soft tissues have sunk more deeply into the spaces of the skeleton beneath.

For the investigation of the skeleton specimens were macerated in Eau de Javelle, and vertical and horizontal sections ground down, and some examples were incinerated. To the naked eye the surface of a macerated-out skeleton or of a dried specimen like that encrusting the *Dendrophyllia* (Pl. 32, fig. 5, 5a) has a very finely porous appearance, the meshes being barely visible.

The meshes are about $\cdot 18$ to $\cdot 22$ mm. in total diameter, i. e. four and a fraction to a millimetre, the actual spaces or holes

TEXT-FIG. 3.



Surface of calcareous skeleton. The large dark circle is the mouth of a worm-tube. \times about 40.

being about $\cdot 12$ to $\cdot 15$ mm. across, and the walls about $\cdot 04$ to $\cdot 06$ mm. thick. The number of tubercles round a mesh varies from four to seven or eight, five or six being the average number. Occasionally two meshes are combined into one larger oval one, with ten to twelve tubercles.

The tubercles are about 75μ high and 75μ broad at the base, and are covered with very minute sharp-pointed conules about 10μ high and 16μ broad at the base, but varying both in shape and size. The point of the conule is generally nipple-shaped and may lean over a little to one side. Again the

conules may be rounded at the summit, or more elongated than usual. A little below the surface the uppermost tabulæ are visible, each with a central, circular or oval hole or nearly closed slit (Pl. 35, fig. 16). Sometimes the tabula is merely a rim or ledge round the inner wall of the calcareous tube, and the central hole is correspondingly large; or, again, the tabula may be imperforate. The tabulæ show five or six sutural lines radiating from the central hole or slit to the circumference.

On the surface of the specimen incrusting the *Dendrophyllia* there are numerous small circular holes due to worm-tubes, and here and there among the ordinary meshes of the *Merlia* are larger ones nearly .5 mm. in diameter, with a shallow floor, on which there are radiating ridges and even a central columella-like knob, the whole somewhat resembling a very small coral calycle. The large meshes are here simply due to the effort of the sponge to repair the lesion caused by the presence of the worm-tubes, the openings of which are found just beneath the floors of the supposed calycles.

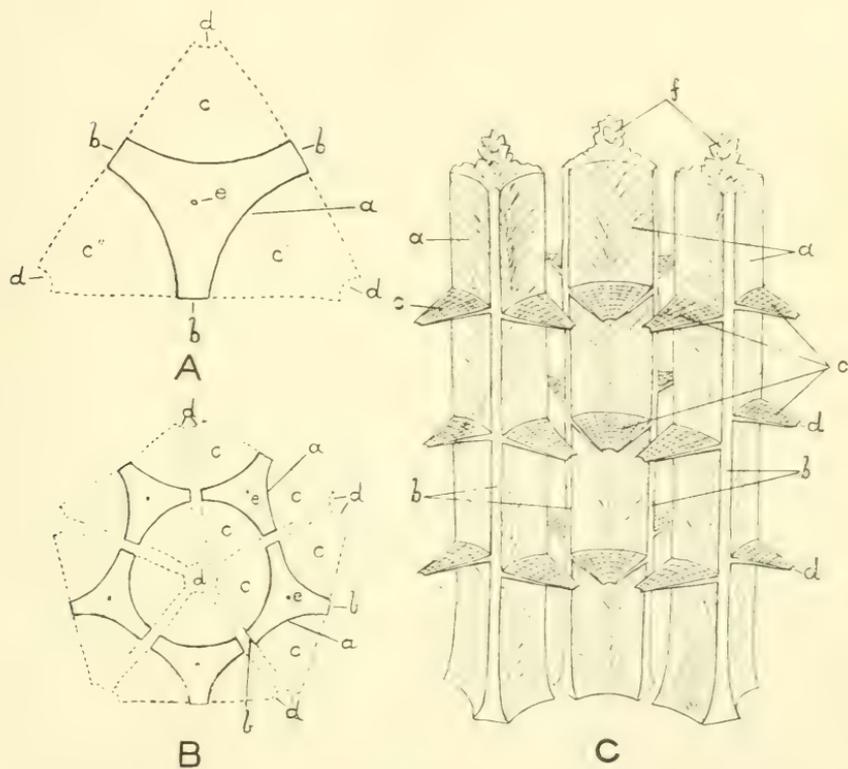
A vertical section of the skeleton shows parallel vertical tubes divided up by tabulæ (Pl. 35, fig. 17). Often the tabulæ form a series of parallel horizontal planes at regular intervals of about .15 mm., but the regularity is frequently disturbed, as will be obvious from fig. 9 on Pl. 32, showing the soft tissues which filled the crypts.

The magnified surface view of the skeleton (Pl. 35, fig. 16) shows three flanges or wings radiating from the base of each tubercle and meeting opposing flanges from neighbouring tubercles. A sutural line marks the junction of the opposing flanges, and the most prominent radial sutures on the tabulæ are continuous with those between the flanges. A vertical section shows the interflange sutures to be continued as vertical lines down the walls of the tubes from surface to base.

The tabulæ are about .015 mm. thick. On both surfaces, in addition to the radial sutures there are fine concentric markings confined to those surfaces, the thickness of the tabula being homogeneous. The radial sutures typically

run from the central region to the vertical sutures between the flanges and are continuous with them, though occasionally this arrangement is a little obscured. Fig. 19, Pl. 35, shows a thick ground-down section in balsam as viewed by powerful

TEXT-FIG. 4.



- A. Diagram showing a structural unit of the calcareous skeleton. Horizontal section at level of three tabulæ. *a*. Curved surface. *b*. Suture of flange. *c*, *c'*, *c''*. Three segments of tabulæ. *d*. Margin of portion of central hole in tabulæ. *e*. Central vertical axis below apex of a tubercle. B. Diagram showing five structural units joined to form one tube and tabulæ, and parts of five other neighbouring tubes and tabulæ. Horizontal section at level of tabulæ. Lettering as before. C. Three structural units in elevation (diagram). Lettering as in A. *f*. Tubercle.

oblique reflected light. The walls of the tubes were here, i. e. at the base of the sponge, very thick, but, both the hori-

zontal tabular and vertical (or columnar) sutures were beautifully lighted up.

The disposition of these vertical and horizontal sutures clearly shows how the skeleton has been built up. The structural unit is a broadly based pillar with three vertical longitudinal wings or flanges (which unite with the flanges of three juxtaposed columns), and with three vertical longitudinal curved surfaces or flutings which form a part or segment of the walls of three tubes, and lastly with tiers usually of three horizontal triangular tabular segments in one plane, the number of tiers varying with the number of tabulæ. (See diagrams A, B, C in text.)

It was a surprise to find that there was no continuous floor such as was depicted (in vertical section) in my original figure.(7) The seeming existence of a floor in certain places is apparently due to the section cutting through the thick outspread base of a vertical wall.

It is certain there is no continuous basal lamina at the periphery of thin spreading crusts, but occasionally I have seen what appear to be complete floors at the base of some of the basal crypts. The floor of *Merlia* is formed by the shells, coralline algæ, Foraminifera, etc., on which the sponge is growing. The vertical walls of the lowest crypts, often thickened and spread out a little at their bases, are intimately fused with the foreign foundation.

Under a fairly high power the skeleton shows a finely fibrillar structure (Pl. 35, figs. 16, 17). When the skeleton is viewed from the surface, the fibrillæ are seen to radiate out from below the base of each tubercle towards the longitudinal sutures, where they meet but do not blend with the fibrillæ of opposite flanges (Pl. 35, fig. 16). Superposed zones of fan-like bands of striæ radiate upwards and outwards from the vertical axes of the columns of the skeleton and pass into the tabulæ and walls of the tubes (Pl. 35, fig. 17).

An English colleague was engaged, at the time this was being written, in the attempt to show that *Merlia* was a Foraminiferan. He had only a dried skeleton, coming, I

believe, from the West Indies, and two of my specimens of the real *Merlia* from Porto Santo, on which to base his conclusions. If I had had such scanty material, I also might easily have come to the same conclusion, because *Merlia* often grows on a spreading crust of *Polytrema* or other Foraminifera, and the cancellated structure of the latter appears to belong to the *Merlia*; but having had hundreds of specimens to deal with, I have found *Merlia* growing on shells, on gneiss, on spreading laminae of thin incrusting Corallines, etc. Foraminifera with a superficial reticulate pattern with nodal tubercles are not uncommon. Dr. Harmer showed me one he had found among the "Siboga" Polyzoa. Professor Stanley Gardiner collected a similar species incrusting *Lithothamnion* from Providence Reef. I myself dredged a *Carpenteria*-like species off Christmas Island. In all these instances the Foraminiferal nature is clearly shown by the finely perforated floor at the base of each mesh. At one time I myself thought *Merlia* was a Foraminiferan, not only on account of the calcareous skeleton, but also because the masses of crypt-cells (in glycerine) looked like lobose pseudopodia, the maltha in which they were imbedded being invisible.

At the growing edges of the sponge and in whole young specimens there is simply a delicate network of calcareous bars. In the case of young specimens on thin bivalve shells one can often see through the whole structure. The soft sponge tissues cover the calcareous network, which has no basal lamina, and there is no trace of any other organism whatsoever. Even this strong circumstantial evidence in favour of the theory that *Merlia* is a sponge is unimportant in comparison with the positive evidence afforded by the soft tissues.

The growing edge of the skeleton shows the delicate polygonal network, with half- or open polygons at the edges, and here and there isolated small lumps or even bars (Pl. 38, fig. 5). In the older parts the slender bars of the delicate meshwork have increased in height and the meshes have become pits, and still later tubes with one or more tabulae.

The growth of the skeleton is much influenced by the conformation of the base. When growing on shells or worm tubes with ridges, the sponge makes squarish oblong meshes with parallel or concentric arrangement (Pl. 35, fig. 20).

This last figure of a specimen macerated in Eau de Javelle shows well also the earliest beginnings of the skeleton before bars or tubercles are formed. Here, in places, little smears of calcareous scales are beautifully distinguished on the reddish surface of the worm-tube. These glistening bands have a crystalline appearance as if a brush dipped in strong solution of sugar had been drawn over the surface and allowed to crystallise. The individual scales have a finely punctate structure, and each one shows a little elevation which corresponds, I believe, to the nucleus of the cell which, made the scale (Pl. 35, fig. 21). Viewed as opaque objects, the fine points and the little nuclear hump in each scale strongly reflect the light. I was unable to detach the glistening scales from the worm-tube so as to examine them by transmitted light under a high power.

The calcareous skeleton of *Merlia* is formed of calcite. Dr. G. T. Prior found that the specific gravity was 2.65. That of pure calcite is 2.7, the difference being due to a certain amount of organic matter remaining in the *Merlia* even after being finely ground and macerated. The specific gravity of aragonite is much higher, being 2.9, and, moreover, Meigen's test gave no reaction.

(3) THE SOFT TISSUES AND CELLS.

Merlia is semi-transparent, and its tissues are of a soft texture, but, at the same time, rather tough. The bright red colour resides in the granules of certain cells—the amœbocytes. The colouring matter is soluble in alcohol, in which it forms an orange-yellow solution. The cells will be described under the following headings: (A) Collencytes, and ground substance or maltha; (B) gland-cells and cuticle;

(c) canalar epithelium; (D) myocytes; (E) scleroblasts; (F) choanocytes; (G) amœbocytes; (H) tokocytes.

(A) Collencytes or Connective-Tissue Cells.

The connective-tissue cells or collencytes (Pl. 37, fig. 2) are finely granular with an oval vesicular nucleus devoid of a distinct nucleolus, and with branching processes which anastomose with those of other collencytes to form a network. The ground substance or maltha at first sight seems almost homogeneous, but in good preparations under a high power it shows a very finely fibrillar structure. When bundles of fibrillæ are cut across they form finely granular areas in section (Pl. 34, fig. 4).

(B) Gland-cells and cuticle.

The sponge surface is often coated over with a very thin layer of structureless cuticle, apparently the product of elongated granular cells vertically orientated just below the surface (Pl. 37, fig. 4). In these cells there is a minute dark body like a nucleolus, but situated just outside the vesicular nucleus.

(c) Canalar Epithelium.

A remarkable feature of great interest, and, I believe, unique, is the absence of a surface layer of epithelium. In all the best preserved material I found the cells at the surface precisely the same as those in the body of the sponge, i. e. they were branching collencytes well separated from each other by the maltha in which they were embedded. Knowing how difficult it often is to see the outlines of surface epithelial cells, at first I concluded that the cuticle—usually, but not always present—was an epithelial layer. Further, in one or two very contracted specimens, which had not been properly fixed, the collencytes had become so pressed down in the

maltha as to resemble an epithelium when viewed from the surface. In some very thick vertical sections, well preserved and stained, it was possible to see a considerable area of the surface as well as the depth of the sponge, and to note the entire absence of any trace of an epithelium. Accordingly, *Merlia* in its adult condition affords a confirmation of the theory put forward by Haeckel and confirmed by the embryological researches of Maas, Yves Delage and Minchin, that the sponge is a two-layered organism. *Merlia* has an "exoderm" and an "endoderm," but no mesoderm.

Although there is no epithelium on the surface of the sponge the canals have an epithelial lining, which apparently consists of cells of the same nature as the collencytes in the maltha, for sometimes a cell on the surface of a canal has branching processes extending back into the maltha.

(D) The Myocytes.

The myocytes form concentric or radial groups round the incurrent and excurrent orifices. The myocytes have a granular plasma, the granules being coarser than those of the collencytes, and the nucleus is vesicular and without a nucleolus. They vary greatly in shape, but generally have branched prolongations—indeed, they do not differ greatly from the collencytes. Sometimes the branched prolongations surround an orifice and fuse, so that the myocyte becomes a porocyte (Pl. 37, fig. 3). Further, one or more small, curved, fusiform myocytes surround the apople of each flagellated chamber.

(E) Scleroblasts.

The scleroblasts are very finely granular, and with a clear spherical nucleus. Pl. 36, fig. 2 shows these cells both in longitudinal and transverse sections of spicule bundles. Pl. 36, fig. 10 shows a scleroblast of one of the clavidiscs.

(F) Choanocytes.

(See under "Canal System," Section 1, and Pl. 33, figs. 8-12.)

(G) Calcigenous Amœbocytes or Calcocytes.

It is to these cells that the extraordinary character of *Merlia* is due, for they are calcigenous, and it is their function to build the calcareous skeleton.

Quite apart from their lime-forming function, the arrangement of these masses of cells in the form of moniliform cylinders is a very remarkable feature.

Before I had understood the structure of *Merlia* I called these cells crypt-cells, because many of them are found in spaces of the calcareous skeleton covered over by tabulæ. As I am now practically certain that these cells which fill up the crypts also build those structures, I suggest the designation "calcocyte," which is in keeping with the cytological terms now in use. Since these lime-forming cells of a siliceous sponge constitute a special and unique phenomena, I think they should be distinguished by a separate name from the telmatoblasts, or better, telmatocytes, which form the cement in *Pharetron* sponges. (8)

The lime-forming cells of *Merlia* are exceptionally deserving of the designation "amœbocyte," for they exhibit a remarkable variation in shape in accordance with their position in the sponge and the kind of work they have to do. Before settling down to build the skeleton and in places where they are not compressed in an enclosed space they are more or less elongated (Pl. 38, fig. 1). When caught in the isthmus between two crypts they may be vermiform (Pl. 36, fig. 3, and Text-fig. 2). In the crypts, where the cells are pressed against each other, they are massive and cylindrical or pyriform (Pl. 36, figs. 1-4), excepting on the outer surface of the crypt masses, in which situation they become flattened (Pl. 34, fig. 6*a*, and Text-fig. 5). When forming the bars of the young calcareous skeleton or the inner smooth walls of crypts

they form large flat plates with the nuclear region projecting out from the free surface (Pl. 36, figs. 6, 7, 8).

Whatever their disguise, they possess certain common characters which enable them easily to be recognised. They are crammed with large orange-coloured pigment-granules, the colour being retained for a long time in specimens preserved in glycerine or formalin. Further, they have a large, soft, spheroidal or oval, vesicular nucleus and a distinct nucleolus. The granules, which stain easily and deeply, vary apparently according to the state of the metabolism—that is to say, in the early stages in the history of a calcocyte the granules are often of unequal size, but when they are in a position to lay down lime they become more or less uniform.

When the granules are pressed out of a cell and viewed under a high power slightly out of focus they appear as uniformly light discs, and gradually, as they are brought into focus, a dark point appears in the centre surrounded by a light circle; on focussing down further the dark point enlarges till the circle becomes uniformly dark. The appearance of the dark point in the light circle is of some importance, because it is seen all over the surfaces of the calcareous skeleton, where the ends of the fibrillæ give this appearance, and even in the interior of the conules on the tubercles (Pl. 35, fig. 22, and Pl. 36, fig. 9). The granules appear to be the essential calcigenous elements.

Wherever the skeleton is being laid down these granular calcocytes abound, and in decalcified specimens they are found in the closest relation with the organic basis of the dissolved skeleton.

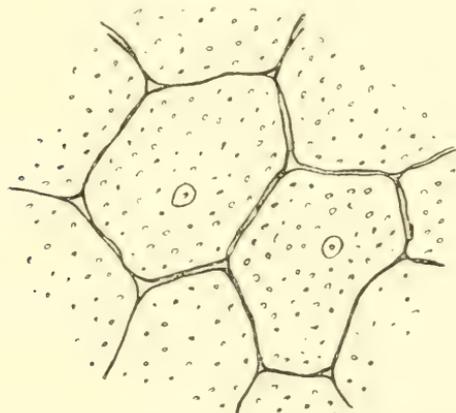
Pl. 34, fig. 3, shows a decalcified tubercle and side of a polygonal mesh with the calcocytes closely applied.

Fig. 1 on Pl. 37, which represents the extreme growing edge of the sponge, is especially interesting, for it shows the calcocytes planning out the first foundations of a polygonal mesh on the surface of the shell on which the *Merlia* is growing. The calcareous products of calcocytes in this position are flakes or scales (see Section 2B, and Pl. 35, fig. 21).

I believe the whole skeleton to be made of calcified calcocytes. Fig. 22 on Pl. 35 shows a camera lucida drawing by Mr. Highley of a conule, which seems to me to be nothing else than a petrified calcocyte. For the conule fits like a cap on the rounded surface to which it is attached; also the edges of its base are rounded and show a lobose process. Further, there is an appearance of an oval nucleus in the interior, and the fine structure shows the granules each with the dark central point.

In the crypts and on the bars of the polygonal network the

TEXT-FIG. 5.



Flattened calcocytes on the surface of a mass of crypt tissue.
× 750.

surface calcocytes are spread out in the form of plates (Pl. 34, fig. 6, and Pl. 36, fig. 1, both in vertical section), and the nucleus forms a little hump-like projection on the surface facing the soft tissues.

The supposed epithelium on the surface of the masses of crypt-cells is composed simply of flattened-out calcocytes of the same nature as the massive cells in the interior of the mass. Fig. 1 on Pl. 36 is drawn from sections made from material in which the fixing fluid did not well penetrate. The large nuclei of the plate-like calcocytes are shrunk and compressed, and cut into vertical sections. Fig. 6 on Pl. 34 shows much better the

vertical sections of the calcocytes on the surface of a crypt; here the nucleus is pressed outwards or away from the skeletal face of the cell to form a little mound on the opposite face. The most difficult part of this investigation was the discovery of the precise nature of the surface of the masses of crypt-tissue. This difficulty was partly due to the slowness with which fixing reagents penetrated into the nearly inaccessible crypts, and partly, I think, to actual variation in the condition of the surface-cells owing to functional causes. Pl. 34, fig. 6, shows a well-fixed Flemming preparation, and Pl. 36, fig. 1, a badly fixed osmic-picro-carminic one. Sometimes flattened surface-calcocytes are very well shown; at other times one can make out only a diffused layer of granules, the boundaries of cells being invisible.

There is a possibility that the large inner cells may be trophic stores, but in my opinion the surface crypt-cells are amoebocytes of the same nature as the inner ones, and also I think they are calcigenous, and not by secretion, but by actual transformation.

Fig. 6 on Pl. 36 shows the calcocytes forming an epithelium-like layer closely moulded to one of the calcareous bars of a polygonal mesh at the growing edge of a crust of Merlia.

The theory that the calcocytes become wholly transformed into calcified lumps or scales is confronted with one difficulty, viz. to account for the fan-like, fibrillar structure of the skeleton. I can only suggest that possibly the fibrillation is a secondary change. A decalcified section of a tubercle (Pl. 34, fig. 3*b*) shows radiating, fibrillar structure apparently corresponding with that of the hard skeleton.

À propos of the calcocytes, it only remains to say that the cylindrical, moniliform masses of these cells are simply basal prolongations of the sponge body in which they have accumulated. The cells are imbedded in the finely fibrillar maltha, just as are the rest of the tissues of the sponge. Occasionally the maltha in the crypts contains collencytes, scleroblasts, and spicules, and, very rarely, undeveloped wedge-shaped

collar-cells, such as occur in the youngest specimen. The reasons for this sharp separation between hypersome and hyposome now seem to me obvious. The calcocytes migrate down and accumulate in the base of the sponge, leaving but little or no room for the other elements, which can only expand upwards. The continued growth of the soft tissues thus separated into upper and lower, or superficial and basal, within the confined space of calcareous pits or tubes which have gradually been forming, leads to an hour-glass constriction and forming of tabulæ, with still more complete separation of the hyper- and hyposomal elements (see Section 5 below).

Often the calcocytes migrate to the periphery of a crypt, leaving the central part of the maltha clear and free of cells, and giving to the mass of cells the appearance of being arranged as a columnar epithelium against the crypt-wall and surrounding a cavity (Pl. 34, fig. 4). Partly for this reason I was led to think that *Merlia* might be a coral of some sort, since both the hard and soft tissues "conspired" to produce this impression.

(H) Tokocytes.

At one time I thought that the cylindrical masses of calcocytes in the crypts were gemmule cells, but unless they were capable of dissolving the calcareous skeleton, in which most of them were interred and cut off from the world, it was difficult to understand what could be the object of forming gemmule cells in nearly closed crypts, roofed in by tabulæ with central holes or slits sometimes only from 1 to 3 μ in diameter.

Just below the choanosome in one section are some granular cells with the nucleus and nucleolus larger even than in the calcocytes (Pl. 34, fig. 8). Such cells may be egg-cells. The specimen from which the section was made was captured in May.

(4) YOUNG STAGE OF MERLIA.

The youngest example found was in the form of a little red

spot, about 2 mm. in diameter, on the inner surface of a shell. The figure (Pl. 32, fig. 12), magnified twenty-five times linear, shows the delicate polygonal network below the surface of the sponge. There are no crypts at this stage, but simply the slender strands or bars of the wide-meshed network, with small tubercles at the nodes.

A vertical decalcified section, highly magnified (Pl. 38, figs. 1-3), shows a layer of cells all over the sponge at, or rather just below, the surface. The cells have processes which branch dendritically into innumerable slender fibrillæ or filaments. The fibrillæ passing to the surface form a thin, surface feltwork, which shows as a thin line in the section.

Bundles of fibrillæ pass down from the cells of the upper surface, and up from the basal cells. Occasionally the opposing filaments meet and almost form a central, horizontal lamina, especially near the periphery of the sponge, where the upper and lower surfaces are very close together. The waving masses of branched fibrils almost resemble the flames of a conflagration as conventionally depicted.

The calcocytes are elongated, and mostly congregated near the base of the crust and orientated at right angles to the base, like a field of stakes or hop-poles. Many of these cells are embraced between bundles of fibrillæ passing up from the basal branched cells.

The flagellated chambers are not yet formed, but the collar-cells are present as small wedge-shaped cells which here and there show a tendency to be arranged in half circles (as seen in section). Further, some wedged-shaped cells appear to be prolonged at their acute angle into a filament which joins on to a main filament, like the main stalk and secondary stalks of a pinnatifid leaf. Apparently the main stem and lateral stems represent the beginnings of the canal system. I have not been able to determine the nature and relations of these branched filaments; possibly they are the processes of connective-tissue cells.

Scleroblasts and their spicules are abundantly present.

The little grooves or arches at the base of the section are

the gaps left by the dissolved bars of the calcareous skeleton. In this situation a few calcocytes are seen in the walls of the arches.

(5) THEORY OF CONSTRUCTION OF THE CALCAREOUS SKELETON.

The skeleton is formed by the granular amœbocytes or calcocytes. The youngest stages of the skeleton (Pl. 35, figs. 20, 21) consist simply of microscopic scales, flakes or lumps laid down on the surface of the shell or other body which *Merlia* incrusts.

Within the growing edge of a flat, thin, spread-out specimen of the sponge the edge of the skeleton is visible in the form of slender bars forming a polygonal network, the polygons being incomplete at the extreme edge. In a still younger stage, visible only under fairly high powers, the slender bars are no more than smears or streaks of flakes, scales or lumps, somewhat higher at points where the tubercles will be formed. In course of growth the smears become ridges, and the polygonal outlines grow into circular pits with a rim of tubercles, and finally the pits become tubes with tabulæ varying in number from one to five, but commonly with one or two.

I believe that this complicated structure can be accounted for in the following way: *Merlia* is a thin incrusting siliceous sponge which has acquired the character of forming a calcareous skeleton. This sponge, like many other Tetraxonid sponges, is a modification of the simple "rhagon," i. e. of a thin-walled flattened sack with the choanosomal folds branching out all round. The mode of growth is that of dichotomous branchlets extending out from centre to periphery and possibly anastomosing with or in close juxtaposition to neighbouring branches (see radiating spokes on Pl. 33, fig. 1). At some epoch in its history—a point to be discussed later—the metabolic cells, the amœbocytes, became fed up with carbonate of lime, and either underwent calcification or re-secreted the lime. These cells would necessarily occupy the spaces in the sponge between the choanosomal branches, especially at points of

bifurcation.¹ Accordingly heaps of amœbocytes would accumulate at these points, and would extend along lines at right angles to the choanosomal branches till they met neighbouring ridges, in such a way as to enclose polygonal or circular areas, over the edges of which the choanosomal branches would extend (and between the tubercles or points of greatest heaping). At last the shallow meshes would become pits into which the heavy amœbocytes loaded with calcigenous granules would migrate. The heavy masses of loaded amœbocytes in the pits would tend to stay there, but the choanosome and ectosome would continue their growth in the direction of the surface. A tendency to a cleavage into two zones, hypersome and hyposome, would gradually become accentuated, especially in cases where the sponge can increase considerably in depth.

The result of the pull between opposing forces would be to cause in the little cylinders of soft tissue an hour-glass constriction. The continual growth of cells and tissues in a confined space would fill in the space round the hour-glass neck so that the "glasses" would become two closely approximated cylinders, between which the amœbocytes, or rather, calcocytes, would form a tabula leaving only an isthmus of tissue. Often even this little isthmus is nipped in and finally cut through by the total closure of the tabula, and the calcocytes below become cut off and buried in their crypt.

Obviously with varying factors there will be varying results. With uniform migration of calcocytes over a flat surface there is a tendency to peripheral extension rather than to growth in depth, and a uniform pull between hypersome and hyposome will lead to uniformity in size of crypts and to deposition of tabulæ in horizontal planes, and so on.

¹ The laying down of calcareous bars in the earliest stages before the flagellated chambers are formed would seem at first to furnish an argument against the theory that the form of the calcareous skeleton has been, so to speak, moulded by the choanosome. It must be remembered that siliceous spicules are found, too, in the youngest stage, and these must originally have been developed later than the flagellated chambers. By "choanosome" is here meant chiefly the canal system and flagellated chambers.

A simple model such as a wide india-rubber tube attached at one end and stretched so as to form a "node"—which could be tied with string—may serve to represent hyposome and hypersome. If air be forced in at each end, the two sections will become cylinders tending to approximate at the node. The pumped-in air will represent the growing tissues and cell-masses. Perhaps the rubber tube should be encompassed by a hard cylinder corresponding to the calcareous pit or tube.

Where growing protoplasm surrounds itself with a nearly closed calcareous cell or wall this opposition between the pull of basal inertia and that of centrifugal growth force tends to bring about the formation of a tabula or its equivalent.

In cases where the soft tissues in a tube are lifted bodily up and secrete a new floor at their base the comparison with what takes place in *Merlia* does not quite hold. The hour-glass-shaped siphonoglyphe of *Tubipora* and the cystiphragms in certain *Monticuliporas* appear to be due to special kinds of pulls or strains on the epithelial and other soft tissues.

The varying style of construction of the calcareous skeleton is due, I believe, to the fact that the calcocytes either settle down and deposit heaps of lumps (the conules of the tubercles) or—in the case of crypt-walls and tabulæ—layers of flakes. When crowded in the crypts the actively secreting calcocytes are spread or squeezed out flat and make smooth walls, filling in irregularities between the already formed conules. Sometimes from difficulties of terrain, such as the presence of steep ridges or shells, the little builders form thick conical main pillars conulated from base to summit and with scarcely any flanges or tube-walls.¹

¹ The researches of Koch and Bourne seem to prove that the skeleton of stony corals is formed as a secretion from calicoblasts. Heider and Maria Ogilvie, on the other hand, have concluded that this skeleton is the product of calcified cells. At present the former theory is in the ascendant. I have given evidence, though not so much as I could have wished, to show that the almost coral-like skeleton of *Merlia* is built up of calcified cells. The amœbocytes of a sponge are so very different from the epithelial cells of a coral that it is doubtful whether the knowledge of the mode of skeleton formation in *Merlia* would throw light on the question of stony coral formation.

Porto Santo is a little island about six miles long from east to west, with a small islet, Cima,¹ at its south-east corner, and another, Ilheo de Baixo, at its south-west corner. Baixo, which is a volcanic island, is extremely interesting geologically, for it has beautifully preserved coral reefs sandwiched between layers of basalt. The Madeirans use the coral for lime, and the English name of the island, viz. Lime Island, is of some significance from the point of view of a theory I am about to suggest in explanation of the peculiar feature of Merlia, viz. that this little sponge may have acquired its lime-forming character at the epoch when the coral reefs of Baixo were being formed, that is to say, during the Miocene period.²

There would probably have been a larger amount of carbonate of lime in solution and of fine particles of that substance in suspension in the sea-water in that area than at present.

Possibly the metabolic cells of Merlia became fed up (if a vulgar colloquialism may be permitted in the classic pages of this journal) with calcareous matter, and either re-secreted it, or became wholly calcified into lumps or flakes.³ Nature, always selecting what is helpful for survival, ended in making use of this property to elevate the delicate little crust—so liable to be overlaid by other organisms—and to form shelter-pits for the soft tissues. Support and shelter are two very important factors in sponge economy, as witness the boring sponges, which have acquired the character of working their way into shells and limestone. Merlia constructs its own shelter and supports. Morphologically the calcareous skeleton is wholly on the basal surface of the sponge, and, indeed, outside of it.

¹ Cima above. Baixo (cf. basis) low. The Portuguese refer to a location to the east as cima and one to the west as baixo, because their own mountains are east and their coasts west.

² It would be interesting to discover Merlia incrusting the shells, corals, etc., so abundant in these reefs. It seems to me very probable that it will be found there.

³ Compare Haeckel's theory of the origin of calcareous and siliceous sponges respectively on calcareous and siliceous oozes.

If it be asked why other siliceous sponges, especially those living in coral-reef-areas, do not form calcareous skeletons, the reply would be that it is too much to expect to be able to explain why any apparently spontaneous variation takes place in any living organism, because the ultimate molecular and chemical factors elude the power of analysis. As Weismann writes (12)—(but à propos of the trophic effect of functional stimulus)—“We are here face to face with the fundamental phenomenon of life, metabolism; and since we do not understand the cause of this, we are not in a position to say why it varies in this way or that according to the stimulus.” Possibly the orange-coloured protoplasmic granules of the calcocytes happened to acquire under favourable conditions just that molecular constitution that rendered carbonate of lime attractive, but apparently under penalty of becoming petrified. Later the “penalty” of the individual cells became transformed into an advantage for the common good.

The theory that *Merlia* constructed its calcareous skeleton in comparatively recent times is rendered plausible by the fact that no fossils, even with a superficial resemblance to the latter, occur till Palæozoic times. In any case the resemblance between *Merlia* and certain Palæozoic fossils may be purely homœomorphous.

If, as I believe, *Merlia* is a siliceous sponge¹ which has taken to forming a calcareous skeleton, then this sponge furnishes a good example of the hereditary transmission of an acquired character, and further, a striking instance of the tendency, which Herbert Spencer (9) first called attention to, of one part of an organism to increase in size and importance at the expense of the rest.

Weismann gave the name “intra-selection” to this ten-

¹ Whether *Merlia* acquired its calcigenous properties in Silurian or in Miocene times, it is difficult to regard it as any other than a siliceous sponge related to *Desmacellinæ*. No spongologist would believe that *Merlia* got its tylostyles, sigmata, trichodragmata and occasional toxæ by a process of convergent evolution.

dency. It is surprising to realise that the extensive crust of *Merlia* on the *Dendrophyllia* is the product of a very thin incrusting siliceous sponge, and, again, it is equally surprising to observe the great bulk of the masses of calcocytes in proportion to the rest of the tissues.

The English colleague already mentioned, who is, I believe, publishing a report on the dried calcareous skeleton of an organism which he thinks is *Merlia*, considers it to be a Foraminiferan. I think the specimen comes from the West Indies. Assuming the skeleton to be that of a *Merlia*, in which case it will be part of a sponge, the existence of *Merlia* at Porto Santo may have been due to embryos being carried by the Gulf Stream. On the north shore of the island Columbus collected *Entada* beans, which led him to speculate on the existence of land beyond the western horizon. The currents which carried the *Entada* beans might also carry the *Merlia* embryos which would settle on the first submarine peaks they would come to after their long voyage.

The theory that *Merlia* is a local survival from the periods when the coral reefs of Ilheo da Baixo were laid down seems to me more probable, however, than that of the West Indian origin of the sponge.

(6) SYSTEMATIC POSITION OF MERLIA.

The calcareous skeleton may be a character of no great importance from the systematic point of view.

The canal system—in spite of its novel or hymenopylous character—and the siliceous skeleton undoubtedly resemble in many respects the types commonly found in Monaxonellid sponges.

The calcareous skeleton is, I believe, an accidental and acquired character due to the activities of cells usually concerned in the metabolism of food and not occupied with skeletogenous functions, these latter belonging to a wholly different type of cells—the scleroblasts. *Merlia*, then, is a Monaxonellid

sponge, and further, there need be no hesitation in placing it in the family Haploscleridæ. At one time I had regarded the clavidiscs as modified chelæ, on account of the existence of two minute knobs on one of the "teeth" of a developing form, but it soon became evident that the affinities of these spicules were with the sigmata. If the free ends of the diancistra of Hamacantha were united it would become a clavidisc. Merlia, with its tylote megascleres, its rhabdides, trichodragmata, sigmata and toxa, is nearly related to the Desmacellinæ, a sub-family into which Lundbeck (11) places the three genera, Biemma, Desmacella, and Hamacantha. The scattered vertical tufts visible through the surface of Merlia in whole decalcified specimens somewhat resemble similar tufts at the oscular and poral surfaces of Biemma rosea Fristedt (11). Occasional horizontal bundles of spicules lying on the tabulæ in Merlia might be compared with the deeper-lying spicular network in B. rosea.

The presence, however, of clavidiscs and of a peculiar type of canal system in Merlia necessitate the founding of a new sub-family, viz., Merlinæ, which should be placed next to Desmacellinæ.

It would be an interesting experiment to cultivate Merlia in lime-free sea-water. If the sponge survived such deprivation the calcocytes would probably at first continue to heap up or build a soft skeleton similar in shape to the hard one, but later, being unburdened with lime, would resume their original functions as ordinary amœbocytes.

(7) ON SOME RESEMBLANCES BETWEEN THE CALCAREOUS SKELETON OF MERLIA AND CERTAIN PALEOZOIC ORGANISMS.

The calcareous skeleton of Merlia undoubtedly presents striking superficial resemblances to certain Palæozoic fossils, such as certain Monticuliporas, and to Rhabdopora stromatoporoides from the Devonian of Gerolstein (Pl. 38, figs. 7, 8, 9). In each case there is a surface polygonal

network with tubercles at the nodes, and tabular floors. In *Rhaphidopora* the tabulæ have a central hole and radial (? sutural) lines. In a former paper on *Merlia* I pointed out that these resemblances might be merely homœomorphous; at the same time I hoped that *Merlia* might throw light on the problem of the nature of *Monticulipora* and kindred organisms that have been placed by various authors in widely different groups; and I was rash enough to state that there was now more evidence in favour of the theory that some *Monticulipores* were sponges than in that of any other theory concerning the nature of these fossils.

After re-examining the large British Museum collection of fossils, and sections of *Monticulipora* and allied forms, especially the American series presented by Prof. Ray Bassler, I doubt whether *Merlia* is going to throw light on the problems concerning the nature of these organisms. It would, indeed, be an astounding paradox to state that some of the Palæozoic so-called Tabulate corals (Nicholson) or Polyzoa (Bassler) might prove to be the product of siliceous sponges, but the theory here put forward concerning the nature of *Merlia* is just such a paradox. At any rate the fact must be taken into account that it is possible for a siliceous sponge to make a tubular tabulate calcareous skeleton with certain resemblances to some of the Palæozoic *Monticuliporas sensu lato*. If *Merlia* had been found fossil, Nicholson would certainly have classed it among the Tabulate corals. Nickles and Bassler would, I think—but I write subject to correction—have placed it among the Polyzoa, possibly in a new sub-order, or in the Trepostomata, though not in the family *Monticuliporidae* Nicholson (emend Ulrich) (10), seeing that *Merlia* has no cystiphragms, mesopores, or acanthopores. The zoologist who would propose to classify a recent *Merlia* among the Foraminifera would naturally place the fossil *Merlia* in that group. I myself at first described the dried skeleton as that of a Pharetron sponge. Certainly no palæontologist or zoologist would have regarded such fossils or dried recent skeletons as products of siliceous

sponges. Yet in this instance I may say, in view of my own error, that truth is stranger than fiction.

It seems to me unlikely that *Merlia* could form anything else than a thin crust, i.e. that it, or forms allied to it, could have formed a laminate or branched growth, for instance, but sometimes the living sponge grows over a dead crust of its own kind.

What has happened in one instance may have taken place on other occasions. Amœbocytes of Tetraxonid sponges may have become calcocytes under favourable conditions at any epoch, and these calcocytes may have formed calcareous skeletons, just as *Merlia* has done.

(8) SUMMARY.

Merlia is a vermilion coloured incrusting Monaxonellid sponge belonging to a new sub-family—*Merlinæ*—of the Haploscleridæ. Large granular amœbocytes (calcocytes) have constructed a basal calcareous skeleton formed of vertical tubes divided up by horizontal tabulæ. The tubes are built up of columns, each with three vertical wings which unite with wings of neighbouring columns to form tubes. This mode of construction was probably primarily determined by the disposition of the branches of the choanosome which led to the deposition of amœbocytes at the points of bifurcation of the lines of flagellated chambers.

Apparently the calcocytes become wholly transformed into lumps, conules or flakes.

The calcareous skeleton shows certain resemblances, especially at the surface, to certain Palæozoic fossils, classed among "Tabulate corals" or Polyzoa.

There is no dermal epithelium, and the canal system is hymenopylous.

The sponge has been found in 60–90 fathoms off Porto Santo Island and Madeira.

A few more words remain to be said. It has been denied that *Merlia* is a sponge. I can only say that my opinion

whether right or wrong, is based on the prolonged investigation of abundance of good material, whereas other opinions seem to me to be founded mainly on *à priori* considerations.

I have examined over 500 specimens of *Merlia* and have always found the tissues of the sponge in most intimate association¹ with a calcareous structure, which grows as the sponge grows. Granular amœbocytes varying in shape according to circumstances are found everywhere in contact with the skeleton, and apparently in continuity with the organic matrix of the same. Also there is a curious similarity in appearance between the granules of these superficially placed cells and the surface view of the ends of the fibrillæ of the skeleton. The amœbocytes in the upper part of the sponge have the same fundamental characters as those in the interior of the crypts.

Amœbocytes are found deep down in crypts nearly closed over by tabulæ, and it is incredible that these large masses of cells could have worked their way down through the almost closed slits which are found in many tabulæ. Apart from these mechanical difficulties, one cannot imagine, from the point of view of common-sense, why the under surface of a supposed parasitic sponge should send down cylindrical moniliform masses of granular cells into the empty spaces of a foreign organism, thereby carrying out a seemingly useless and exhausting procedure. In young sponges on delicate shells, well stained and perfectly transparent, it can be clearly seen that there is not the least trace of any other organism than the sponge (on the shell). Weltner writes—and not unnaturally—of the calcareous structure as that of an unknown organism in which a sponge has settled. If this be so the said organism has preserved its incognito in a marvellous manner. The theory that *Merlia* is a sponge that has formed both a siliceous and a calcareous skeleton seems to me the only one possible, and further, the theory that it is a siliceous

¹ Of course, it is usual for a parasite to be closely associated with its host, but I trust that this investigation will make it clear that *Merlia* is not an instance of an association of this nature.

sponge that has taken to forming carbonate of lime one that is extremely probable.

Personal Note.—The finding of Merlia was associated with a curious incident which I trust may be of sufficient interest to be worth mentioning. I went out dredging daily at Porto Santo in a small whaling boat with Senhor Noronha and a crew of seven men. As day after day passed without result, I reluctantly decided to give up the search and return to Madeira. On the ninth day of dredging I secured as usual several large tubfuls of various specimens, but apparently Merlia was not amongst them. One of the tubs contained coarse sand, and I had told the men to throw it overboard in order to refill with more likely material, such as shells, pieces of rock, etc. I had arranged to leave the island that evening, and shortly after landing from the dredging I sent my luggage down to the beach. While the men were carrying away my boxes I casually picked up a small worm-tube out of the tub of coarse sand which had been brought ashore after all. Great was my pleasure on recognising in the fading light the long-sought-for Merlia. I at once altered my plans and went next morning to the spot whence the sand had been dredged, and secured a number of living specimens of the little sponge.

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EXPLANATION OF PLATES 32-38,

Illustrating Mr. R. Kirkpatrick's paper "On *Merlia normani*: a Sponge with a Siliceous and Calcareous Skeleton."

PLATE 32.

Fig. 1.—*Merlia normani*. Piece of shell incrustated with three small specimens. Natural size.

Fig. 2.—Specimen on coralline. Natural size.

Fig. 3.—Specimen on shell. $\times 3$.

Fig. 4.—Specimen on volcanic block. Natural size.

Fig. 5.—Specimen, dried and mostly denuded of soft tissues, incrusting *Dendrophyllia*. Natural size.

Fig. 5*a*.—Part of same. The larger circular holes are due to presence of worm-tubes. $\times 5$. (See also Text-fig. 3, p. 373).

Fig. 6.—Macerated and incinerated specimen. $\times 15$.

Fig. 7.—Part of surface of sponge showing oscule surrounded by a ring of pores. $\times 50$.

Fig. 8.—Another part of surface showing irregular distribution of pores. $\times 50$.

Fig. 9.—Decalcified thick vertical section of sponge showing several storeys of masses of crypt-cells or calcocytes; sponge much contracted at surface. $\times 50$.

Fig. 10.—Vertical section of well-expanded specimen. *a*. Subdermal spaces. *b*. Flagellated chambers. *c*. Crypt-cells or calcocytes. *d*. Long

siliceous spicules. *e.* Clavidiscs. *f.* Tubercles of calcareous skeleton. *g.* Skeletal wall showing zones of fibrillæ fanning out. $\times 125$.

Fig. 11.—Crypt-cells in glycerine. $\times 1500$.

Fig. 12.—Youngest specimen (*A*) forming a minute red spot on a shell. $\times 25$.

PLATE 33.

Fig. 1.—Upper surface of decalcified specimen showing "nodal" masses, *a*, of flagellated chambers joined by radial bands, *b*, of flagellated chambers. *c.* Spaces left by decalcified tubercles. $\times 100$.

Fig. 2.—Under surface of another specimen (decalcified). *a.* Masses of choanosome filling uppermost spaces of calcareous skeleton. *b.* Radial "spokes" joining these masses. *c.* Spaces left by decalcified tubercles. *d.* Crypt cells. $\times 120$.

Fig. 3.—Decalcified vertical section of well-expanded specimen (showing only one mass of crypt-cells). *a.* Subdermal space. *b.* Flagellated chambers. *c.* Soft tissues (in uppermost crypts). *d.* Soft tissues, mostly calcocytes, in a lower crypt. *e.* Bundles of siliceous spicules. *f.* Clavidiscs. *g.* Inhalant pore. $\times 140$.

Fig. 4.—Flagellated chambers. *a.* Apopyle in sphinctrate membrane. *b.* Sphincter "muscle"-cell. $\times 750$.

Fig. 5.—Flagellated chambers. Lateral view. $\times 750$.

Fig. 6.—Flagellated chamber, convex surface, showing prosopyles between stellate bases of collar-cells. $\times 1600$.

Fig. 7.—Section of a flagellated chamber. $\times 2425$.

Figs. 8-11.—Collar-cells in different conditions of contraction, drawn from the same section. $\times 1800$.

Fig. 12.—Collar-cell showing flagellum passing down to nucleus. $\times 1800$.

Fig. 13.—Growing edge of young specimen. *a.* Flagellated chambers. *b.* Radiating tufts of spicules. *c.* Clavidisc. $\times 175$.

PLATE 34.

Fig. 1.—Section of a small decalcified specimen showing gradual development of calcareous skeleton from edge to centre of the crust (the skeleton being represented by spaces in figure). $\times 25$.

Fig. 2.—Surface of a young specimen. *a.* Oscule. *b.* Pores. $\times 425$.

Fig. 3.—Decalcified tubercle and upper edge of wall of skeleton, showing large granular cells (calcocytes) and radiating fibrillar structure of organic matrix. *a.* Granular cells. *b.* Radiating fibrillæ in body of tubercle. *c.* Flagellated chamber. $\times 375$.

Fig. 4.—Transverse section of mass of crypt-cells (calcoocytes). *a*. Fibrillæ of maltha. *b*. The same cut across. $\times 540$. (N.B.—Calcoocytes in crypts are, for convenience, termed crypt-cells.)

Fig. 5.—Portion of same. $\times 2000$.

Fig. 6.—Edge of mass of crypt-cells, showing cut edges of surface ones forming a flat epithelium (*a*), with the large oval nucleus forming a hump on inner aspect facing cell-mass. $\times 1025$.

Fig. 7.—A crypt-cell showing spherical nucleus and nucleolus. $\times 3000$.

Fig. 8.—Granular cell in an uppermost or open crypt, with very large nucleus and nucleolus, *a*. ? Tokocyte (egg-cell). $\times 3000$.

PLATE 35.

Fig. 1.—Tylostyles. $\times 525$.

Fig. 2.—Trichodragma. $\times 750$.

Fig. 2*a*.—Rhaphides. $\times 750$.

Fig. 3.—Clavidisc, most frequent shape. $\times 750$.

Fig. 4.—Broken clavidisc showing axial canal. $\times 1000$.

Fig. 5.—Clavidisc with narrower rim, deeply situated in the sponge. $\times 750$.

Fig. 6.—Developmental form of clavidisc, with ends joined by slender filament. $\times 750$.

Fig. 7.—Incomplete clavidisc, like a sigma. $\times 750$.

Fig. 8.—Clavidisc with crossed ends. $\times 750$.

Fig. 9.—Young twisted form of clavidisc—abnormal. $\times 750$.

Fig. 10.—Very slender, simple sigmata. $\times 750$.

Figs. 11–15.—Abnormal spicules, all from one specimen. Fig. 11, tylostyle with three knobs. Fig. 12, tylostyle with double curve. Fig. 13, toxa-shaped rhaphide. Fig. 14, clavidisc with flat discs in place of keyhole sinuses. Fig. 15, slender sigmata with knobs at each end. All $\times 750$.

Fig. 16.—Calcareous skeleton, surface view, showing surface tabula, with central hole, or slit, which may be quite closed. The tabula show concentric circles and radial sutures radiating from centre to the inter-flange suture. $\times 130$.

Fig. 17.—Vertical section of skeleton, showing several crypts, one series being laid open and two others closed over (by vertical flanges or wings). $\times 130$.

Fig. 18.—Base of skeleton, showing some tabulæ nearly closed, which

roofed over crypts which had been full of calcocytes, later macerated out. $\times 100$.

Fig. 19.—Ground-down basal section with soft tissues remaining. Thick walls of tubes as seen by strong, oblique reflected light, showing well how vertical flanges or wings join to form walls of tubes. *a*. Tabula. $\times 160$.

Fig. 20.—Calcareous skeleton of *Merlia* on surface of carinate worm-tube, macerated in Eau de Javelle, the youngest parts of skeleton being lumps and scales. $\times 100$.

Fig. 21.—Calcareous scales on surface of worm-tube (see fig. 20) having appearance of calcified flattened cells, with a nuclear hump and granular structure. $\times 425$.

Fig. 22.—A conule at base of tubercle having the appearance of a petrified amœbocyte, fitting cap-like on rounded surface below, with rounded basal rim and lobate basal process, with oval nucleus and granular structure. $\times 1500$.

PLATE 36.

Fig. 1.—Part of mass of crypt-cells. *a*. An isthmus of tissue. *b*. Flattened crypt-cells in section, much contracted, showing sections of nuclei. *c*. Connective tissue cell. $\times 1000$.

Fig. 2.—Part of sponge tissue at bottom of an upper or open crypt. *a*. Collar-cells. *b*. Longitudinal bundles of spicules with their scleroblasts. *c*. Transverse section of spicule bundle. $\times 1500$.

Fig. 3.—A neck or isthmus joining upper part of sponge to a mass of crypt-tissue. *a*. Large granular crypt-cells. *b*. Connective-tissue cells (collencytes). $\times 1500$.

Fig. 4.—Large granular crypt-cells. $\times 1500$.

Fig. 5.—Three calcocytes on summit of a tubercle. Slightly separated by pressing down cover-slip; separation indicated by **Y** line. [Note.—The view is in projection; the cells extend some distance vertically down.] $\times 2200$.

Fig. 6.—Growing edge of skeleton. Calcareous bar covered with layer of calcocytes. $\times 650$.

Fig. 7.—One calcocyte from those depicted in fig. 6, showing granular contents (each granule with dark central spot), large oval nucleus, and the nucleolus. $\times 4000$.

Fig. 8.—Two flattened granular cells. $\times 2220$.

Fig. 9.—Calcareous skeleton, showing granular appearance (due to ends of fibrillæ) at surface, and fibrillar structure in section. $\times 1800$.

Fig. 10.—Clavidisc with its scleroblast. *a*. Nucleus. $\times 1450$.

PLATE 37.

Fig. 1.—Growing edge of a specimen of *Merlia* (osmic-picro-carminé preparation in glycerine) showing elongated calcocytes planning out calcareous skeleton on smooth surface of shell. *a.* Calcocytes. *b.* Flagellated chambers and excurrent canal. *c.* Siliceous spicules. *d.* Edge of sponge. *e.* Connective-tissue cells (collencytes). $\times 750$.

Fig. 2.—Collencytes. $\times 2425$.

Fig. 3.—Myocytes arranged radially and concentrically round a pore. $\times 1450$.

Fig. 4.—Gland-cells. $\times 2425$.

PLATE 38.

Fig. 1.—Vertical section of very young specimen figured on Pl. 32, fig. 13, decalcified. *a.* Spaces left by dissolved skeleton. *b.* Elongated calcocytes. *c.* Connective-tissue cells, with finely and abundantly branched processes. *d.* Young wedge-shaped collar-cells. $\times 1000$.

Fig. 2.—Vertical section of same showing a tylostyle and a bundle of siliceous spicules cut transversely. $\times 1000$.

Fig. 3.—Vertical section of same nearer to edge of sponge than in fig. 1. $\times 540$.

Fig. 4.—Part of a collencyte (mostly cut away) showing many tufts of branching processes. $\times 1500$.

Fig. 5.—Growing edge of a specimen. *a.* Edge of sponge. *b.* Calcareous skeleton. $\times 120$.

Fig. 6.—The tuning-fork spicule from the original dried specimen of *Merlia*. $\times 1050$.

Fig. 7.—*Monticulipora* (*Heterotrypa*) *moniliformis* Nich. (from Devonian of Ontario), surface, showing polygonal network and tubercles. Copied from Nicholson's 'Palæozoic Corals,' "*Monticulipora*," 1871, pl. i, fig. 1*a*. $\times 18$.

Fig. 8.—*Rhaphidopora* (*Chætetes*) *stromatoporoides* Roemer. Devonian of Gerolstein. "Tangential section" (but ? surface view.—R.K.). From Nicholson and Foord, 'Ann. Mag. Nat. Hist.' (5), xvii, pl. xvi, fig. 5, showing polygonal network, tubercles, and tabulæ, some with central hole and apparently with radial sutural lines. $\times 20$.

Fig. 9.—The same as fig. 8, deeper transverse section. $\times 20$.

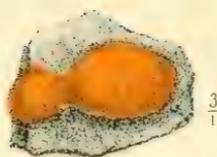


Fig. 3

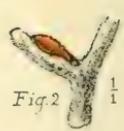


Fig. 2

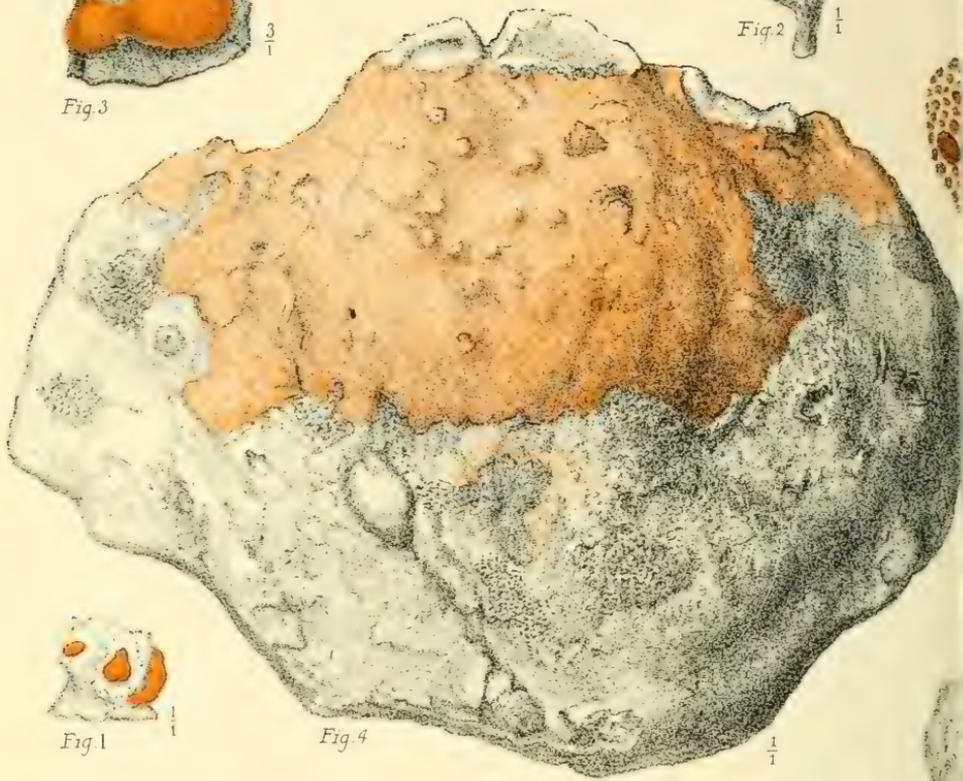
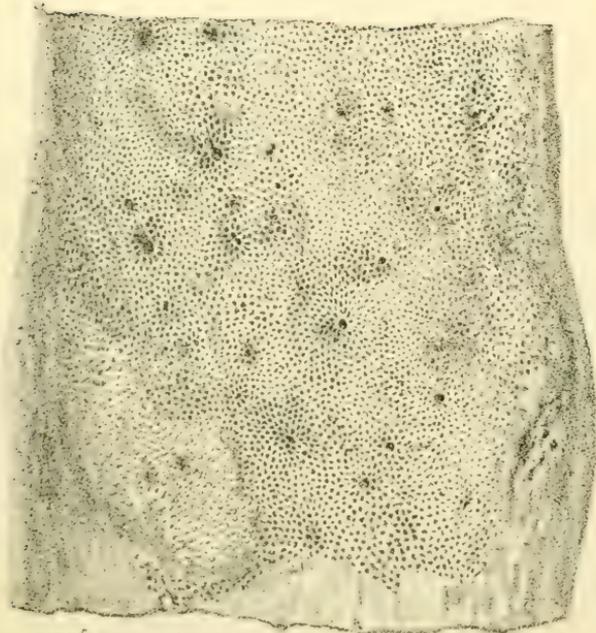


Fig. 4



Fig. 1



5 a

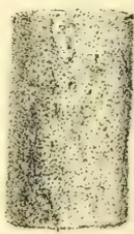


Fig. 5

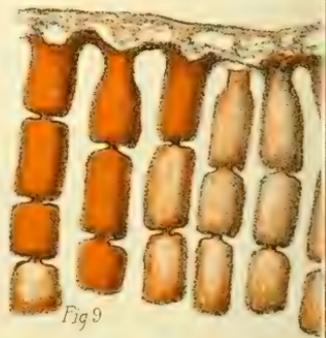


Fig. 9



Fig. 11

$\frac{125}{1}$

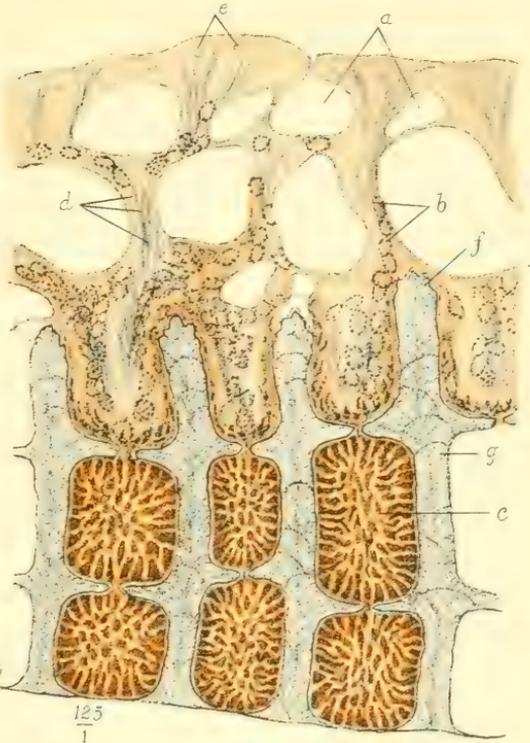
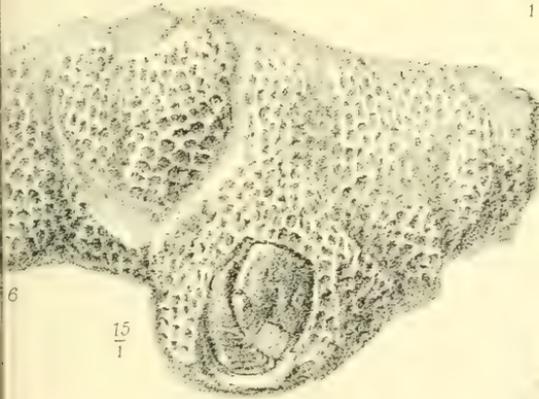


Fig. 10

$\frac{125}{1}$



$\frac{15}{1}$

Fig. 8



$\frac{50}{1}$



$\frac{50}{1}$

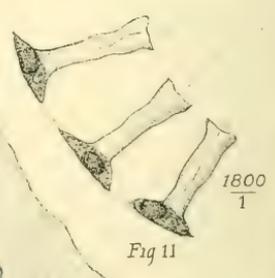
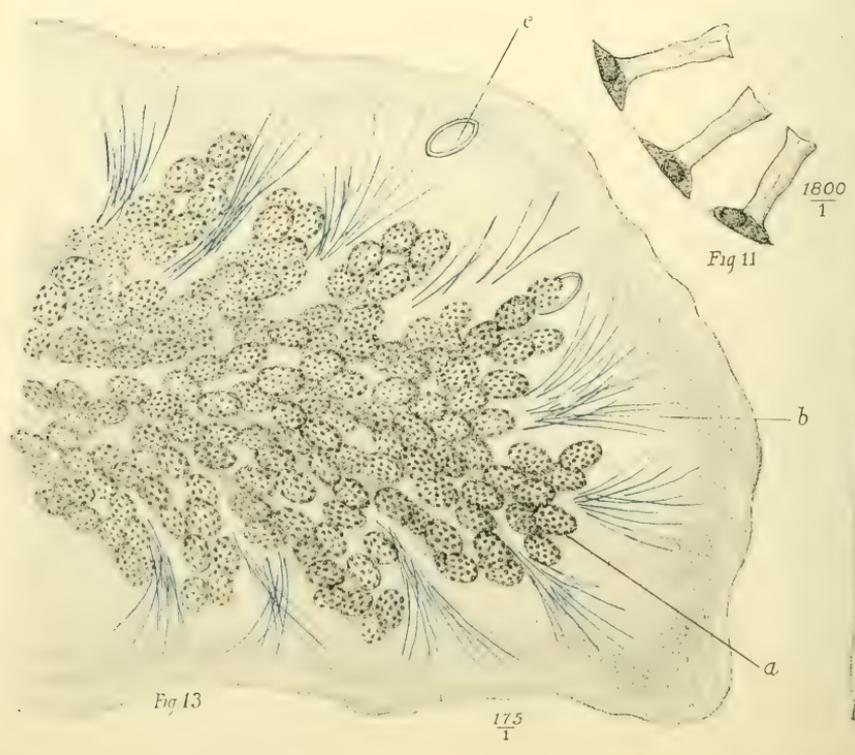
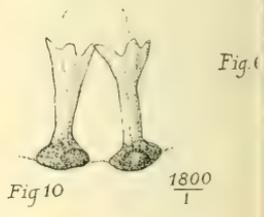
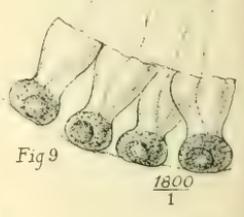
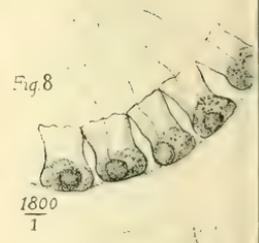
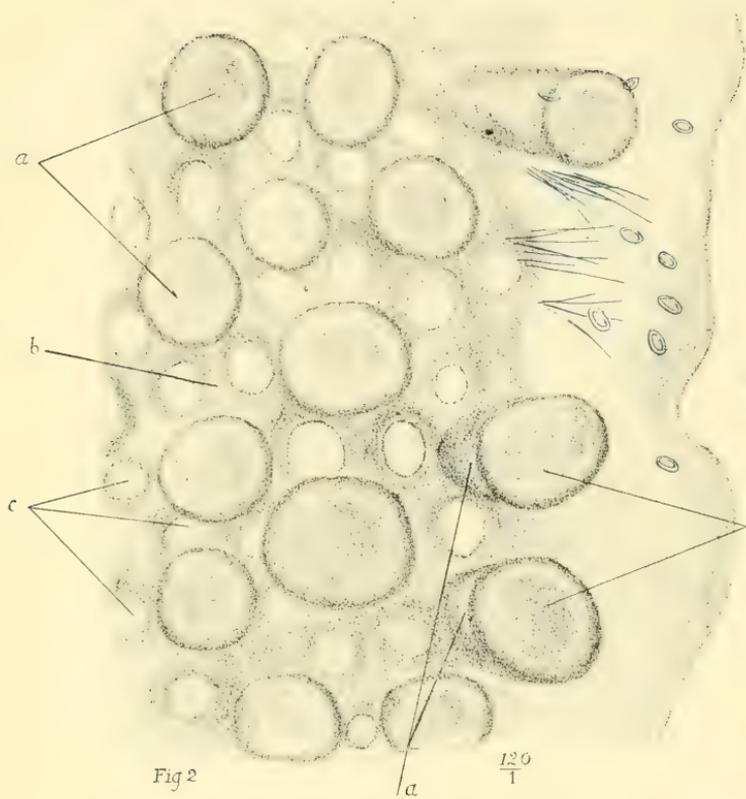
Fig. 7

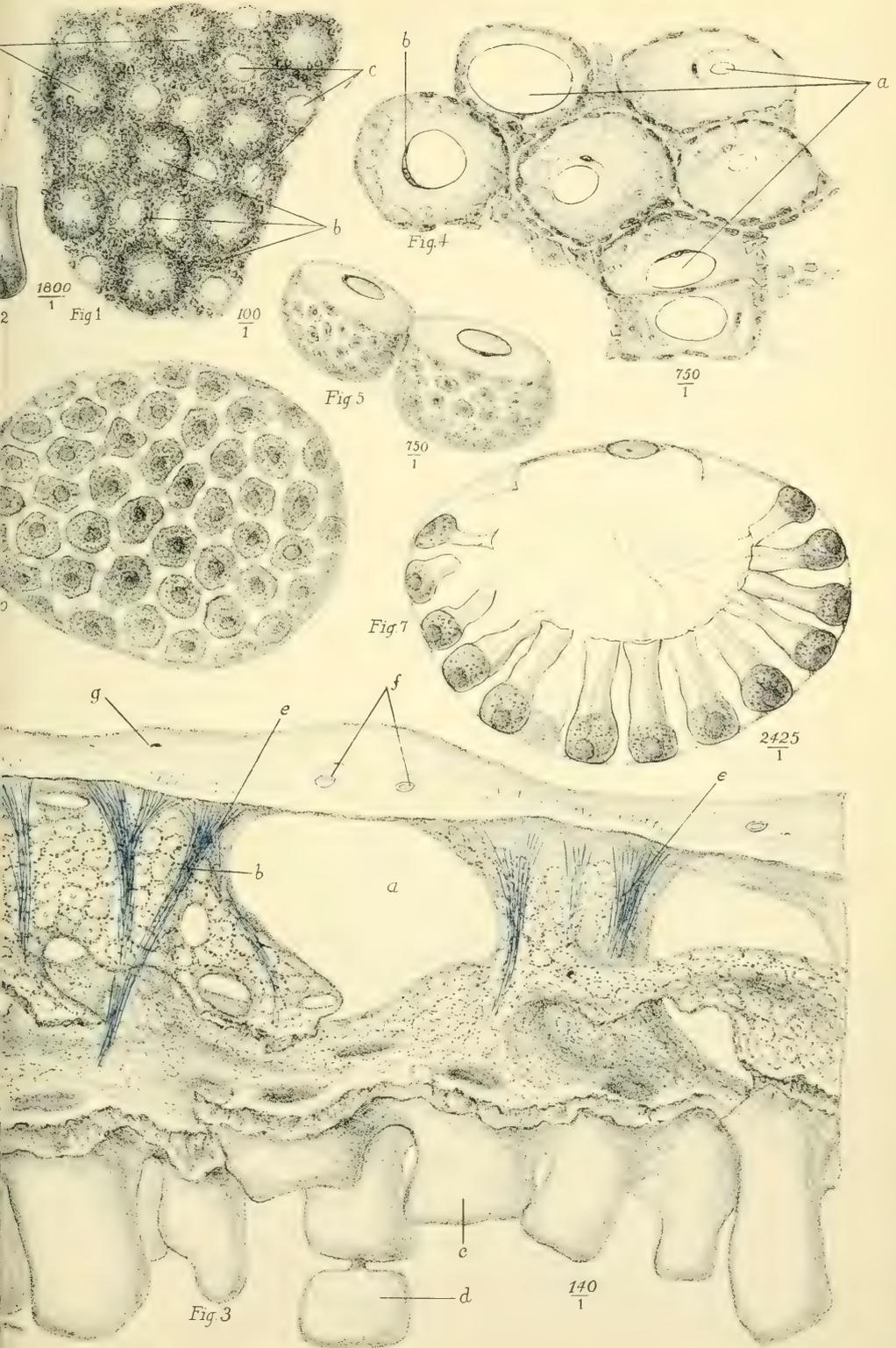
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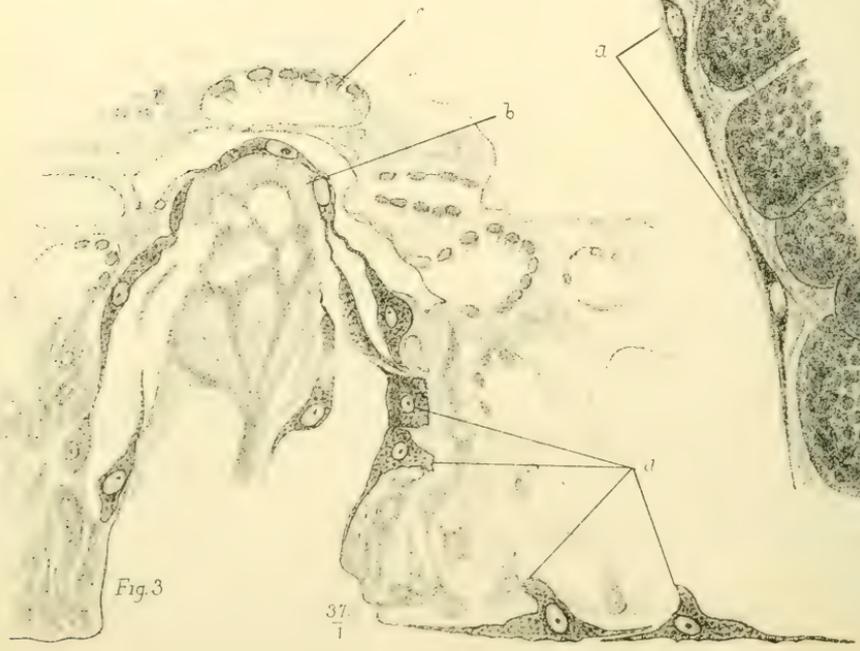
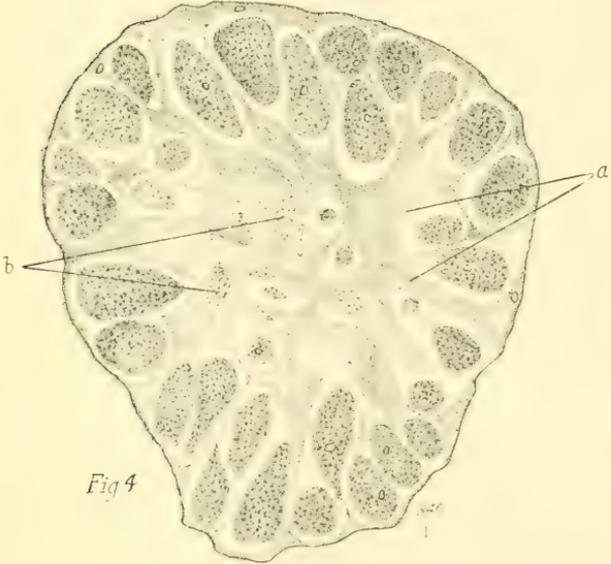
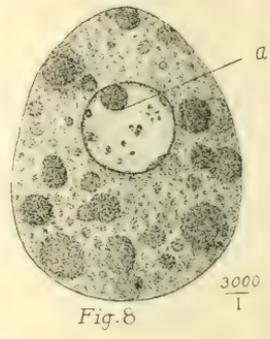
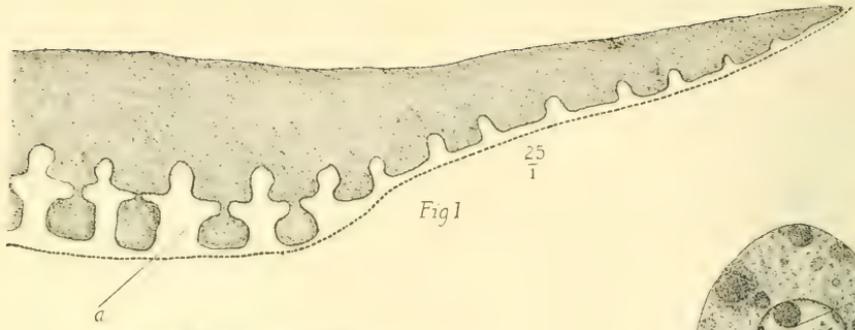


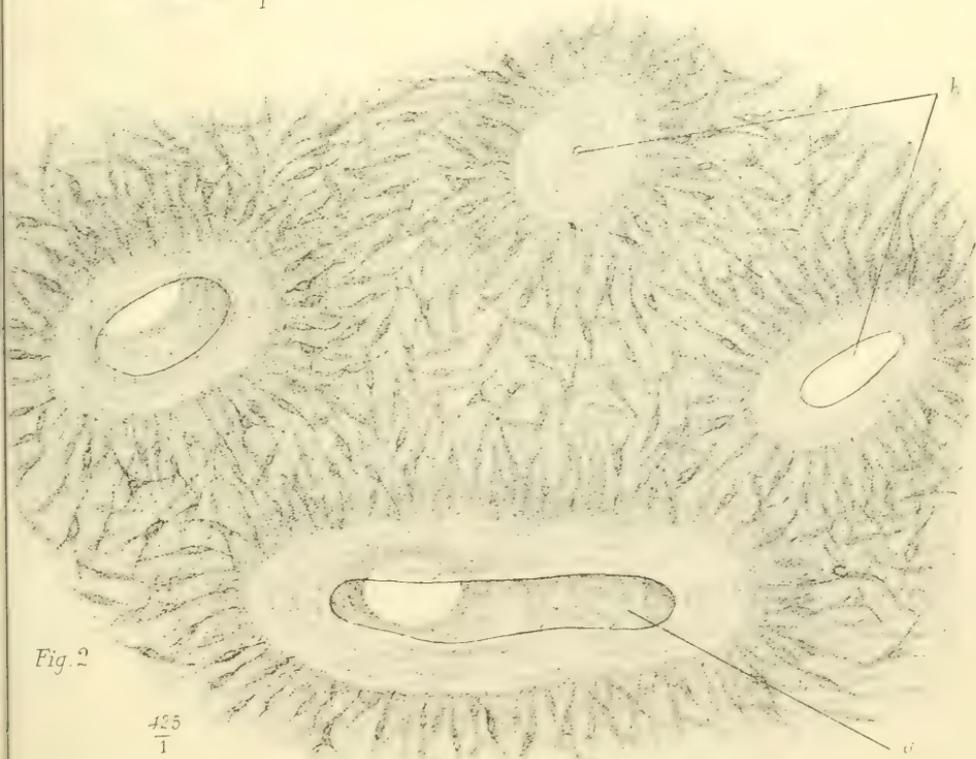
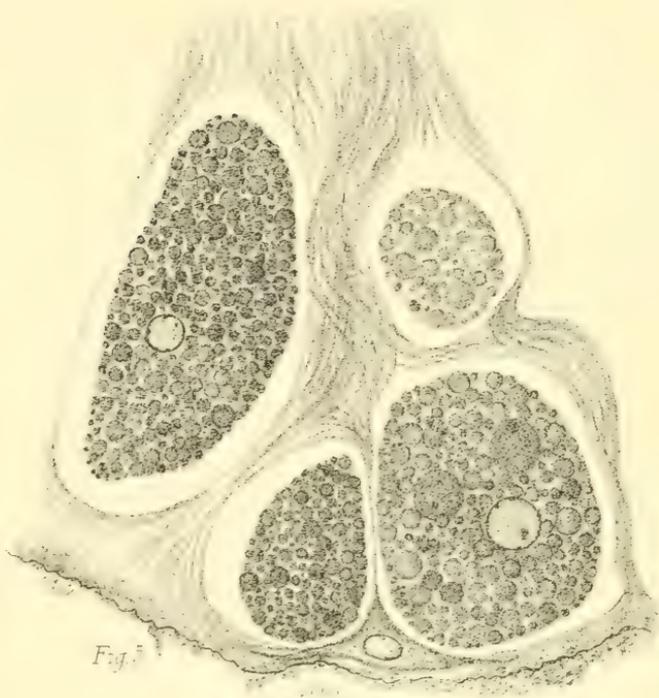
Fig. 12

$\frac{25}{1}$









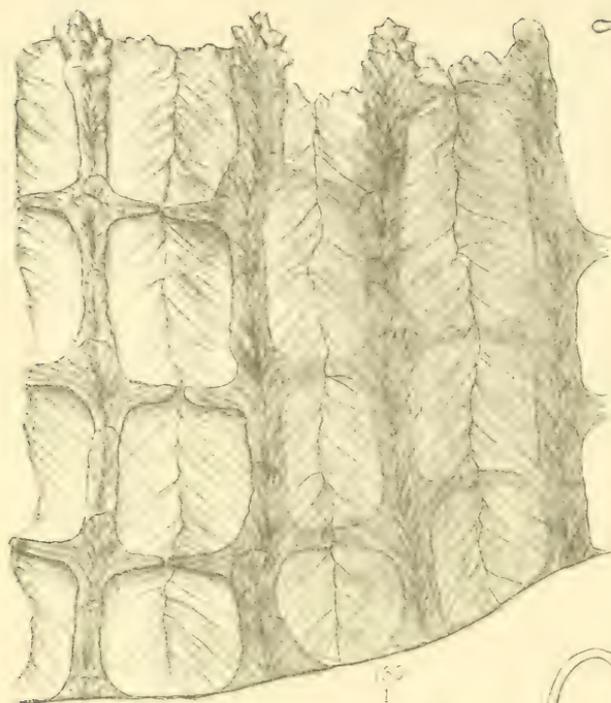


Fig. 11

150
1



Fig. 7

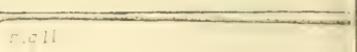


Fig. 11



Fig. 14



Fig. 14



Fig. 3

700
1

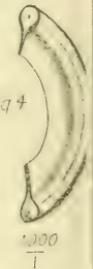


Fig. 4

1000
1



Fig. 5

50
1



Fig. 8

700
1

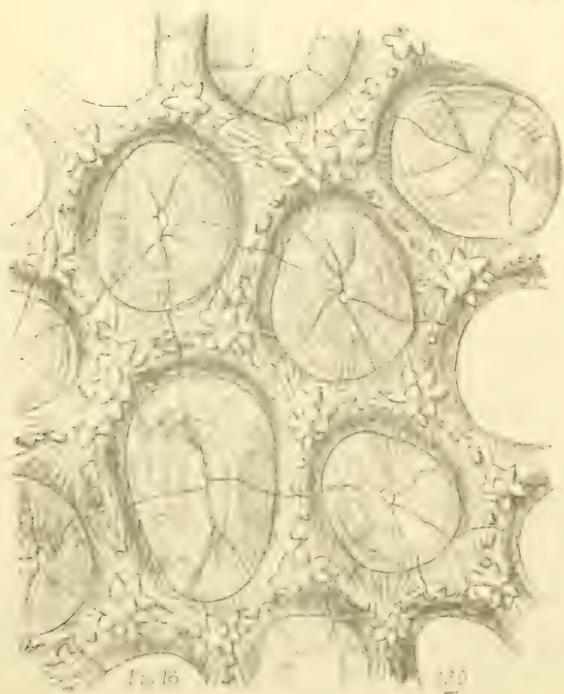


Fig. 16

120
1



Fig. 2

200
1

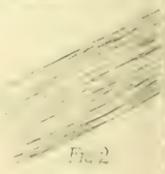


Fig. 2

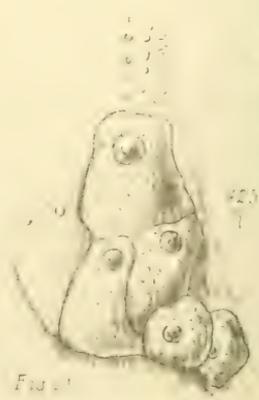
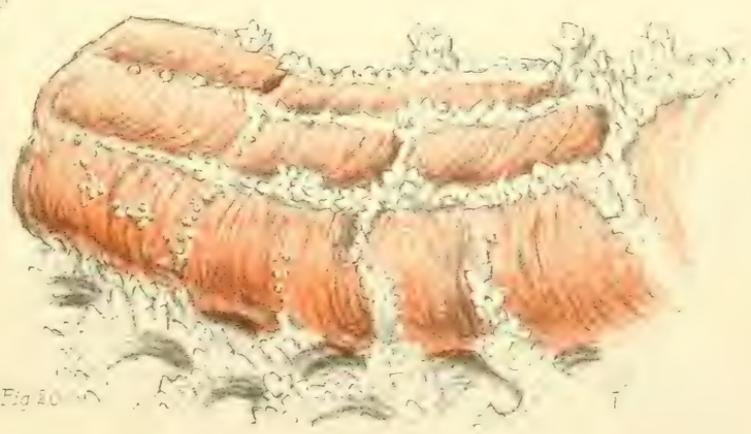
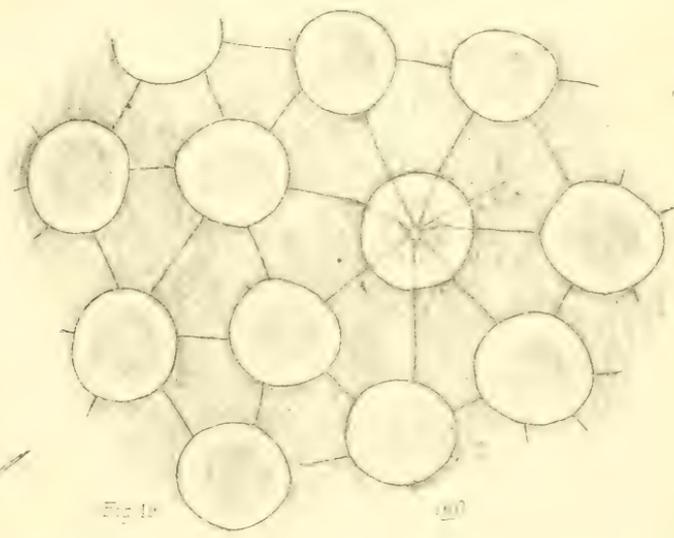
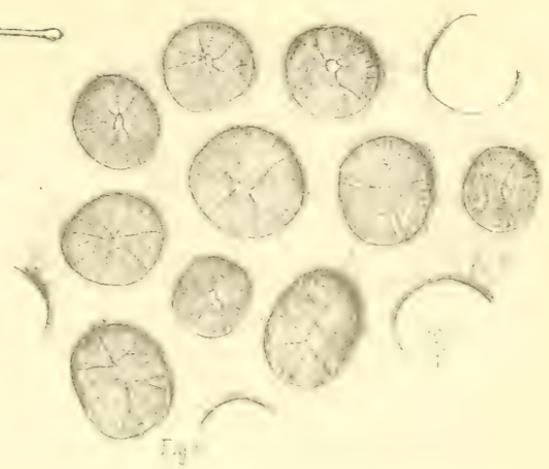
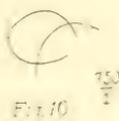
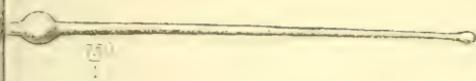


Fig. 1

420
1



Fig. 1



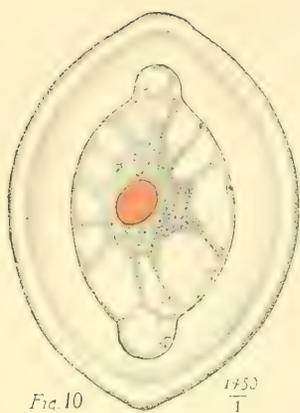


Fig. 10

$\frac{1750}{1}$

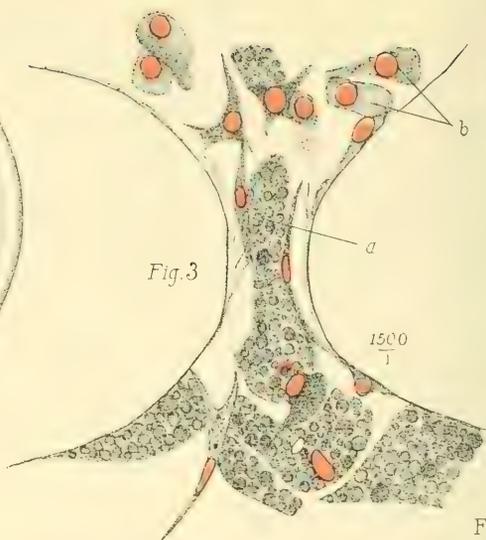


Fig. 3

$\frac{1500}{1}$

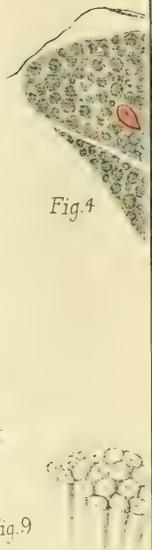


Fig. 4



Fig. 8

$\frac{2200}{1}$

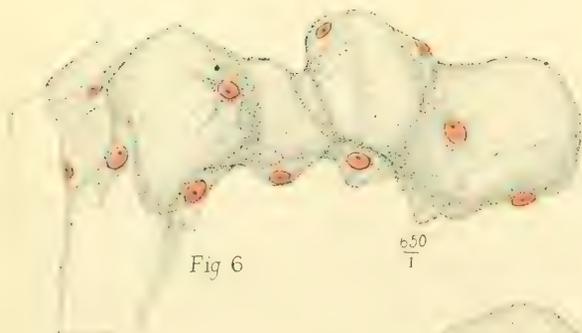


Fig. 6

$\frac{650}{1}$



Fig. 7

$\frac{4000}{1}$

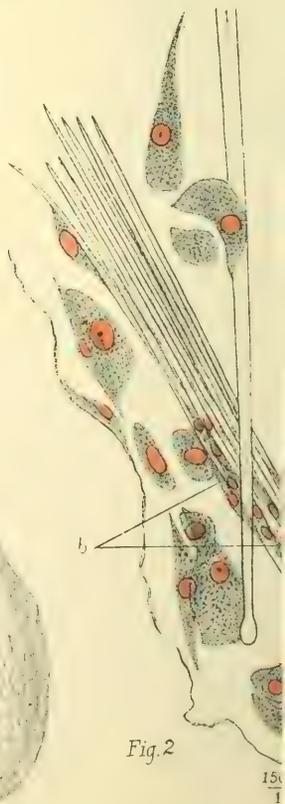
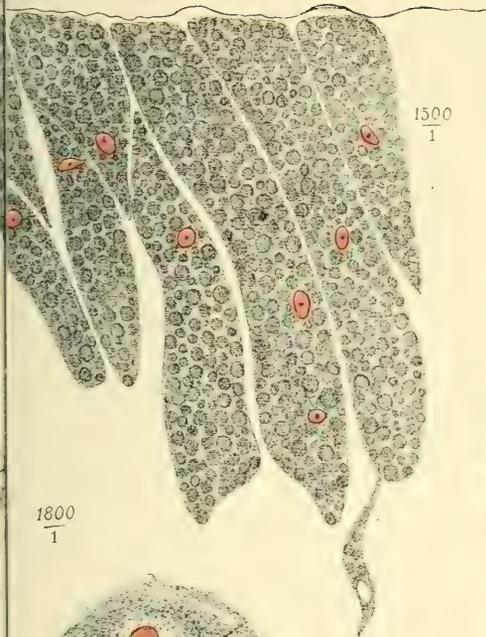


Fig. 2

$\frac{1500}{1}$



1800
1

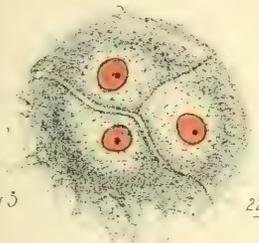


Fig. 5



Fig. 1

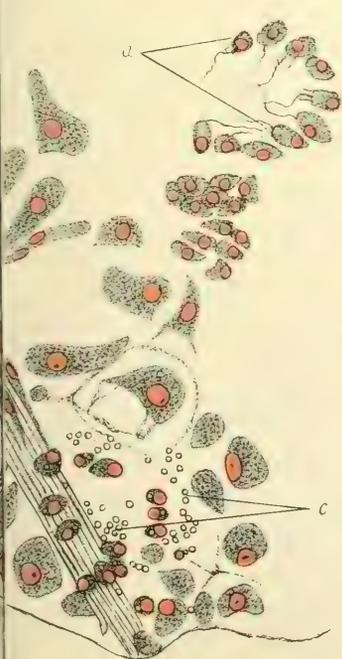
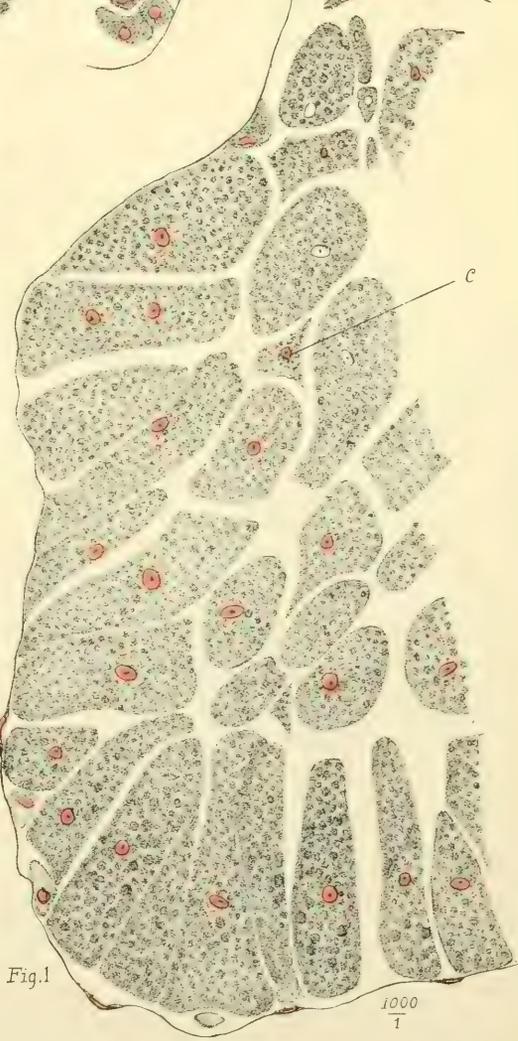




Fig. 1

125
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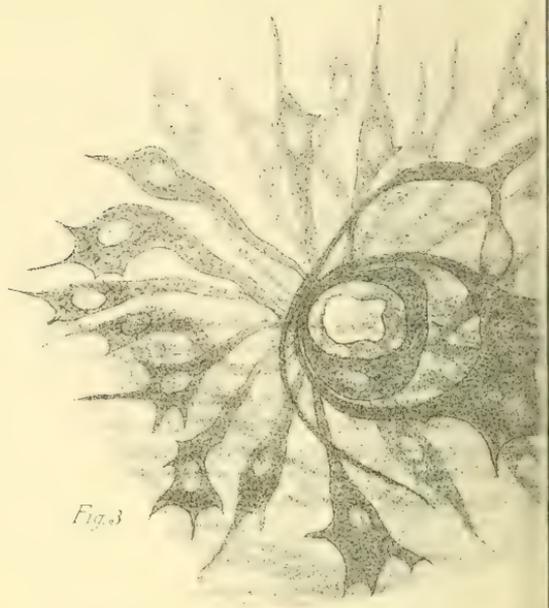
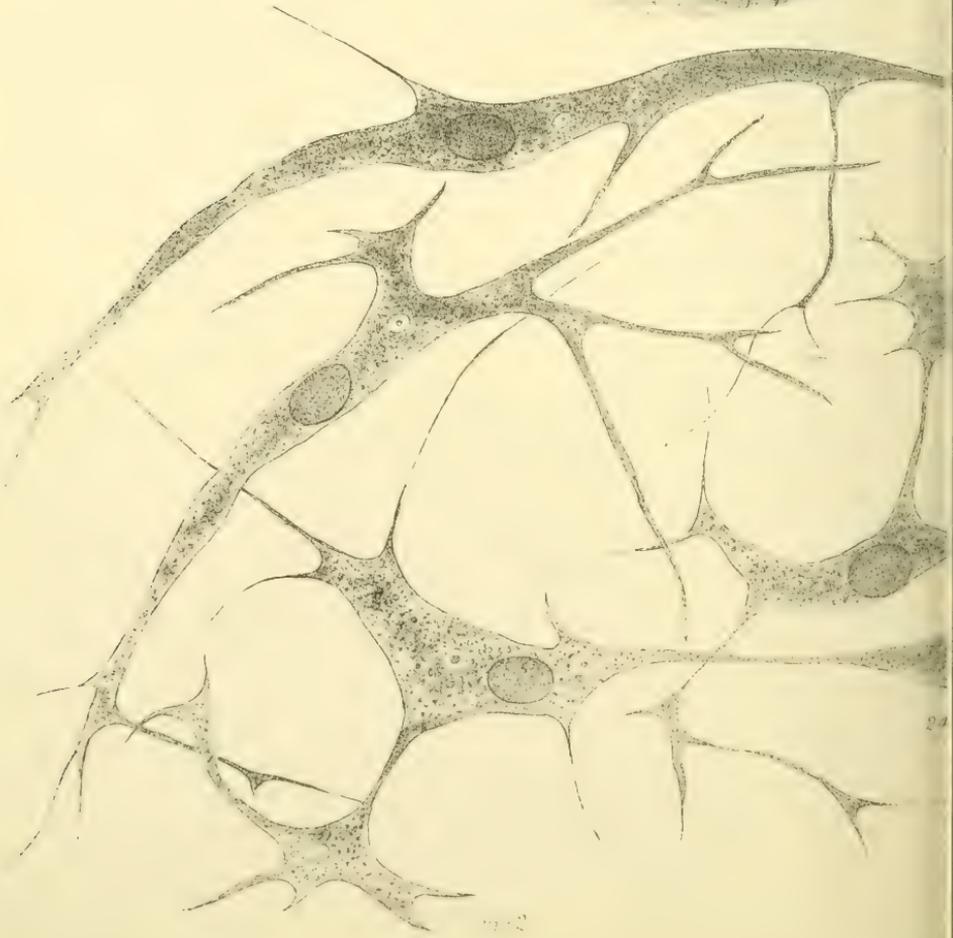


Fig. 3





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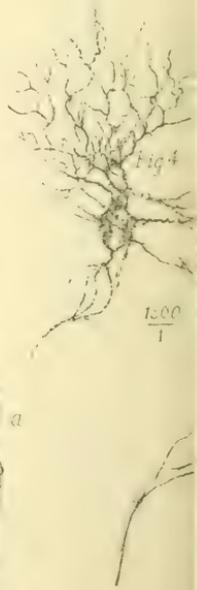
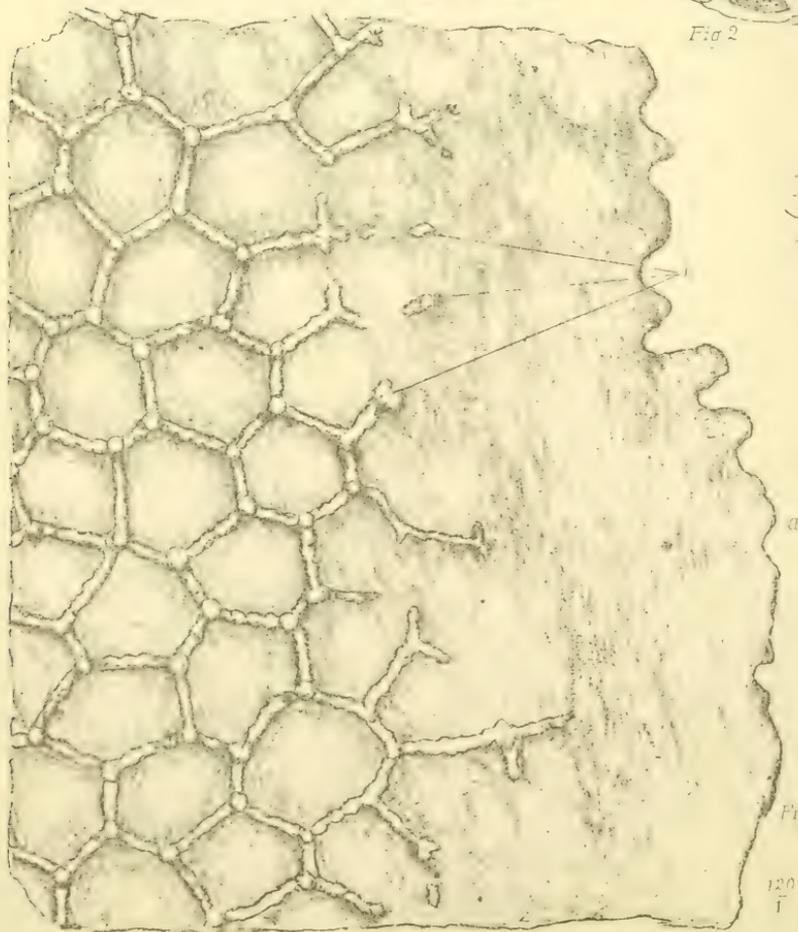
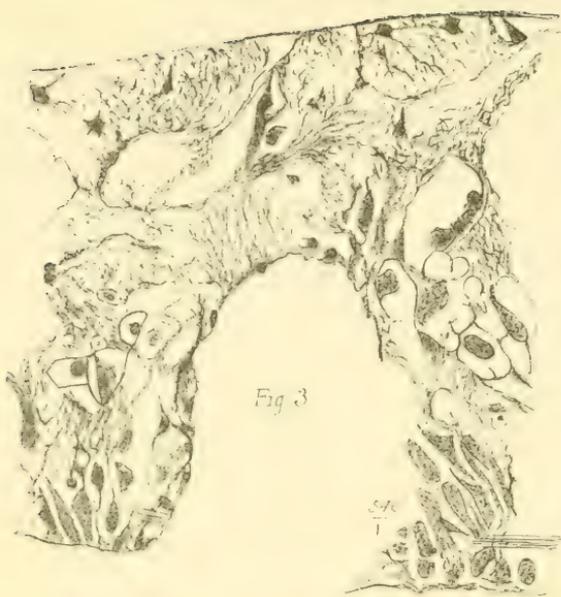
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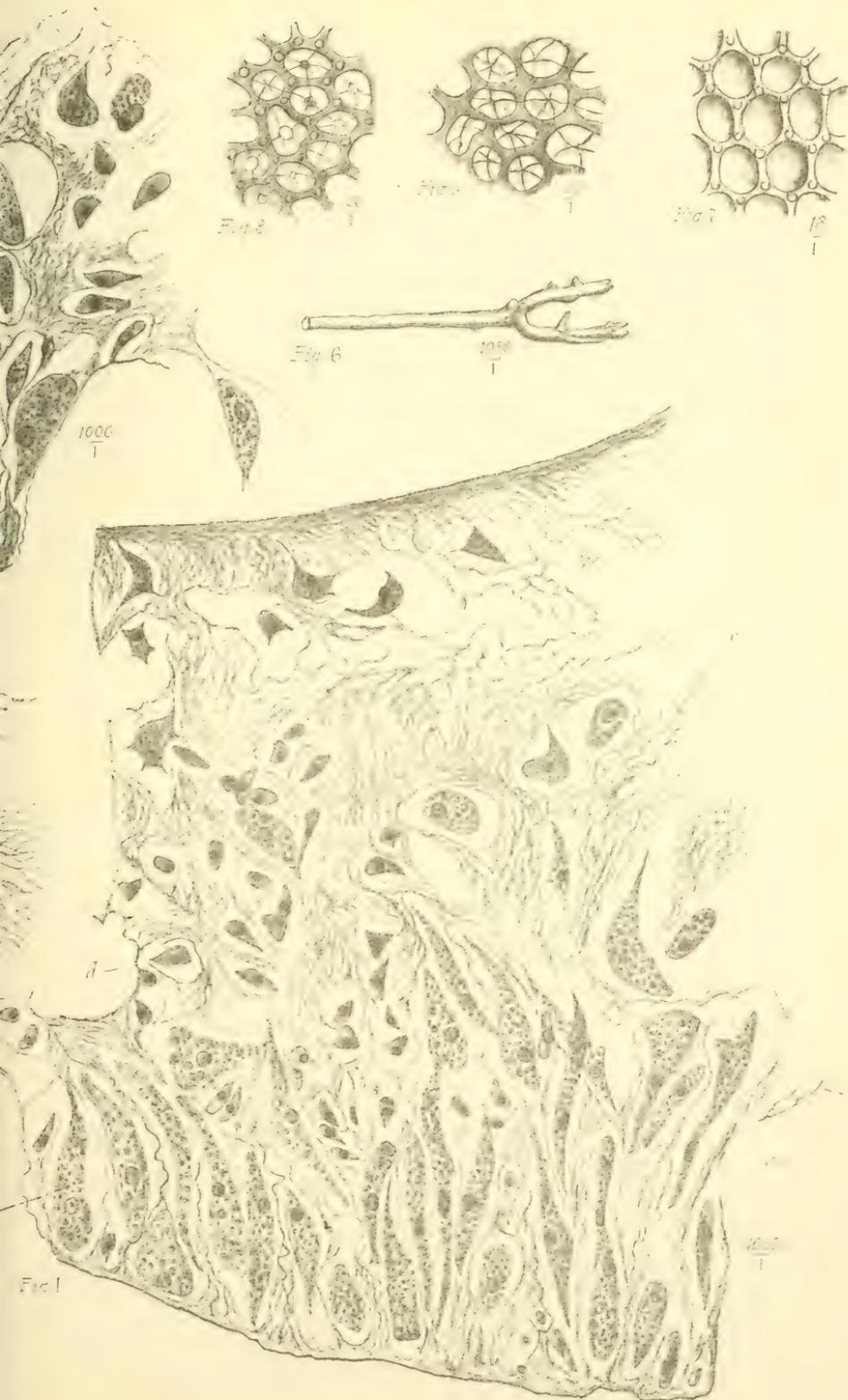
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