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Some Problems of Reproduction: a Comparative Study of Gametogeny and Protoplasmic Senescence and Rejuvenescence.

By

Marcus M. Hartog, M.A., D.Sc., F.L.S.,

Professor of Natural History in the Queen's College, Cork.

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I. Introductory.

THE curious phenomena preceding the maturity of the ovum in Metazoa have been the object of much study and the groundwork of much theory during the last fifteen years. Unfortunately processes occurring in this highly specialised group have been assumed to be typical of all organisms; the authors who have put forward explanations of what they have seen here have too often sought to extend these explanations to other groups, where the facts are different; they have created homologies where none such exist in Nature, and over-looked those which lay under their eyes. In this way proto-plasmic changes of various origin and functions occurring in Protozoa, Protophytes, and Higher Plants, have been inter-preted as excretory processes, in order to make them fit in with the replacement theories of fertilisation, founded almost exclusively on the formation of the polar bodies in the Metazoan ovum.

A careful study of the accessible materials in the gigantic storehouse of facts bearing on this subject has led me to the views which will be found in the following pages, namely:

(1) That the most general, but not universal, feature un-derlying the preparations for fertilisation is the specialisation

of gametes by rapidly repeated divisions of a cell—the gametogonium;

(2) That the alleged nuclear excretions in the Metazoan egg and the Ciliate "gamete," &c., represent true gametes arrested in their development;

(3) That the so-called "excretions" of protoplasm in plants are of various kinds, many of which are homologous neither with the former process nor with one another;

(4) That the use of the rapid preliminary divisions is a purely physiological one; that is, to induce by exhaustion the same reproductive incapacity that would otherwise require a long series of slowly repeated divisions.

On these lines we can account for all the facts, from the simplest cases of the formation of isogametes to the most peculiar phenomena of oogeny and spermatogeny; phenomena which the sexual replacement theories of Minot, Balfour, and van Beneden, on the one hand, and the more complex replacement theory of Weismann, on the other, only profess to explain in the higher groups. The views here put forward are essentially a development and extension of what we may term the "morphological theory" of polar bodies, first enunciated by Giard, Bütschli, Whitman, and Mark,[1] and advocated especially by the Hertwigs. It will not seem strange that this view has never had full justice done it when we reflect that it is to men who have worked especially at the Metazoa that we owe the greatest debt for shaping our biological theories; and that our gratitude has, perhaps, led us to be too unquestioning in our attitude of discipleship to such respected masters as Balfour, van Beneden, and Weismann.[2]

The exposition of the processes of gametogeny naturally leads

[1] Mark was the first definitely to express the view that the polar bodies represent abortive ova; see "Maturation, Fecundation, and Segmentation of Limax campestris," in 'Bull. Mus. Comp. Zool. Harv. Coll.,' vol. vi, 1881.

[2] I may say that I never doubted that some replacement theory of fertilisation was sufficient to cover the facts, till I read and meditated over Maupas's account of the conjugation of the Ciliata; and this it was that first weakened my belief, not only in replacement theories, but also in the "preliminary excretion" theory on which the others were founded.

on to a consideration of the causes of the individual repro-
ductive incapacity of most gametes; of the senescence of the
individuals produced in the later generations of a long cycle of
fissiparous reproduction ; and conversely of the rejuvenescence
induced by karyogamy and other processes, which are classified
and discussed. A special section is devoted to those modes of
rejuvenescence which have been confounded under the name
of " parthenogenesis," but which we may term " paragenetic ;"
and a general summary of conclusions closes the present
study. To keep the exposition clear and continuous I have
abstained as far as possible from controversy.

I wish to express my thanks to Professors Mark, Guignard,
Strasbürger, and Weismann for their communication of
reprints of valuable papers not otherwise readily accessible
to me.

I have not deemed it necessary to give references for facts to
be found in every text-book, though I have verified as far as
possible every statement from the original authorities. In all
other cases I have cited my authority in a foot-note.

The following is a list of the text-books and papers of general
interest to which I may refer the reader.

BOOKS—
 'Essays upon Heredity and Kindred Problems,' Weismann (Engl.
  Trans.), 1889.
 ' Evolution of Sex,' Geddes and Thomson, 1890.
 ' Studien über Protoplasmamechanik,' Berthold, 1886.
 ' Handbuch der Botanik,' Schenk (in progress).
 ' Pflanzenfamilien,' Engler and Prantl (in progress).
 ' Fungi, Mycetozoa, and Bacteria,' De Bary (Engl. Trans.), 1887.
 ' Physiology of Plants,' Vines, 1886.
 ' Befruchtung und Zelltheilung,' Strasbürger.
 ' Neuere Untersuchungen,' Strasbürger, 1884.
 'Protozoa' (in Bronn's 'Thierreich'), Bütschli.
 ' Comparative Embryology,' Balfour, 1880.
 'Introduction to the Study of Embryology,' Haddon, 1887.
 ' Traité d'Embryologie' (Ed. Fr.), O. Hertwig, 1891.
 ' Forms of Animal Life,' Rolleston and Hatchett Jackson, 1888.

MEMOIRS—

"Sur la Multiplication des Infusoires ciliés," Maupas, in 'Arch. de Zool. Exp.,' 1888.

"Le Rajeunissement karyogamique chez les Ciliés,' Maupas, in 'Arch. de Zool. Exp.,' 1889.

"Karyokinesis and Fertilisation," Part II, Waldeyer (Engl. Trans.), in 'Quart. Journ. Micr. Sci.,' Dec., 1889.

"Vergleich der Ei- und Samenbildung bei Nematoden," O. Hertwig, in 'Arch. f. mikr. Anat.,' vol. xxxvi, 1890.

In my terminology I have used the word g a m e t e to designate a cell which fuses with another, cytoplast with cytoplast and nucleus with nucleus, and z y g o t e for the cell produced by their union; p r o n u c l e u s and g a m e t o n u c l e u s, indifferently, to denote the nucleus of a gamete, without any connotation of imperfection; g a m e t o g o n i u m and p r o g a m e t e to express, from slightly different points of view, a cell which divides to form gametes or (rarely) passes into the state of a gamete; s p e r m a t o g o n i u m[1] and o o g o n i u m, s p e r m a t o g a m e t e and o o g a m e t e for male or female gametogonia and gametes respectively: f a c u l t a t i v e and o b l i g a t o r y gametes are discriminated according as they retain the primitive cell privilege of multiplying by fission (independent of karyogamy) or have lost it altogether. I use the phrase g a m e t a n g i u m for the apocytial chamber in which gametes are developed. C y t o - p l a s t designates the cytoplasm considered as a unit in contrast to the nucleus.

By a p o c y t i u m I mean a mass of protoplasm which is habitually plurinucleate, cell division remaining in abey- ance; apocytial plants may be c o n t i n u o u s; or s e p t a t e by partitions, which always, however, separate multinucleate masses of protoplasm. Apocytial structures which unite like gametes are termed g a m e t o i d s, and the product of their union a z y g o t o i d. I have freely used Maupas's ad- mirable term k a r y o g a m y to comprise all forms of union of gametes involving fusion of their nuclei, since f e r t i l i s a t i o n and f e c u n d a t i o n are altogether inappropriate to cases of

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[1] Corresponding with the "spermatospore" of Blomfield, not necessarily the "spermatogonium" of La Valette St. George.

isogamy. By endogamy I imply the union of gametes from a single gametogonium, by exogamy their union with those from other oogonia exclusively. Portions of the nucleated or non-nucleated protoplasm, left out in schemes of gametal formation, are termed epiplasm. I have found it useful to introduce the following notation to denote relationship among nuclei, viz. to use a given letter for the parent nucleus, and the same letter with that figure as an index which denotes the number of bipartitions that have taken place, to denote a brood-cell issuing from those bipartitions; thus if N be a nucleus, N^5 will denote a brood nucleus of the 5th bipartition of N; the notation is abbreviated, N^a denoting $N \div 2^a$.

II. Typically Agamic Reproduction.

Before examining into gametogeny we must note the existence of a group of organisms which appear to be essentially agamous. I refer to that of Mycetozoa, including the Monadineæ of Cienkowsky, the Acrasieæ, and the Myxomycetes proper.

In the Monadineæ, a group relegated with exceptional liberality by the zoologists to the botanical "sphere of influence," we have a very primitive group, most species having the three forms of Mastigopod, Myxopod, and Cyst, beside a Resting Stage, which is never preceded by karyogamy. The adult forms may become plurinuclear or fuse into plasmodia like the Myxomycetes, but no nuclear union takes place; nay, even in the plasmodia further nuclear divisions may occur.

In the true Myxomycetes, plasmodial fusion always precedes spore formation. Possibly, as has often been suggested, plasmodial formation has led to the various modes of karyogamy. The nuclei pass freely from place to place in the plasmodium, and may eventually be far removed from what was their original cytoplast; and the cytoplastic elements again undergo a reorganisation by their fusion, which we may term plastogamy. In this way is fulfilled what I regard as the object

also of karyogamy—the association of nucleus and cytoplast that are strangers to each other.

We may fairly adopt the view that multiple isogamy, where the fusion of the nuclei follows that of the cytoplast, was originally derived from plasmodial formation, and that binary isogamy and the higher forms of karyogamy are further stages in the upward path.

In the ACRASIEÆ, the individuals produced by fission simply aggregate together without fusion before passing into a resting state, as a fructification in which their cellular individuality is retained. The only explanation I can suggest for this is that it indicates the loss of a primitive formation of plasmodia at this stage; the Acrasieæ would be an apoplasmodial or apoplastogamous group, a degenerate offshoot of the M y x o m y - c e t e s.

III. The Modes of Karyogamy as illustrated in Protophytes.

Undoubtedly the lowest forms of life that present us with cases of karyogamy are the FLAGELLATES; and in the PHYTOMAS-TIGOPODS or Green Flagellates we may study its modes from isogamy to complete sexual differentiation. From the colonial Phytomastigopods we can trace an almost unbroken line upwards past the Thallophytes, which in their asexual reproduction so often revert to the lowly Flagellate type; up through the Archegoniata to the Gymnosperms and true Flowering Plants at the top of the scale. We distinguish the following modes of karyogamy :—(1) ISOGAMY—(a) EUISOGAMY, (b) EXOISOGAMY ; (2) ANISOGAMY ; (3) HYPOOGAMY ; (4) OOGAMY.

(1) ISOGAMY.—This is the simplest mode of karyogamy. In this process cells exactly similar fuse as gametes, cytoplast to cytoplast, nucleus to nucleus; a single nucleated cell, the zygote, being the produce. In most cases only two gametes unite in binary isogamy; more rarely three or more may fuse in multiple isogamy. In many of the Phytomastigopods, and some of those simple filamentous or thalloid Algæ that

seem hardly more than colonies of Phytomastigopods in the resting state, such as Algæ Confervoideæ, and such apocytial forms as Cladophoreæ and many Siphoneæ, in these I say the gametes differ in no appreciable respect from the ordinary swarmers or zoospores, save that they are often smaller; they are usually formed by the segmentation or rapidly-repeated binary fission of the cell-body of the gametogonium. In some cases these isogametes, failing conjugation, may develop like ordinary zoospores; they are facultative[1] gametes; but in most cases they have lost this power of independent growth, and are obligatory gametes.

In some cases all the vegetative cells assume the character and function of gametogonia; in other cases we may distinguish clearly between " brood cells " (gametogonia or zoosporangia) on the one hand, and " colonial" or vegetative cells on the other.

Apart from other differentiations we may discriminate two grades of isogamy, which we shall term (a) EUISOGAMY, (b) EXOISOGAMY.

(a) In EUISOGAMY, each gamete has the power of uniting with the other, irrespective of its origin ; nay, in some cases, as in Pediastreæ, the gametes of a single gametogonium habitually conjugate with one another, thus forming endogamous unions.

(b) In EXOISOGAMY, a gamete will not conjugate with another of the same brood, but will only mate with one from a different gametogonium at least. This phenomenon would be difficult to demonstrate in the free Flagellates ; but it occurs in some of the lowest Confervoids, such as Ulothrix. In this genus the gametes, facultative though they be, are strictly exogamous. In the Volvocine Pandorina the gametes of different broods vary in size, and the small and middle-sized ones will pair indifferently with one another, quite independently of their size, but on the condition that the two gametes belong to distinct broods. About the largest gametes there is some doubt.

[1] I believe De Bary first introduced the terms ' facultative' and ' obligatory' in treating of parasitic fungi, &c.

Among the marine Siphoneæ, Dasycladus evinces a yet more subtle difference; for Berthold has shown[1] that the gametes of one brood may refuse to pair, not only with one another, but also with gametes of certain other plants, while they do so with others; and yet he failed to make out any distinguishing character in the plants themselves.[2]

(2) ANISOGAMY.—This is the second stage of karyogamy, where the gametes are similar in form, but of two sizes, large and small respectively; and in their union a micro- always pairs with a mega-gamete. This may be regarded as the lowest form of sexual differentiation, the smaller more active microgamete being the male, the larger more passive megagamete the female. Exogamy is a necessity here, for each brood is all of one kind, large or small, as the case may be. If the gametogonia do not show a corresponding difference of size (which they do sometimes), the differentiation of the gametes is effected by the smaller number of bipartitions in the segmentation which produces the megagametes. Thus in Chlamydomonas pulvisculus the megagametes are produced in twos or fours, the microgametes in eights.

We shall note below the transitional conditions between isogamy and anisogamy presented by the genera Ulothrix and Pandorina.

(3) HYPOOGAMY or HYPERANISOGAMY.—This is our third stage, recognised but not named by previous writers. The gametes are similar, but differ not merely in size but in behaviour; for the megagamete absolutely goes to rest before the microgamete comes to unite with it. This process occurs in slightly different modes in two of the lower groups of Olive Seaweeds, Cutleriaceæ and Ectocarpeæ. The Ectocarpeæ are remarkable for the fact that their microgametes, as well as the megagametes, are facultative, or capable of independent

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[1] 'Botanische Zeitung,' 1880, 648. Possibly the explanation is that the ultimate offspring belonging to the same cycle derived from a single zygote will not conjugate together any more than they will in certain Ciliate Infusorians; and that the individuals that showed this mutual sexual incapacity had this blood relationship.

growth failing conjugation, though the microgametes, indeed, are said to form but weakly plants. We know of no other case where a well-differentiated male cell retains this power of independent growth or parthenogenesis in the strictest sense.

(4) OOGAMY.—This term has been freely applied to cases of anisogamy and hypoogamy. It is, however, better restricted to those cases in which the megagamete is neither ciliate nor flagellate, but motionless, or at the outside slightly amœboid; while the microgamete or spermatozoon is most frequently a free-swimming cell, and consequently retains its primitive mastigopod form in the majority of cases, including the highest Metazoa. The megagamete is usually termed an ovum or egg in animals; but there are profound differences, morphological and physiological, between the immature metazoon egg as a progamete and the egg after the expulsion of the polar bodies as a true gamete; and I shall henceforth designate the egg in this latter stage an "oogamete" or "oosphere," reserving the words "egg" and "ovum" loosely for all stages.

In most cases of oogamy the microgamete is very minute, reduced to a "resting" nucleus, with just enough cytoplasm to cover it and carry it up to the oosphere. This reduction in size finds a curious parallel in the reduction of the male Rotifer, a bag with sexual organs, and just enough other organs to enable him to find and fertilise the female, the organs of nutrition being completely absent.

The lowest oogamous groups are certain Volvocineæ belonging to Phytomastigopods, and the Confervoid genera Œdogonium and Cylindrocapsa.

(5) SIPHONOGAMY.—Yet another mode of reproduction has to be noted, combined with any of the preceding, that where the gametes reach and unite, not by ordinary locomotion and as naked cells, but by a protoplasmic outgrowth of the gamete or gametogonium, protected by a cellulose tube. This is termed SIPHONOGAMY by Engler; it is a mere distinction in the mechanism of karyogamy, for it is associated with isogamy in some Conjugatæ, anisogamy in others of this group and

Chlamydomonas pulvisculus, with oogamy in the Peronosporeæ, and in Gymnosperms and Flowering Plants.

IV. Comparative Gametogeny in the Vegetable Kingdom.[1]

I propose now to examine, as completely as materials will allow, the types of the modes in which gametes are differentiated in the vegetable kingdom, from the Protophytes upwards. I have not followed any published classification; nor have I aimed at absolute completeness in examples, but only in types.

A. Protophytes and Cellular Algæ.

Many of the lower types have been treated incidentally in the foregoing section, and I need not revert to them. In Chætophoraceæ and Ulvaceæ the zoospores are frequently 4-flagellate, the isogametes 2-flagellate. This would seem to indicate that the segmentation which would otherwise have formed zoospores has been pushed a stage further in the formation of gametes, i. e. that the gametes are formed by the bipartition of the nascent zoospores. Multiple union is not rare in this group. In several members the conjugation of what are supposed to be gametes has not been observed, but their nature is a matter of inference from comparison with allied forms; possibly these doubtful gametes are exogamous, and could find no suitable mates in the specimens under observation.

To Ulothrix I have referred above (p. 9). The formation of zoospores and gametes is peculiar : the nuclear divisions are completed before the cytoplasm divides; the vacuole becomes excentric and peripheral, surrounded by a layer of cytoplasm which takes no share in the divisions of the rest of the proto-

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[1] I have not confined myself strictly to gametogeny, but have described the formation and fate of the zygote wherever it was necessary for the elucidation of the true character of the gametes.

plasm which form the zoospores or gametes; and the vesicle so formed persists and is usually expelled with them. But this formation does not always take place, and no share is taken by the nucleus in it. A similar vacuolar bladder is formed in certain Siphoneæ. The zoospores vary in number, owing to the number of nuclear bipartitions that produce them; the smallest (of several sizes, however) and most numerous conjugating exogamously as (facultative) isogametes. Dodel-Port relates[1] that he has seen copulation between small active swarmers and larger more sluggish ones, though they usually conjugate with those of the same size. So that we have here a combination of isogamy and anisogamy.

CYLINDROCAPSA is oogamous. The oosphere is formed simply by the enlargement of a single cell, and is facultative. The spermatogonia are formed by the rapid transverse fissions of the vegetative cells; and the cell-body of each divides to form two spermatozoa.

CHLAMYDOMONAS PULVISCULUS, referred to above as anisogamous, shows a transition to the siphonogamy of the group we shall next examine, for the gametes come to rest, surrounded each with a cell-wall, and the microgamete " creeps " into the cell-chamber of the megagamete to fuse with it therein.

The DESMIDS, CONJUGATES, and DIATOMS are forms permanently enclosed in their cell-walls, and destitute of cilia or flagella, never even forming zoospores. In these conjugation is altogether isogamous, or in certain Conjugates (Spirogyra) the male is only distinguishable by its slightly smaller size, and by passing, like that of Chlamydomonas pulvisculus, into the megagamete cell-chamber to form the zygote therein. In other cases siphonogamy also occurs, but the zygote is formed at the junction of the tubes emitted by the isogametes. In Diatoms the gametes leave their shells to conjugate as naked cells.

In the Desmid Closterium lunula and certain Diatoms

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[1] "Ulothrix zonata," in 'Pringsheim's Jahrbücher,' vol. x, 1876, p. 539.

(Epithemia, Amphora) two cells (so-called "frustules") approach as "progametes," and division takes place in each; and their offspring conjugate two and two, in the young state consequent on recent fission.

In the Conjugate Sirogonium the gametes are somewhat unequal, and are separated by septa from sterile cells; the male is formed from the vegetative cell by the marking off of two sterile cells, one on either side, the female by marking off of a single sterile cell.

Facultative gametes occur in all divisions of this group, or rather a vegetative cell, instead of assuming the character of a gamete, assumes that of the zygote, constituting the azygo-spore of De Bary.

In the genus Volvox a colony is formed by the segmentation of a single reproductive cell; at an early stage of this process certain cells undergo no further division, though they continue to increase in size. These large cells may behave as—
(1) Parthenogonidia, which after the maturity of the colony begin segmenting on their own account to reproduce a fresh colony; (2) "Oogonia," which on their maturity, without further division, assume the character of oospheres; "Spermatogonia" which at maturity undergo rapid segmentation and are resolved into the numerous spermatozoa, which are biflagellate like the colonial cells. Eudorina, another Volvocine, is anisogamous, the megagametes being flagellate. All the cells of the sexual colonies are fertile, either all becoming spermatogonia and segmenting into spermatozoa, or all assuming directly the function of oospheres.[1]

The Confervoid Oedogoniaceæ are also oogamous. Here, on the bipartition of a vegetative cell, the one daughter-cell enlarges to form the oosphere, the other is sterile. We may perhaps regard the latter as a gamete arrested in its development. The apical protoplasm of the oosphere grows upwards at one side pushing the cell-wall into a short beak, soon perforated, or merely forms a hole at this point. The cytoplasm con-

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[1] The case of Pandorina will be treated below, p. 72.

cerned in this process degenerates into mucus, while the rest rounds off, awaiting fertilisation. This process is obviously a purely adaptive one, destined merely to favour the approach and entrance of the spermatozoon.

Similar "excretions" occur elsewhere for similar purposes; thus in Peronosporeæ, Saprolegnieæ, and Chytridieæ the asexual zoosporange forms a beak for the discharge of the zoospore; in Chytridieæ the protoplasm which fills this beak undergoes degeneration; while in Saprolegnieæ it is retracted and absorbed into the body of protoplasm not yet fully differentiated into zoospores. In the Chytridian Woronina polycystis, which I have studied myself, the formative protoplasm of the beak contains a nucleus, and appears to be a zoospore degraded for the purpose of opening a gate to its sisters.[1] Hence neither physiologically nor morphologically can excessive stress be laid on such a degradation, whether of cytoplasm or nucleated protoplasm.

The spermatogonia of Oedogonieæ are formed by repeated bipartitions of the vegetative cells, and are short and discoid. In each spermatogonium the protoplasm rounds off and divides to form two naked spermatozoa; or this division is suppressed, and the protoplasmic body of the spermatogonium escapes as a single spermatozoon. The spermatozoa have, like the asexual zoospores, a subapical ring of cilia, but are smaller. In certain species the discoid cells produce not spermatozoa, but so-called "androspores," of similar character but of different fate. For the androspores settle on the base of the oogonial cell-wall and develop into "dwarf male" plants; all the cells of these (save sometimes a sterile basal cell) are spermatogonia, and behave like the same organs in the other species. This is the first case in which we find sex anticipated by or reflected back upon individuals or organs antecedent to the gametogonia.[2]

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[1] As stated by me in a communication to Section D of the British Association at Leeds, 1890, Report, p. 872.

[2] The colonies of Eudorina and some forms of Volvox only produce gametes of one sex, but have no other peculiarity to distinguish them.

COLEOCHÆTEÆ are also oogamous; the oosophere essentially resembles that of Oedogonieæ, but it is the direct transformation of a superficial tissue-cell: its fertilising beak is prolonged into a slender tube open at the apex, the "Trichogyne;" the formative cytoplasm contained therein degenerates as in Oedogonieæ, while the rest of the protoplasm in the body of the oogonium rounds off into the oosphere.

The spermatogonium undergoes two binary divisions, and the cell-body of each of the cells so formed escapes as a biflagellate spermatozoon. In certain species the four daughter-cells of the spermatogonium are budded off at its apex, and though no information has been given we may well believe the process to be that following as found in the spore formation of Basidiomycetes. The nucleus of the basidium divides by two mitoses, and each of the four nuclei so formed migrates, accompanied by cytoplasm, into a bud formed at the apex, which becomes shut off by a septum from the now sterile and enucleate basidium.[1] That we are fairly justified in interpreting budding here as a modification of segmentation is obvious when we reflect on the behaviour of meroblastic ova or the zygote of Noctiluca (infra, p. 41) in their segmentation.

In the MELANOPHYCEÆ, or Olive Seaweeds, we have seen the transition from isogamy through anisogamy to hypoogamy. True oogamy occurs in the highest order of this group, the FUCACEÆ.[2] In these the oogonial nucleus divides by successive mitoses into eight gametonuclei. In Fucus each of these attracts round it one eighth of the cytoplasm, so that there are eight oospheres. In Ascophyllum four of the nuclei pass to the centre, and four lie at equal distances nearer the surface of the cytoplasm. The cytoplasm aggregates around the four peripheral nuclei to form four oospheres, leaving the four central nuclei as rejection-bodies in the

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[1] Possibly, however, the formation may take place as in the Floridian genus Chondria (infra, p. 30).

[2] The following account is taken from Oltmanns' "Beiträge zur Vergleich. Entwicklungsgesch. der Fucaceæ," in 'Sitzungsber. d. Berlin Akad.,' 1889, p. 587.

centre. In Pelvetia six of the nuclei pass towards the equator of the oogonium, and two lie towards the ends of its axis. The cytoplasm separates into two oospheres, each containing one of the axial nuclei, while the six equatorial nuclei are left out as rejection-nuclei. Finally, in Halidrys and Himanthalia, and also Cystoseira, seven of the nuclei pass to the periphery as rejection-nuclei, while the eighth remains in the centre of the cytoplasm of the oogonium which is thus directly resolved into the single oosphere. Oltmanns, who gave the first correct account of these processes, rightly remarked on their close identity with the formation of polar bodies in the Metazoa. It is obvious that the formation in Fucus is primitive, and that the advantages due to the increased size of the oospheres in the other genera are obtained by the abortion of half, three quarters, or seven eighths of them, and that this physiological advantage is a relative gain and not an absolute necessity.

B. Apocytial Forms.

1. Green or Algal Types.

CLADOPHORA has septate filaments with many nuclei in each joint, and forms zoospores, ordinary or isogametal, by the resolution of these apocytia into uninucleated swarmers. In both cases a portion of the cytoplasm is eliminated at the boundaries of these cells, and takes no further part in the living processes. From Berthold's description[1] it appears certain that he is correct in regarding this excretion of "epiplasm" as derived from a primitive formation of cell-wall, a process which has been lost in the evolution of the group. We may note that this confirms the propriety of our using Vuillemin's term "apocytial" instead of Sachs' "non-cellular."

The gametes of Cladophora are biflagellate, the ordinary zoospores quadriflagellate, a distinction which we interpret as

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[1] Op. cit., p. 302. He expressly notes (p. 305), in opposition to Dodel-Port, that such formations, occurring also in asexual spore-formation, can have no relation to fertilisation-processes as such.

indicating the formation of the gametes by a binary fission of potential zoospores.

The SIPHONEÆ form continuous apocytia, parts of which may become partitioned off as gametangia.

ACETABULARIA and BOTRYDIUM are exo-isogamous; their gametes are formed in gametogonia, which are formed by the resolution of an apocytium into cells, and have the character of resting spores. The gametogonium is at first uninucleate, at least in Botrydium, judging from the original figure,[1] and must become plurinucleate before being resolved into the gametes. In that of Acetabularia the protoplasm surrounds a central vacuole, and the cytoplasm immediately round this persists without taking any part in the gametoparous segmentation, but serves by its turgescence to liberate the gametes. These are exogamous, and in Acetabularia may form multiple unions—two to six.[2]

BRYOPSIS is anisogamous. Here gametangia are cut off, males and females being on distinct plants. According to Berthold, repeated bipartitions of the nuclei precede the resolution into gametes. There is a formation of epiplasm between the gametes, as in Cladophora, besides a central bladder, as in Acetabularia. The reproductive organs of Codium appear to be similar in most respects.

DASYCLADUS forms its gametes also by the resolution of an apocytial gametangium into cells ; but here a fusion of several vegetative nuclei constitutes each single gametonucleus, a process of which we shall find other instances in this group. We now learn that the view that a gametonucleus or pronucleus differs from an ordinary one in being reduced either by preliminary mitosis or "excretion" expresses no universal law. We have adverted above to the peculiarly strong exogamy of this genus.

In DERBESIA it is stated[3] that no sexual process is known;

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[1] Of Rostafinsky, in 'Bot. Zeit.,' 1877.

[2] If the gametogonia be kept long in a resting state, the bodies they produce behave like ordinary zoospores, and will not conjugate.

[3] Welle, in ' Engler and Prantl,' op. cit., i, § 2, p. 129.

but the zoospores, formed by the resolution of the protoplasm into uninucleated cells, have nuclei constituted by the fusion of several vegetative nuclei.   We may, however, regard this multiple fusion of nuclei as a karyogamic process, and the zoospores as zygotes issuing from multiple endogamy. This process is a further development of the gametogeny of Dasy-cladus; and we shall find that the Saprolegnieæ and Pero-nosporeæ have similar relations with one another.

SPHÆROPLEA,[1] like Cladophora, has chambered filaments, and each chamber is subdivided by protoplasmic septa, in which alone the nuclei lie, from one to four in each.   The plant is oogamous.   The spermatozoa are formed in distinct chambers to the oospheres; to form the former rapid nuclear fission takes place in each protoplasmic septum, which is finally resolved into the numerous uninucleate spermatozoa, the parietal protoplasm being also used up in the process.

The oospheres are also formed by the rounding off of the protoplasm into numerous uninucleated spheres without epi-plasm; perforations are formed in the wall of the tube to admit the spermatozoa, but how or at what stage is not stated.   The oosphere-nucleus is formed by the fusion of several nuclei. Rauwenhoff writes,[2] " The number of nuclei seems to diminish; each aggregation of protoplasm possessing three or four chromatophores, ... while I only found one or two nuclei. When there were two, they were closely appressed; when a single one, it was large and elongated.   In either case the nucleoli had disappeared; the chromatic elements were visible as dots or rods, aggregated in an irregular figure.   To all appearances, then, several nuclei fuse into one."   This account might almost fit word for word the phenomena seen in the formation of the oospheres of Saprolegnia.

[1] "Zur Kenntniss der Algengattung Sphæroplea," in ' Berichte d. Deutsch. Bot. Gesellsch.,' vol. i.

[2] "Rech. sur le Sphæroplea annulina," in 'Arch. Néerland.,' vol. xxii, 1888.   This later paper I only saw during the correction of the proofs.   I had, in the MSS., anticipated the probability of nuclear fusion in the oospheres, despite Heinricher's statement to the contrary.

VAUCHERIA is continuous and oogamous. The spermatangium is the distal end cut off from a short lateral tube. From the number of spermatozoa formed here, as compared with the vegetative nuclei of the ordinary protoplasm, it is certain that nuclear fission must precede spermatogeny; according to Berthold[1] there is here a formation of epiplasm comparable with that of the sporangia and gametangia of Cladophora.

The " oogonium " is formed primitively as a lateral outgrowth; it contains at first numerous nuclei, the number of which is finally by fusion reduced to one.[2] A beak for the passage of the spermatozoa is formed as in Oedogonium, and the formative plasma undergoes mucous degeneration. Schmitz calls this mucified plasma of the beak a Richtungskörper (polar body), and says it contains " numerous small nuclear fragments abstricted from the numerous nuclei of the young oogonium."[3] If this meagre statement be accurate (and it is all we have), it would seem that nuclear divisions to form gametonuclei take place, and half the offspring pass into the epiplasm, and the other half fuse to form the pronucleus of the single oosphere—a process comparable with that of Peronospora (infra, p. 22).

2. Colourless or Fungal Types.
(a) Phycomycetes Zoosporeæ.

It is convenient to consider separately the Phycomycetes with flagellate spores, comprising Chytridieæ, Ancylisteæ, Monoblepharis, Peronosporeæ, and Saprolegnieæ, as Zoosporeæ, in opposition to the higher group Aplanosporeæ, which never form organs of locomotion, and which comprises only Entomophthoreæ and Mucorini.

ANCYLISTEÆ[4] are in appearance oogamous, the entire con-

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[1] Op. cit., p. 305.

[2] According to Schmitz, " Die Zellkerne der Thallophyten," in ʻSitzungsb. d. Niederrh. Ges. zu Bonn,' 1879.

[3] This is contained as a mere by-statement in a foot-note (on p. 225) to his paper, " Untersuchungen über die Befruchtung der Florideen," in ʻSitzungsb. d. Berl. Akad.,' 1883. A full account is much to be desired.

[4] My account of Ancylisteæ and Olpidiopsis is taken from Zopf,

tents of the female plant condensing into one or more
"oogonia" (?), that of the male into "antheridia," which
open one into each "oogonium;" the whole of the male proto-
plasm passes over into the oogonium by siphonogamy, and
the two plasmas fuse to form the zygote. Though we have
no information as to the cytology of this group, it would seem
probable that either gamete has a nucleus formed by the fusion
of several vegetative nuclei, and that the zygote is also uni-
nucleate. No epiplasm is formed.

CHYTRIDIEÆ present conjugation in various modes; in many
cases the "gamete" contains all the protoplasm of the plant,
which we know to be apocytial in the vegetative state; but we
have no evidence as to the nuclei of the so-called gametes and
zygote. Conjugation has been observed in four genera:
Polyphagus by Nowakowski,[1] Olpidiopsis by Zopf,
Zygochytrium and Tetrachytrium by Sorokin.[2]

In the two former genera all the protoplasm goes into the
gametoid. In Polyphagus the gametoids are formed by two
distinct plants somewhat different, and the union is siphono-
gamous. In Olpidiopsis the gametoids are differentiated by
the separation of a single apocytium into a larger part, the
"oogonium," and a smaller part, the "antheridium;" the latter
is at first completely shut off by a cell-wall which becomes
perforated, admitting the whole of the protoplasm of the an-
theridium to enter and fuse with that of the oogonium, as in
Ancylisteæ. In both genera the zygote is a "resting spore."

ZYGOCHYTRIUM forms gametoids on outgrowths of its myce-
lium which conjugate in (monœcious) pairs, like those of
Mucor.

TETRACHYTRIUM forms, in terminal enlargements of the
mycelium, numerous one-ciliated zoospores which are euisoga-
mous, conjugating in pairs as soon as they leave the sporangia.

"Zur Kenntniss der Phycomyceten," in 'Nov. Act. Ac. Leop. Carol.,'
vol. xlvii, 1885.
    [1] In Cohn's 'Beiträge,' vol. iii.
    [2] 'Bot. Zeit.,' 1874, p. 308.

The last two genera have a mycelium unusually well developed for this group, and are regarded by De Bary as doubtful members thereof.

MONOBLEPHARIS[1] forms its spermatozoa by the resolution of the protoplasm of spermatangia into one-flagellate cells, differing only in their smaller size from the vegetative zoospores, and therefore possibly formed by bipartition of potential zoospores. The protoplasm of the oangium, after forming a terminal aperture, contracts and rounds off into the oosphere; which, if uninucleate, has probably, from its size, formed its gametonucleus by the fusion of many vegetative nuclei. No epiplasm is apparently formed.

The PERONOSPOREÆ are oogamous and siphonogamous. The only species in which the formation and union of the gametes has been fully studied is Peronospora parasitica; and I shall utilise Wager's careful description[2] of the sexual processes in this species, having had the opportunity of verifying its accuracy on the original specimens. The spherical " oogonium" (or rather oangium) is cut off from the tubular hypha by a basal septum if terminal, by two if intercalar; it is apocytial, containing numerous nuclei. Each nucleus undergoes repeated bipartition; and the majority of the nuclei so formed pass into a peripheral layer of protoplasm, thus constituting the so-called " periplasm;" three of them pass to the middle of the central mass of protoplasm, the so-called " gonoplasm," and fuse therein into the single pronucleus. The " antheridium " (or spermatangium) is an ovoid enlargement of a hypha, closely applied by its apex to the oogonial wall and cut off by a basal septum; it contains several nuclei, which like those of the "oangium" multiply by mitosis; it emits a tube which perforates the oogonial wall and passes through the periplasm to open just at the surface of the gono-

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[1] Cornu, "Monographie des Saprolegniées," in 'Ann. Sci. Nat. Bot.,' ser. 5, vol. xv.

[2] "On the Structure of the Nuclei in Peronospora parasitica," in 'Annals of Botany,' vol. iv (1889).

plasm; a small portion of granular protoplasm carries down a single male nucleus to fuse with the female one. The gonoplasm secretes an inner coat to the zygote, around which an outer one or "epispore" is secreted by the periplasm. The nuclei present in this layer can hardly be essential to the formation of the epispore, since no nuclei are present in the layer of epiplasm which does similar service to the endogenous spores of the Ascomycetes. We can only regard the nuclear divisions in "oogonium" and antheridium as phylogenetic reminiscences of the formation of gametes by cell division; the periplasm is thus equivalent to a number of degenerated gametes which have taken on the function of epispore formation; the multitude of gametes are sacrificed to the few. Obviously what we here term the "oogonium" is neither morphologically nor physiologically the exact equivalent of a single oogonium in cellular, as distinguished from apocytial plants, but represents an apocytium of oogonial cells; and the antheridium has a similar relation to the spermatogonium of cellular plants.

The processes in those SAPROLEGNIEÆ[1] that have been fully studied mark a distinct step further in the same path. The oangia are at first filled with multinucleated protoplasm; vacuoles appearing and enlarging bring the nuclei closer together, and they soon fuse in pairs, a process continued until their number is materially reduced; while the mitoses observed in Peronospora do not take place. The primitive nuclei are vesicles with a central chromatin mass supported by a "linin" or nucleo-hyaloplasma network. In fusion of the nuclei the chromatin masses long remain distinct, but are smaller and take up stain less readily, and the nuclear wall at this stage ceases to stain, so that the fusion nuclei have the look of vacuoles in the cytoplasm containing a variable number of chromatic granules. During this stage the true vacuoles unite to form a large central cavity into which fresh vacuoles open, so that the protoplasm forms a thick

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[1] The following account is largely taken from an original paper of my own "On the Cytology of Saprolegnieæ," still incomplete and unpublished.

mantle around the central space. The protoplasm then becomes aggregated into distinct masses, the oospore " origins," projecting into the vacuole and united by a continuous peripheral mantle, which thins gradually as its substance becomes taken up into these masses. Finally, the connecting mantle gives way ; the masses separate and round off; after a short rest they become amœboid, and some of their blunt, non-nucleated processes become abstricted. Very soon, however, they are taken up again by the masses which abstricted them, and these masses round off into the " oospores." The very same abstriction and resumption of non-nucleated processes of cytoplasm takes place in the formation of the asexual zoospores ;[1] it is probably a process derived from cell-wall formation, analogous to what we have seen in Cladophora, but in a yet more reduced condition. The oospores after coming to rest soon become surrounded by a cellulose wall, thickened by successive internal deposits ; each finally possesses a single nucleus in the resting state, i. e. spherical, with a single central sphere of chromatin. The complete fusion of the nuclei takes place in Saprolegnia as early as the first formation of the masses or oospore origins, while in Achlya it may be deferred till after the formation of the spore membrane, for young oospores are frequently binucleate.

The antheridium is also multinucleate, and lies closely appressed to the oogonium ; on the rupture of the protoplastic mantle of the oogonium and separation of the oospores it emits tubes which grow into the cavity of the oogonium, and abut against the oospores just before they form a cell-wall or during this process ; but they do not enter the oospore, open, or emit any fertilising bodies.[2] Their contents are

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[1] See Rothert, "Entwicklung d. Sporangien bei den Saprolegnieen," 1888 (this paper was an advance publication of 'Cohn's Beiträge,' vol. v, 1890 ; it was abstracted and criticised by me under the title " Recent Researches on the Saprolegnieæ," in ' Annals of Bot.,' vol. ii, 1888.

[2] I have now fully satisfied myself that the contrary statements of Pringsheim (in ' Sitzungsber. d. Berl. Akad.,' 1882) are erroneous, being based partly on the intrusion of parasites as shown by Zopf, partly on the postmortem appearances produced by unsuitable reagents.

granular protoplasm, with small nuclei formed by the division of those of the antheridium. Antheridia may be present or absent in one and the same species without making the slightest difference to the formation of the oospores.

The homology of the antheridium with that of Perono-spora is obvious and complete. The oangia behave very differently; the mitotic nuclear divisions are suppressed; there is no differentiation of gonoplasm and periplasm, but the number of the nuclei is reduced by successive fusions, and the whole protoplasm becomes finally resolved into uni-nucleated spores. The usual statement made is that this group is "parthenogenetic;" but the process is different from true parthenogenesis, which means the independent evolution of a single gamete. Here the formation of gametes remains completely in abeyance; instead of nuclear divisions we have nuclear fusions, "Karyosymphysis" if not Karyogamy re-placing Karyokinesis. What I believe to be the true inter-pretation of the facts is this:—We have a case of multiple endogamous union of potential gametes; the pre-liminary nuclear divisions occurring elsewhere have been sup-pressed as useless in anticipation of the subsequent fusions; the process of fusion of three nuclei to form the female pronucleus in Peronospora_has advanced here to the fusion of so many, that the part to be played by the male nucleus has been cut out as unnecessary.

If we remember how reckless Nature is of wasting sperm-atozoa, and that in the related Peronosporeæ all the anther-idial nuclei save one are wasted, we shall see that the utilisation in Saprolegnieæ of all the oangial nuclei is positively an economy, even though the males (antheridia) continue to be formed and die, going to absolute waste without fulfilling any functions whatever; in some species, however, few or no antheridia are formed. The oospores are then endogamous zygotes, not parthenogametes; their rejuven-escent nucleus is the product of the fusion of many closely-related nuclei, not of two of different origin as in ordinary cases of binary isogamy. The relations of Peronosporeæ and

Saprolegnieæ find a parallel in those of Dasycladus and Derbesia (supra, p. 18).

Marshall Ward sought[1] to identify the extrusion of protoplasm by the forming oospores with the formation of polar bodies in the Metazoa; and the resumption of these masses he regarded as probably connected with the alleged parthenogenesis of the group. The latter view, and indeed the former also, break down now that we know that both processes occur in the formation of the asexual zoospores; they find their explanation in the comparison with the zoospore formation of Cladophora.

(b) Phycomycetes Aplanosporeæ.

The Entomophthoreæ present conjugation between their hyphæ; as a rule the conjugation takes place in the horizontal tube uniting two hyphæ, like the cross-bar of an **H**, and the zygote forms a spherical enlargement in the middle.

Unfortunately we know nothing of the cytology of the process in the majority of the species; it has only been studied in Basidiobolus,[2] which is by exception truly cellular (while the other genera are apocytial), and which is peculiar in its conjugation.

In this genus two adjacent cells of a hypha grow out sideways at their adjacent ends, which turn up side by side, and the nucleus of each enters its turned-up tip. Either nucleus divides by mitosis; the upper daughter-nucleus passes into the extreme tip, and is cut off as a sterile cell; the lower nucleus, with all the protoplasm of the cell, fuses with its fellow to form the zygote. Here we have the best parallel with polar-body formation to be found in isogamous conjugation. If we compare this with the conjugation of Closterium or Epithemia (supra, p. 14) we find the interpretation obvious: the first cells are progametes, and on approaching produce the

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[1] "Observations on Saprolegnieæ," in 'Quart. Journ. Micr. Sci.,' vol. xxiii, pp. 282 (note) and 291.

[2] By Eidam, in 'Cohn's Beiträge,' vol. iii.

gametes by binary fission.   Owing to the subterminal position of the nucleus in the cell at the time of mitosis one daughter-nucleus has too little cytoplasm around it for further evolu-tion, and so forms an arrested gamete, while its sister gets the lion's share, and enters into karyogamic union with the
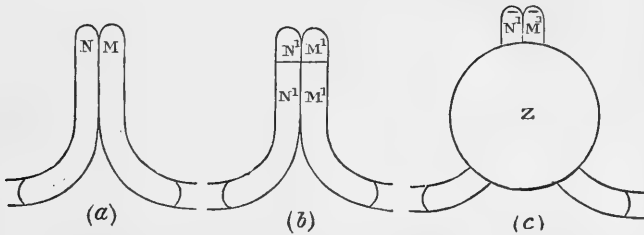
FIG. 1.—Conjugation of Basidiobolus: a, early stage; b, division of each cell to form active and arrested gametes; c, formation of zygote. I use here the nuclear notation explained above.

corresponding gamete of the other pair.   So we describe the process thus : the adjacent cells are progametes, each of which by unequal divisions forms two gametes, the apical one arrested, the other functional.

The MUCORINI, in the common phrase, form zygotes by the isogamous union of gametes, save in one species, Mucor heterogamus, Vuillemin, which is anisogamous.   But this description is not at all accurate from the present standpoint of cytology ; for "gametes" and "zygotes," so called, are not cells, but apocytia of plurinucleate protoplasm in a cell-wall, which we may term "gametoid" and "zygotoid" re-spectively.   Of the nuclear changes involved in the conjuga-tion we know as little as in the Chytridieæ.   Several possibilities are open :

(1) The nuclei of the respective gametoids may unite two and two within the zygote in exogamous union ;

(2) The nuclei may unite in pairs or otherwise, irrespective of their origin ; or, finally,

(3) Rejuvenescence may ensue, as in plasmodial formation,

by the nuclei wandering into foreign protoplasm, and by the plastogamy of the cytoplasm.

"Azygospores" or "pseudo-zygospores" may be formed by such gametoids as fail to conjugate. But we must be content to leave the true nature of this paragenetic process undetermined so long as we are in ignorance of the nuclear processes involved.

(c) Higher Fungi.

The hyphæ of the higher Fungi are transversely septate, with usually multinuclear chambers; by lateral tubular outgrowths above and below the septa, which fuse and become pervious to form loops, or so-called "clamp-connections," protoplasm with or without nuclei can pass from one chamber to another; but to what extent migration does take place we do not know.

The lower Ascomycetes show apparently a siphonogamous union, the larger "ascogonial" cell (or gametoid?) ultimately growing out into the sporiferous asci; but we really know nothing of the cytology of the process.

In certain closely-allied Lichens an oogonium similar to that of other Ascomycetes is formed, which, however, emits a trichogyne like that of Florideæ, but repeatedly septate. Spermatia (probably uninucleate) are also formed here by abstriction from special hyphæ, and conjugate with the trichogyne, after which the ascogonium buds off asci. Here, again, we know nothing of the nuclear relations.

The majority of Ascomycetes and all the Basidiomycetes are completely apogamous, so far as is known.

In Ustilagineæ from the spores is formed a rudimentary mycelium; this divides into four gametal cells, which conjugate by loop or clamp connections; or else usually four elongated uninucleate sporidia are formed which conjugate in pairs, forming H-shaped unions with their fellows or neighbours. Fisch states that there is no nuclear fusion;[1] but there is certainly plastogamy. After conjugation one (or both?) of

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[1] According to Vines, 'Veg. Phys.,' p. 618.

the zygotes emits a tube which may abstrict spores or grow into a vegetative mycelium. This germination of the gametes may in some cases take place facultatively independently of conjugation.[1]

PROTOMYCES resembles Ustilagineæ in the behaviour of its gametes; but they are formed endogenously in large numbers in the spore, leaving a quantity of non-nucleated epiplasm.

In UREDINEÆ Massee has described a process of siphono-gamous[2] conjugation between a larger "oogonium" and a smaller antheridium comparable to that of the lower Asco-mycetes. The only cytological observations of importance given are that the oogonium is uninucleate before fertilisa-tion, and contains several small nuclei on the third day.

C. HIGHER THALLOPHYTES.

1. Florideæ.

The Red Seaweeds stand apart from the other Thallophytes in many respects, and we follow Falkenberg[3] in regarding them as distinct from the true Algæ. Their male reproduc-tive cells or "spermatia" are all but motionless, and scat-tered by local currents. Their female cells, "carpogonia" or "procarpia," are usually permanently fixed in the thallus.

They emit a trichogyne which does not open, and is ab-stricted after receiving the male pronucleus by conjugation with the spermatium, and transmitting it to the female pro-nucleus in the base of the "carpogonium."

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[1] It is noteworthy that the formation of gametes here takes place at a stage in no way homologous with the sexual organs of the (more primitive) Asco-mycetes. It would seem, indeed, probable that this gametal process has originated de novo as a specialisation of and advance upon the free anasto-moses formed between contiguous young hyphæ in so many of the higher Fungi. There is, indeed, no reason why such processes should not originate afresh at a different stage in forms that have become apogamous by the complete loss of a sexual process at the usual stage.

[2] "On the Presence of Sexual Organs in Æcidium," in 'Ann. of Bot.,' vol. ii, 1888.

[3] In his monograph of the Algæ in Schenk's 'Handbuch der Botanik.'

The BANGIACEÆ differ, however, little in the essentials of gametogeny from those protophytes which they resemble so closely in vegetative growth. In Bangia the spermatogonium undergoes repeated bipartitions accompanied by cell-wall formation; finally, the cell bodies undergo two further bipartitions, and become free as four naked spermatia. The oogonia produce oospheres by bipartition, which are cells with a narrow anterior receptive apex and a dilated base containing the nucleus. A spermatium sticks to the narrow end (trichogyne), and conjugates with it, and the spermato-nucleus passes down, we may suppose, to fuse with the oonucleus. The basal protoplasm then contracts to form the zygote, while the upper part takes no further share in its life. The trichogyne is here obviously comparable to the beak in Vaucheria or Œdogonium. PORPHYRA differs in minor points only from BANGIA.

In the true FLORIDEÆ the spermatia[1] are formed, not by ordinary fission, but by budding and abstriction, and are uninucleate. In every case they are borne on a persistent basal cell, whose nucleus divides, one daughter-nucleus passing into the budded cell, the other remaining in situ. We may distinguish two modes, which can again be subdivided. 1. The budded cell undergoes (one or) two mitotic divisions to form four spermatia, all of the same generation, and grandnieces of the basal cell: (a) the divisions are crucial and the spermatia are collateral, resting on the basal cell (Polyides); (b) the divisions are horizontal, and the spermatia form a vertical file (Hypnea). 2. The basal cell repeats the former process of budding, so that the spermatia are of successively lower generations, and the youngest is sister to the ultimate basal cell: (a) the buds are collateral and all spring directly from the basal cell (Chondria); (b) the buds are intercalated in turn between the basal cell and the next older, so as to form a basipetal file like the conidia of many Fungi (Melobesia).

[1] The following account is taken from Guignard, "Développement et Constitution des Anthérozoides," in 'Rev. Gén. de Botanique,' vol. i, 1889.

The oosphere or "procarp" has a long trichogyne closed at the apex, and a basal enlargement containing the single nucleus. The spermatium adheres to the trichogyne and opens into it ;[1] its contents with the male pronucleus pass down ; and since at this time two nuclei are seen in the base of the procarp, and only one shortly after, it is certain that the nuclei must fuse in the zygote. The trichogyne is then shut off, its lumen being encroached upon to obliteration by a centripetal growth of its cell-wall close to the base. Before and after fertilisation granules which react to stains like nuclear chromatin are present in the plasma of the trichogyne; and Schmitz identifies these with excreted nuclear fragments on purely à priori grounds, relying on the current theory of fertilisation. But he adduces no real evidence as to their origin and nature.

A peculiar process occurring in many Florideæ is secondary fertilisation, the zygote forming a new karyogamic union as a gamete with an " auxiliary cell" or secondary oosphere ; or the zygote grows and branches with cell division, and its offspring play the part of secondary males to the secondary oospheres (Dudresnaya). In this way sometimes one spermatium indirectly fertilises several secondary oospheres —an economical process as yet unparalleled in nature ; its explanation is probably to be sought in the motionless character of the male cells, and the inadequate adaptations for what we may almost term " pollination."

In CORALLINA the procarps with their auxiliary cells are collected into a disc ; the central cells alone possess trichogynes, and are fertilised by the spermatia ; but as zygotes they have no power of development save to fertilise in turn the peripheral procarps, which have no receptive organs of their own.

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[1] The following cytological details are taken from the account by Schmitz in ' Sitzungsber. d. Berl. Akad.,' 1883, 215.

2. Characeæ.

In CHARACEÆ the spermatozoa are biflagellate; they are formed by the direct evolution of the cell-bodies of short discoid segments of long confervoid filaments; these grow by intercalar divisions, which, perhaps, may be taken to correspond with the ordinary gametogenic fissions of a spermatogonium. The oosphere is formed by the enlargement of the terminal member of a short file of cells. The cutting off of one or three sterile basal cells ("Wendungszellen") by oblique septa suggests the formation of one functional and one or three arrested gametes by one or two divisions of a gametogonium.

D. ARCHEGONIATA.

This group is distinguished by the formation of the archegonium, a cellular flask-shaped structure, with a single oosphere occupying its belly, and a series of canal-cells its neck; the latter degenerate to attract and bring in the spermatozoon.

1. Muscineæ.

In the MUSCINEÆ one single "initial cell" by its divisions forms the archegonium. The earliest divisions separate off the tissue-cells destined to form the wall and stalk of the archegonium from the "inner cell," which gives rise to the oosphere and canal-cells. This inner cell divides according to the accompanying schema (Fig. 2); at its bipartition are

$$
N \begin{cases} N^1 \begin{cases} N^2 \begin{cases} N^3 \\ N^3 \end{cases} \\ N^2 \begin{cases} N^3 \\ N^3 \end{cases} \end{cases} \text{ \&c., neck canal-cells.} \\ \\ N^1 \begin{cases} N^2 \ldots \ldots \text{ belly canal-cell.} \\ N^2 \ldots \ldots \text{ oosphere.} \end{cases} \end{cases}
$$
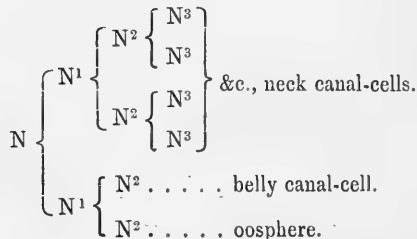
FIG. 2.—Schema of divisions of inner cell of archegonium in Muscineæ.

formed two cells, a basal "central cell" and an apical "neck

canal-cell." The latter elongates with the neck of the growing archegonium which it occupies and fills, and by repeated bipartitions forms a file of cells (a multiple of four) which are also termed " neck canal-cells." The proximal cell (central cell) occupies the belly of the archegonium, enlarging with it, but undergoing no further division till its maturity. Then this central cell divides into two, the lower rounding off as the oosphere, the upper undergoing mucous degeneration like the neck canal-cells, and termed the " belly canal-cell." Sometimes these two sister-cells are equal, but usually the belly-cell is much smaller. Obviously both are gametes, the former functional, the latter degenerate.

The next question that arises is this:—What are we to regard as the "oogonium"—the central cell, the inner cell, or the initial cell of the archegonium? The divisions of the last are too closely allied to those which form tissue-cells elsewhere; and of this nature are the majority of its brood-cells forming the wall of the oogonium, so that it would be rather strained to call the initial cell a gametogonium. The second alternative would seem most natural : to regard the inner cell as a gametogonium, and the neck canal-cells as degraded gametes (or rather their offspring). For though the growth of these is concurrent with that of the cells of the neck and accompanied by numerous divisions, yet the horizontal septa are not coplanar with those of the neck wall, and do not complete with these the schema of orthogonal trajectories, which they should do if they belonged to the same system of tissue-cells ; their number is always a multiple of four; and while the cells forming the oogonial wall have a prolonged life, that of the canal-cells is limited, as in most gametes. Again, the antheridium is formed from a single initial cell similar to that of the archegonium, and similar divisions mark off its wall-cells from a single central cell. The latter forms a complex of cubical spermatocytes only, by repeated bipartition, the cell-walls intersecting at right angles ; and the cell body of each spermatocyte is converted into a biflagellate sperma-tozoon, a small portion of the cytoplast remaining unutilised

in the great transformation it undergoes. There can be no
doubt that the "inner cell" of the male antheridium is a true
gametogonium, since all its brood-cells become functional
gametes; and as the "inner cell" of the archegonium is the
homologue of that of the antheridium, we have here an addi-
tional reason for regarding the former as a gametogonium,
and the neck canal-cells as the outcome of degraded gametes;
for we must remember that these are of a lower generation than
the belly canal-cell and oosphere.

2. Vascular Cryptogams.

In VASCULAR CRYPTOGAMS generally the antheridium re-
sembles that of M u s c i n e æ in essentials; but the archegonium
is somewhat different, for the initial cell only forms the neck-
wall and inner cell, the belly-wall being formed by a sort of
epithelial wall segmented off the adjacent cells of the prothallus.[1]
Moreover, the divisions of the inner cell are often symme-

$$N \text{ (inner cell)} \begin{cases} N^1 \text{ (neck-canal cell)} \begin{cases} N^2 \\ N^2 \end{cases} \text{neck-canal cells.} \\ N^1 \text{ (central cell)} . . \begin{cases} N^2 \text{ (belly-canal cell).} \\ N^2 \text{ (oosphere).} \end{cases} \end{cases}$$
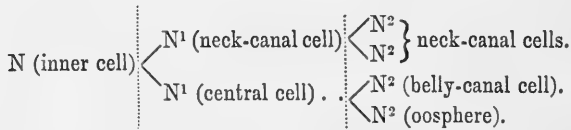
FIG. 3.—Schema of divisions of inner cell of Vascular Cryptogams.

trical, for the primitive neck-canal cell undergoes at most only a
single bipartition.[2] Thus by two bipartitions are formed four
gametes, one functional, three degraded. The parallel with the
formation of the polar bodies in the Metazoan ovum is com-
plete so far as numbers go; and if we had only the Metazoan
ovum and the archegonium of the Vascular Cryptogam to deal
with, we might admit the conclusion, advocated especially by
Maupas, that two and only two mitoses are requisite to dif-
ferentiate the female pronucleus. But in P i l u l a r i a the
central cell remains undivided, according to Campbell, and

[1] Sometimes, however, a basal cell is formed from the initial, and closes
the belly of the archegonium below.
[2] In many cases it remains undivided.

passes directly into the oosphere,[1] and the "law" breaks down in our very next group also.

The spermatozoa of this group are multiciliate, not flagellate; a certain amount of cytoplasm is left over in their differentiation from the spermatocytes.

E. SIPHONOGAMÆ (PHANEROGAMS).

1. Gymnosperms.

In GYMNOSPERMS the pollen-grain or androspore produces a few sterile cells at its base, which are recognised as equivalent to the male prothallus of a Heterosporous Cryptogam. One cell, probably equivalent to the initial of an antheridium, grows out into the pollen-tube; its nucleus divides into two nuclei, one of which is the gametonucleus; or into a number of nuclei; so that the pollen-tube represents an apocytium of microgametes.

The formation of the archegonium is similar to that of the Vascular Cryptogams; the initial cell only forms the neck-wall and inner cell, the belly-wall being formed from the neighbouring cells of the prothallus (so-called endosperm). But the gametogenic processes are still further reduced, for in Conifers and Gnetads the inner cell only divides once, forming directly the oosphere and single canal-cell, while in Cycads the inner cell does not divide, but assumes directly the character of the oosphere,[2] a cytoplastic beak-like process replacing the canal-cell. Thus, in the series of archegoniate plants in Vascular Cryptogams two mitoses specialise the oosphere from the inner cell; in Conifers and Gnetads one mitosis suffices; in Cycads none takes place. This variation is a sure proof that there can be no need of these mitoses to eliminate part of the egg-nucleus and make room for the male, and that the function of these mitoses is not that assigned to them in the "replacement" hypotheses.

[1] "On the Development of Pilularia globulifera," &c., in 'Ann. of Bot.,' ii, pp. 247–8.

[2] According to Eichler, in 'Engler and Prantl,' op. cit., vol. ii, § 1, p. 16.

2. Angiosperms.

In ANGIOSPERMS the processes in the pollen-tube show a still further degeneration. The original nucleus divides into two: (1) a vegetative nucleus corresponding to the multicellular body of the Gymnosperms; (2) a reproductive nucleus corresponding to the gametogonial nucleus of that group: but no cellulose wall separates them; or if a rudimentary cell-wall be formed, it is at once absorbed. The vegetative nucleus seems here to have a function in connection with the growth of the pollen-tube, at the apex of which it lies, degenerating when this growth is completed. The gametogonial or generative nucleus then passes forward into the apex of the tube and undergoes mitosis; one of the two nuclei so formed is the male pronucleus, and passes into the oosphere.[1]

Here we have the two mitoses demanded by popular theory, but an interval of hours or days separates their occurrence; and the morphological explanation—that the first differentiates a prothalliar from a gametogonial nucleus, and that the second (of the latter only) is a gametogenic fission—is absolutely incontrovertible, when we compare the pollen-grain here with that of Gymnosperms, and again with the androspores of Heterosporous Cryptogams.

The FEMALE GAMETE is differentiated in the embryo-sac by a process unrivalled in complexity; while the morphology of the process is still doubtful. In recapitulating this, I will try by full notation to make the relations and fates of the nuclei as clear as possible. The embryo-sac corresponds to that of Gymnosperms, and most morphologists are agreed in regarding it as a megaspore, homologous with those of the Heterosporous Filicineæ that develop into a reduced prothallus bearing few archegonia only. Three mitotic divisions of its

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[1] Strasbürger and Elfving, who first discovered this process, originally reversed the parts of the respective nuclei. In some cases the generative nucleus undergoes two mitoses and so forms four gametonuclei.

nucleus occur as follows :—The first differentiates an Apical (N^1a) and a Basal nucleus (N^1b) respectively. Each of these again divides to form a pair of nuclei nearly superposed, which we term and letter Apical (N^2a), Subapical (N^2sa), Subbasal (N^2sb), and Basal (N^2b) respectively. Each of these four again divides to form a pair of nuclei of the degree N^3. The Apical pair ($N^3a + N^3a$) and Basal pair ($N^3b + N^3b$) are collateral; the Subapical and Subbasal pairs are superposed to form a file of four nuclei, which we name and letter Upper and Lower Subapical ($N^3usa + N^3lsa$), and Upper and Lower Subbasal ($N^3usb + N^3lsb$) respectively. Of the cytoplasm lining the cell-wall and surrounding the immense central vacuole, each nucleus attracts a certain portion so as to form a naked " free cell " somewhat ill-defined, and indeed anything but free. The following schema (Fig. 4) shows the for-
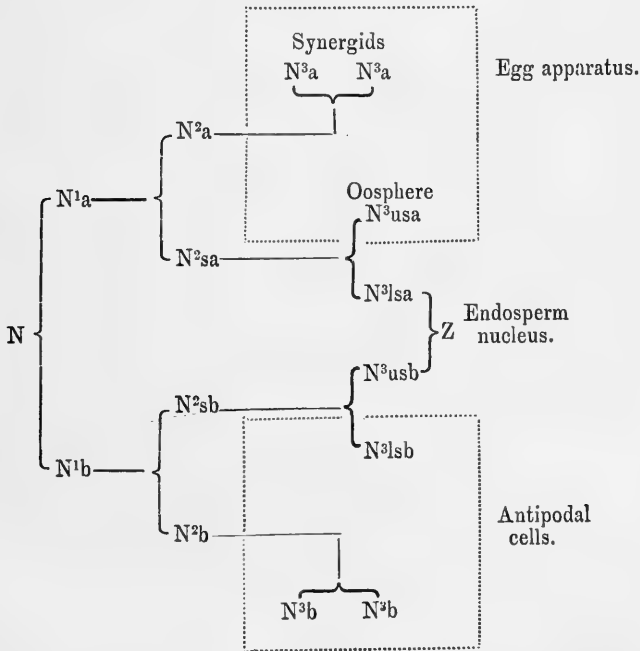
FIG. 4.—Schema of divisions in embryo-sac; the vertical lines separate successive stages.

mation of these cells and their position in the mature embryo-
sac ready for fertilisation ; the dotted vertical lines indicate
successive stages.   The symmetrical relation of the eight
cells formed is in no way exaggerated in the figure.

Of the eight cells so formed not one is capable of ulterior
independent development; though it appears from Stras-
bürger's figures,[1] and those of other authors, that enough
interval is left between the mitoses for the nuclei each time
to resume a resting state.   Hence it cannot be the extreme
rapidity of the mitoses that determines the reproductive in-
capacity of these cells.   The fate of these eight cells is very
different.

(1) The Apical Pair or Synergids, aptly termed Gehül-
finnen (handmaids) by Strasbürger, are utilised as the channel
for the transmission of the male pronucleus, sometimes
undergoing a complete degradation into a gelatinous form of
cellulose.

(2) The Upper Subapical Cell is the Oosphere,
destined on receipt of the male pronucleus to evolve into the
Embryo.

(3) The Lower Subapical and Upper Subbasal Cells
conjugate to form what we may term the Endosperm-cell;
this undergoes repeated divisions, appropriating at each more
and more of the parietal cytoplasm of the embryo-sac, till at
length the brood-cells constitute a continuous cellular layer,
the Endosperm, in which the embryo lies, and destined to
destruction as food for the embryo, either during the matura-
tion of the seed, or later on in the process of germination.
For some time no cell-walls separate the brood-cells, which
thus constitute an apocytium, lining the embryo-sac, and en-
closing a gigantic vacuole which persists in the Coco-nut. When
cell-walls divide up this apocytium, their inner tangential walls
form a continuous lamina, within which remains a layer of
cytoplasm surrounding the central vesicle,[2] just like that of
the gametangium of Botrydium or Acetabularia.   Here

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[1] 'Befruchtung und Zelltheilung,' &c.
[2] Berthold, op. cit., p. 213.

there is no question of giving a mystical significance to this "excretion process."

(4) The Lower Subbasal and the two Basal Cells lie huddled and inert at the base of the embryo-sac, as "Antipodal cells," and finally disappear in the growing endosperm.

The homologies of these structures are somewhat obscure, but the following explanation may be tendered as a fair one :

(1) The four cells of the form N^2 correspond to prothallial cells of a Cryptogam or Gymnosperm ; cells which we know form the common initials of both archegonium-wall (or neck at least) and oogonium.

(2) The Apical cell (N^2a) divides to form an archegonium of two neck-cells only, without any oogonium (such an archegonial neck of two cells is found in Cycads).

(3) The Subapical cell (N^2sa) divides into a superposed pair of which the Upper is the oosphere, of whose significance as a gamete there can be "no possible doubt whatever."

(4) The Lower Subapical cell (N^3lsa) is that which conjugates with the Upper Subbasal (N^3usb) ; as a consequence the cell produced by their fusion rejuvenesces, and by its repeated bipartition forms the endosperm. This structure is comparable to a thallogenous plant of low organisation and limited life ; the endosperm-cell that produces it must be regarded as a zygote, and the two cells that unite to form this zygote are necessarily gametes, whose close kinship, though the most distant possible under the circumstances, may influence the low organisation and limited life of their zygote.[1] Thus the subapical cell producing by bipartition two gametes is a gametogonium ; or we may go further and identify it with an initial cell, producing an archegonium without a neck, and reduced to the oogonium.   Of the two gametes formed by the

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[1] This important identification of the endosperm-cell as a zygote was first made out by Professor Le Monnier, of Nancy, in an unpretentious little note in ' Morot's Journal de Botanique,' vol. i, p. 140 (June, 1887).

divisions of the oogonium both are functional though in different ways.[1]

(5) The Upper Subbasal cell (N^3usb) is one of the gametes of the endosperm; its sister-cell, the Lower Subbasal cell, is, therefore, an arrested gamete; and the Subbasal cell (N^2sb) is a gametogonium like the subapical (N^2sa).

(6) From the symmetry of the embryo-sac we may regard the basal cell (N^2) as equivalent to the apical, and their divisions as homologous; or we may regard the basal pair of cells as the remains of the prothalliar tissue of the Gymnosperms.

The above identifications we may summarise thus: four prothalliar cells (N^2) are formed; of these the two in the mean position (N^2sa, N^2sb) are gametogonia, which by a mitotic division form four gametes, three functional, one arrested. The apical cell (N^2a) forms an archegonium reduced to a two-cellular neck; the basal cell (N^2b) forms two cells constituting a barren archegonium or mere prothalliar cells. I assume that the sister-cell of a gamete is necessarily a gamete, functional, arrested, or degraded; but the same rule does not apply to a gametogonium, which in all but the lowest Protophytes must have tissue-cells, not gametogonia, for its sisters.[2]

From the above explanation one thing is certain, that the endosperm of Gymnosperms is not homologous with that of Angiosperms, though its final function of nourishing the seed be the same.

[1] The relations of position would indeed identify the oosphere with the Gymnosperm canal-cell, and the lower subapical cell with the Gymnosperm oosphore.

[2] From a consideration of Guignard's researches I am now compelled to regard the embryo-sac as morphologically equivalent to a spore mother-cell, and the four nuclei, N^2, as megaspores, which differentiate as in the above statement; for it shows the same nuclear reduction in the prophases of its first mitosis that occurs in the primary pollen mother-cell, which has certainly this value.

V. COMPARATIVE GAMETOGENY IN THE ANIMAL KINGDOM.

A. PROTOZOA.

1. Flagellata.

Our chief knowledge of the gametogeny in the true Flagellata is due to the researches of Dallinger and Drysdale. After repeated acts of fission karyogamic unions occur, always binary.

In CERCOMONAS DUJARDINII and TETRAMITUS ROSTRATUS the gametes resemble the ordinary forms, and are isogamous. BODO SALTANS is anisogamous; the larger gamete, produced by the longitudinal fission habitual in this species, has the specific form; the microgamete is smaller, and produced by transverse fission. BODO CAUDATUS and MONAS DALLINGERII are also anisogamous, the microgamete having the same form as the megagamete, and only differing in size. In DALLINGERIA DRYSDALII the gametes are equal in size, but dissimilar, the one being like the ordinary individuals, triflagellate, the other uniflagellate; so that in this group we already find tendencies to anisogamy, and indications of the specialisation of gametes by peculiar forms of bipartition.

In NOCTILUCA, belonging to the Cystoflagellates, conjugation is isogamous between individuals of the ordinary type. In this case we shall describe the behaviour of the zygote, which affords a most instructive parallel to certain forms of spermatogeny in the Metazoa. The nucleus of the zygote comes to lie peripherally below an elevation of the cytoplasm; and as the nucleus divides, so the cytoplasmic elevation is parted by crucial furrows into hillocks, into which the daughter-nuclei pass one to each. By some eight or nine bipartitions 256 or 512 buds are formed, grouped into a disc-like elevation. By a basal thinning the buds are abstricted as uninucleate flagellates, while the body of the zygote is left, containing a residue of cytoplasm but no nucleus, and obviously incapable

of further vital evolution. In Dallinger and Drysdale's group the whole zygote is resolved into uninucleated spores; and the process in Noctiluca is a mere modification of the same process.

2. Rhizopoda and Heliozoa.

Conjugation is known to occur in some members of this group; the gametes appear to be ordinary individuals; but we have no full knowledge either of the antecedent or consequent processes.

3. Gregarinida.

The details of conjugation in this group are not fully made out, but that of OPHRYOCYSTIS is interesting. In this genus the apocytial body is first resolved into uninucleate cells, which conjugate. The two nuclei are stated not to fuse, but to give rise to six, two of which unite to give rise to a zygote-nucleus, around which the cytoplasm aggregates, while the other four nuclei pass to the peripheral cytoplasm, which degenerates with them. Obviously the conjugating cells are progametes, which only develop gametonuclei afterwards; and of these last, two only are functional : the peripheral cytoplasm and the other four nuclei represent arrested gametes. It would be interesting to know if the tripartite division of the nucleus of each of the conjugating animals corresponds to the schema annexed (Fig. 5), where N, M, represent the

$$N \left\{ \begin{array}{l} N^1 \left\{ \begin{array}{l} \overline{N^2} \\ \underline{N^2} \rightarrow \\ \overline{N^1} \end{array} \right. \end{array} \right. \boxed{Z} \leftarrow M^2 \left\{ \begin{array}{l} \overline{M^2} \\ \underline{M^1} \end{array} \right\} M^1 \right\} M$$
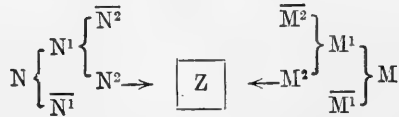
FIG. 5.—Tentative schema to explain the conjugation of Ophryocystis. N, M, are the primitive nuclei of the conjugating pair ; successive stages are separated by dotted lines, and the rejection-nuclei are surmounted by dashes; the square encloses the zygote-nucleus Z, formed by the union of N^2, M^2.

nuclei of the conjugating cells.   In this case the rejection-nuclei would be of different generations, like the first and second polar bodies.

In Monocystis Wolters has shown[1] that the nucleus of either conjugating individual divides into two, a rejection-nucleus which passes out like a polar body, and a gameto-nucleus which fuses with that of the other individual.

4. Radiolaria.

In Collozoum and Sphærozoum anisozoospores (of two sizes) are formed, but are not known to conjugate.   The size of the anisozoospores is inversely proportioned to the number of the brood.   Both are formed by preliminary divisions of the nucleus, and the resolution of the contents of the central capsule into uninucleate swarmers.

5. Ciliata.

a. Free Ciliata and Suctoria.

The conjugation in this group differs from that in any other, for in either gamete two pronuclei are formed, one of which is exchanged for a pronucleus derived from the other gamete; in each gamete the original and derived pronuclei fuse to form a new nucleus; and the two separate without cytoplasmic fusion, and after a time resume their normal life and fissiparous powers.   To understand fully this process we must premise that the nuclear apparatus of the Ciliata is double, consisting of a larger and smaller element, now termed meganucleus and micronucleus respectively.   The meganucleus divides by mere constriction, the micronucleus by a true mitosis, in fission; and there is reason to believe that the meganucleus is the seat of what we may term the physiological properties, the micro-nucleus of the morphological or atavistic properties of the single nucleus of other organisms.   A fact that supports this

[1] In 'Arch. f. mikr. Anat.,' vol. xxxvii, 1891, p. 91.

view is that in conjugation the meganucleus undergoes a disorganisation and is sometimes completely destroyed, while the pronuclei which conjugate are descendants of the micronucleus only. From the conjugation-nucleus is regenerated a complete nuclear apparatus in the exconjugates ; rarely is even a part of the old meganucleus retained and utilised by concrescence with the new one formed from the conjugation-nucleus.

The details are singularly interesting. Two Ciliates approach as apparent gametes, and join by the ventral surface. Their meganuclei undergo fragmentation. The micronucleus in each enlarges and then undergoes three mitotic divisions ; usually three of the four nuclei formed at the second division (which we may letter μ^2) abort as rejection-nuclei ("noyaux de rebut" of Maupas), and they degenerate and are excreted or digested ; while the fourth nucleus (μ^2) divides to produce the two pronuclei (μ^3). The only difference between the three abortive and the one preserved nucleus of the brood (μ^2) is that of position ; it is the nucleus nearest the mouth of the gamete that produces the pronuclei. Again, the pronuclei are absolutely similar in all save position :—that nearer the mouth of the gamete passes over as a migratory pronucleus into the other gamete, the one more remote as a stationary pronucleus awaits the arrival of the migratory pronucleus from the other gamete. For convenience and by analogy we may term the migratory and the stationary gametes "male" and "female" respectively, but we must remember that there is no essential difference between them, and we shall find in Vorticellines, where the gametes fuse completely and only one zygote-nucleus is formed, that it is constituted by the union of two migratory nuclei. After the fusion of the pronuclei is complete the gametes separate. I give a schema of the processes in a single gamete up to this point (Fig. 6).
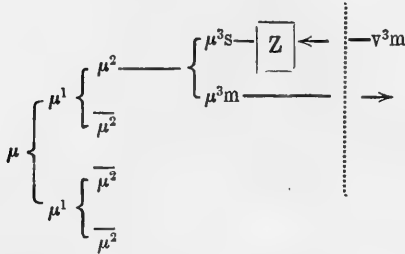
FIG. 6.—Schema of formation of pronuclei in a conjugating Ciliate. $\mu =$ original micronucleus of one of the gametes. μ^3s and $\mu^3m =$ the stationary and migratory (female and male) pronuclei respectively. $v^3m =$ migratory pronucleus from the other conjugate, which lies the other side of the dotted line. The pronuclei are supposed to unite in the square Z. The dash above indicates rejection-nuclei.

Some Ciliates have habitually two micronuclei; in this case both undergo the first two mitoses to form eight nuclei (μ^2), and seven of these abort, leaving only one to undergo the final mitosis; or, again, one of the two micronuclei undergoes the first mitosis only before its brood abort.

The conjugation-nucleus undergoes at least two mitoses, and of the four nuclei so formed in the simplest cases two become mega- and two micro-nuclei, and at the first fusion of the exconjugate one mega- and one micro-nucleus pass to each daughter-individual. Though this is the easiest type to understand it is not the commonest, but the processes, though of interest, are complex and too remote from our subject. Some of the progeny of the zygote-nucleus in certain species are eliminated as rejection-nuclei—a very significant fact.

The main peculiarities of the conjugation in the Ciliata (apart from the formation of rejection-nuclei, to which we shall return) are—(1) the formation of two fertile pronuclei; (2) the separation of the gametes after karyogamic union of the nuclei has taken place without any transference of cytoplasm. After noting that here, at least, conjugation is an essentially nuclear process, we proceed to infer from the coexistence of conditions 1 and 2 that they are correlated phenomena. Let us consider the process after the second mitosis and elimination of the three rejection-nuclei.

The following schema contrasts the conjugation of the Desmid Closterium, and of a Ciliate.

The zygote of the Closterium is totally different from the gametes, whose structure can only be obtained afresh by a very complex reorganisation and after protracted rest. The Ciliate, on the contrary, carries away from conjugation its cytoplast with all its complexity retained, and yet has a nucleus of the same "form" as that of the zygote of Closterium. The process then seems to involve the suppression of formation of proper gametes, in order to gain the advantage of the retention of the cytoplasmic body unchanged.
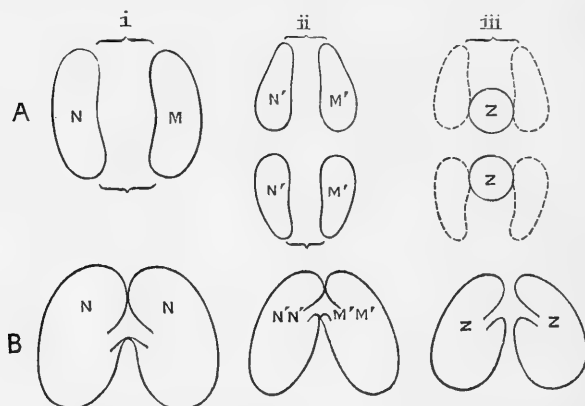
FIG. 7.—Comparisons of three stages of the processes immediately leading up to conjugation in Closterium (A) and Paramœcium (B). In each case two zygote-nuclei of the value $(N' + M' = Z)$ are formed, but the cytoplasts are unchanged in B, and totally altered in A.

Having found a key to the final processes of conjugation we can step back, and consider the first two mitoses with the formation of the rejection-nuclei. We may fairly regard these also as attempts to form a plurality of gametes comparable with the processes observed in certain Fucaceæ, the position in the cytoplast determining which nuclei shall be rejected. Possibly, too, the failing energies of the meganucleus at this stage are insufficient to determine the division of the cytoplast;

indeed, the fact that cytoplastic division remains in abeyance during the absence of a meganucleus in " working order " is an additional argument for regarding this as a directive organ.

We may note that the pairing individuals are in many cases much reduced below the normal size by a rapidly repeated series of bipartitions.

We may then briefly state our view of the homologies of the process of conjugation thus :—(1) The three mitoses of the micronucleus point to a primitive formation of gametes in broods of eight. (2) The fact that no cell division accompanies these mitoses may be the physiological result of the inertness of the now disorganised meganucleus. (3) The formation of two fertile pronuclei in each gamete points to an ancestral stage, in which a binary division of two mutually attached individuals immediately preceded the conjugation of their offspring. (4) The interchange of pronuclei and separation of the gametes are probably modifications of the process indicated in (3), directed to the preservation of the highly organised cytoplast. (5) The pairing Ciliata before the completion of the preliminary mitoses are not true gametes, but progametes.

b. Vorticellinea.

The group of VORTICELLINES, consisting of attached forms, is exceptional in two respects. (1) The conjugating individuals, instead of being similar, are unlike and unequal; the larger individual being of the ordinary attached type, the smaller free, and produced by repeated vertical fissions from an ordinary individual. (2) Instead of the gametes separating ultimately, they fuse, the larger absorbing the smaller into itself; and of the four pronuclei which are formed as in other Ciliates, only two unite to form a single conjugation-nucleus. The preliminary mitoses and formation of rejection-nuclei offer no exceptional character, save that in the male two micronuclei are formed (indicating a further suppressed fission). Two pronuclei are formed in either gamete, and the migratory pronuclei, instead of passing one another, fuse where they meet; while the stationary pronuclei both abort.

This confirms our interpretation of the rejection-nuclei in general, as gametonuclei that have failed to find cytoplasm. We must admit that the fusion of the gametes in Vorticellines, externally resembling a true sexual process, is only derived from the peculiar temporary conjugation of the free Ciliata.

B. Metazoa.

The gametogenic processes in the Metazoa are strangely uniform as compared with the wide range of organic differentiation they present; and it is easy to see that workers impressed by the latter fact should have laid undue stress on the former. In my introductory remarks I have pointed out that, as most of our biological thought has been largely moulded, nay, created by the Metazoan zoologists, a reverence for great teachers has impressed itself in provinces where they had neither the knowledge nor the training to guide theory aright.

1. Spermatogeny.[1]

The spermatozoa may be formed in one of the three following ways.

(a) The most primitive mode, existing in some Sponges, Cœlenterates, Vermes, and possibly other groups, is this : a germinal cell or spermatogonium undergoes segmentation to form a more or less coherent mass of cells, not inaptly termed a " sperm-morula " by comparison with the morula of a holoblastic oosperm. Each brood-cell ("spermatocyte" of La Valette, St. George's) develops usually into a uniflagellate spermatozoon. One of the simplest cases occurs in Ascaris megalocephala, an organism which, for researches on Metazoan gametogeny and karyogamy, has taken the place occupied by the classic Frog in the physiological laboratory : here the spermatogonium by two divisions forms four spermatozoa; but the simplicity is probably derived, not primitive;

[1] Here, as elsewhere in the paper, I have omitted the proper ontogeny of the spermatozoa, or the modifications by which it arises from the spermatocyte, its youngest stage, as foreign to the scope of the inquiry.

and the spermatozoon is amœboid.[1] By the passage of segmentation to budding we have a transition to mode (b).

According to Ebner,[2] in the Rat each spermatogonium divides into four spermatocytes; and a number of the broods so formed contract a syncytial union with an attached and uninucleated cell (Sertoli's cell), which thus plays the part of a nurse to numerous spermatozoa.

(b) The second mode of spermatogeny is that fully studied by Blomfield in Lumbricus. Here the nucleus of the spermatogonium undergoes repeated divisions; the brood nuclei come to the surface of the apocytium so formed and pass into cytoplastic buds, whose ends finally taper into flagella. These uninucleate buds are ultimately, as spermatozoa, abstricted from a central residue of non-nucleated cytoplasm, the " blastophore " of Blomfield. It is uncertain whether a cytoplastic blastophore is left in all cases of spermatogeny by budding; and the differentiation in this case from mode (a) is difficult. Comparing the holoblastic and the centrolecithal ova, the segmentation of the zygote in Euflagellates and in Noctiluca, we can fully realise of how little general import, physiological or morphological, is the presence of the non-nucleated blastophore.

(c) The third mode of spermatogeny occurs in the Sponge Grantia (Sycandra),[3] the Mollusc Helix, and some Vertebrates. Here, at an early stage of gametogenic fission (at the first bipartition in sponges), one nucleus undergoes no further divisions, and can only have a nutritive (or protective) function henceforward. In most cases the spermatogonium is attached by the basal cytoplasm in which this nucleus lies; the other nuclei pass to the free end of the cell, and henceforward sper-

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[1] We may correlate the absence of a flagellum here, as in most Arthropods also, with the absence of cilia in the tissue-cells, and the complete chitinisation of the body.

[2] " Spermatogenese bei Säugethieren," in ' Arch. f. mikr. Anat.,' xxi, p. 236. Unfortunately Ebner's views are contested, and the matter is unsettled.

[3] And in Spongilla fluviatilis; see R. Fiedler, "Ueb. Ei- und Samenbild. bei Spongilla fluviatilis," in 'Zeit. f. Wiss. Zool.,' t. 48. In this group the 'nutritive cell' forms an investment to the brood of spermatocytes.

matogeny goes on by budding as in mode (*b*).   The spermatozoa become free and leave behind a uninucleated blastophore, which is not known to be capable of further growth or division. We may fairly connect this peculiarity with the attachment of the spermatogonia in most cases, the nucleated blastophore serving as an active intermediary for the exchanges between the wall of the tube and the developing spermatozoa.   And this is a character of adaptation, of no morphological, and of minor physiological importance.[1] I annex a schema (Fig. 8) of a spermatogonium of this kind, in which four divisions are supposed to have occurred.   Owing to the fate of one of the

$$2^{4-1} = 8 \text{ spermatozoa.}$$

N[1] . . . . nucleated blastophore.
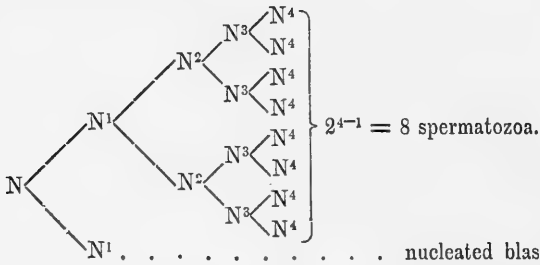
FIG. 8.—Schema of spermatogeny, with formation of nucleated blastophore;
Grantia type.

first two nuclei the number of spermatozoa formed at the nth bipartition of the nucleus is only 2^{n-1}, instead of 2^n, i. e. only half the normal number.

2. Oogeny.

The Metazoan oogonium, the "ovarian egg" or "ovum" of authors, is peculiar in attaining an enormous size, owing to its power of storing up reserve supplies in the form of unorganised yolk-granules to supply the metabolism of the future embryo ; and, correlated with this, it usually possesses an immense

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[1] Our interpretation of the nucleated blastophore, as a nutritive organ rather than as an excretion, is confirmed by the fact that the numerous broods of Rat spermatozoa, formed by mode (*a*), contract a union with a basal cell ; and this syncytium is undistinguishable from the apocytium of the spermatocytes and nucleated blastophore in mode (*c*).

vesicular nucleus, the germinal vesicle, with its chromatic elements concentrated and fused into a spheroidal mass, the germinal spot, supported by a delicate network of "intra-nuclear protoplasm," "nucleo-hyaloplasm," or "linin." In the structure of its nucleus the ovum recalls the only other cells that enjoy a prolonged life uninterrupted by fission, and that attain to an equally large size—somatic ganglion-cells.

The first symptom that marks the maturity of the ovarian ovum and its return to active life is the disappearance of its nuclear wall, and the merging of part of its "achromatin" contents, together with the true nucleoli, in the cytoplasm; while the chromatic elements of the germinal spot become separate as rods. It is to this process and stage that we must refer the elimination of mere trophic elements from the nucleus of the ovum—a process in many ways comparable to the disorganisation of the meganucleus of the conjugating Ciliates. There is every reason to believe that the nucleus of a cell destined to lie quietly feeding and fattening for days, months, years, or decades, must be of a very different character from one that has to undergo rapidly repeated fission; in other words, between a purely anabolic and an essentially katabolic nucleus. If we accepted Geddes and Thomson's view,[1] that there are actually entities that we can term anastates and katastates, we should have to reject their con-clusions, and say that this preliminary disorganisation of the germinal vesicle is the elimination of its anastates: for hence-forward all the phenomena manifested are katabolic, even without the advent of the male; and in ova which are not parthenogenetic the resumption of anaboly is henceforward impossible. This process is to some extent comparable with the elimination of the trophic element of the sper-matogonium, the blastophore, nucleated or non-nucleated; but the parallel is a very remote one, and purely physiological.

[1] A view which I no more accept than I do Sachs's view, that roots are formed at the base of a wallflower and flowers at the top by the migration downwards of "root-forming," and upwards of "flower-forming substances" (Würzel und Blumen-bildende Stoffe).

In the present case the trophoplasm eliminated from the nucleus passes into the cytoplast, and is utilised, not excreted.

The chromatic elements of the nucleus now become more or less free, and in anticipation of two mitoses undergo two successive longitudinal fissions.[1] This is, as O. Hertwig shows, only an anticipation (comparable with precocious segregation in embryonic development) of the subsequent mitoses, which follow one another in rapid succession with no interval of rest in the vesicular state separating the first from the second, as is usually the case between two successive mitoses.

The nucleus is at this time peripheral. A mitotic spindle is formed with its axis concurrent with that of the ovum. A very uneven cell division now takes place, the smaller cell being apparently budded off from the larger, which retains the name of the ovum, the smaller being termed the "first polar body." The nucleus of the first polar body then passes into a resting state in some animals. What we may term the secondary nucleus of the ovum at once forms a second spindle, and a " second polar body" is budded off like the first. The nucleus produced in the egg by this second mitosis is the gametonucleus, the egg being now converted into the oosphere. In most animals[2] the process is made symmetrical by the division of the first polar body into two. In this case the brood consists of four gametes, three arrested and one functional. When three polar bodies are thus formed the nuclei of all four cells are exactly similar and equivalent. The only difference between the polar bodies and the oosphere lies in their cytoplasts. Adopting the usual nomenclature of mother- and daughter-cells, the relationships here may be noted thus : the oosphere and second polar bodies are sisters,

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[1] This account is taken from Ascaris megalocephala. It is by no means certain that the peculiar characters of the two mitoses here are universal, though they have formed the chief morphological base for a very big theory. Similar "anticipated" mitoses, however, occur in spermatogeny also.

[2] Cœlenterates, Molluscs, Vermes, and Vertebrates, according to O. Hertwig.

and both are nieces of the first polar body.   I append a schema
to show these relations (Fig. 9).[1]

(i)   (ii)

N^1——$\Big\langle$ N^2p
              N^2p

              N^2p N^1p

N——N^1——No N^2p

N——N^1——N^2o
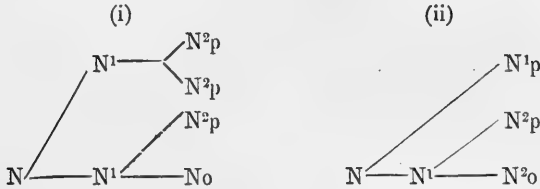
Fig. 9.—i. Schema to show usual formation of polar bodies in Metazoa.
ii. Schema of polar bodies in Ascaris.

Unmistakably these processes point to a primitive condition,
when each ovarian ovum divided in two stages to form a brood
of four oospheres.   Unfortunately the phenomena of the
gametogenic bodies have been made too much a study isolated
from other similar formations, and false interpretations have
been put on the peculiarities of their mitoses to suit preconceived
ideas.   Doubtless had the segmentation of the Metazoan egg
proceeded after the type of Cystoseira (supra, p. 17), in-
genious hypotheses would have been framed to explain what
plasmic properties resided in each of the seven nuclei rejected,
and for what reason each one had to be expelled from the egg
to leave a pronucleus fit for fertilisation.

The remarkable uniformity of oogeny (in the restricted
sense) in the Metazoa as compared with other equivalent
groups is perhaps attributable to the fact that its processes
are usually limited to the short time during which the ovum
is free before fertilisation, and to the fact that it is usually
free at that time : a uniformity of external conditions has
preserved uniformity of results.   The much greater variability
of spermatogeny which takes place under much more varied
conditions supports this view.[2]

[1] Blochmann has found in some Insects that the second polar body divides,
but not the first (" Zahl d. Richtungskörper,'&c.," in ' Morph. Jahrb.,' 1889).

[2] I have omitted the consideration of "paracopulation" in the "winter
eggs" of Cladocera, as described by Weismann and Ischikawa (in 'Zool.
Jahrb.,' "Abtheil f. Anat. u. Ontog. d. Thiere," iv, pp. 155—196).   In

Though we are now considering gametogeny, we must turn aside to note that in most cases of so-called "parthenogenesis" of Metazoa only one polar body is formed, and the ovum, rather a progamete than an oosphere, segments and develops directly. We revert to this process below in its proper place (p. 74).

We must, however, note that in some cases the true oosphere, differentiated by two mitoses (i. e. the formation of both polar bodies), is a facultative gamete, and may develop without fertilisation; this has been demonstrated in Liparis dispar and some other Lepidoptera, and in the Hive Bee (Apis mellifica); and in the last case the produce of the unfertilised oosphere is always a male or drone. This is proof conclusive that the formation of polar bodies is not necessarily an elimination of male elements or "katastates;" on the other hand, it would work in well with the very old view that in bisexual union the "superiority" of one parent determines that the offspring shall be of the opposite sex. But such considerations are outside the limits of our theme.

VI. A GENERAL VIEW OF GAMETOGENY.

Before summarising the results of our systematic survey we have to consider several points of general bearing.

A. The Reduction of the Chromatomeres in Gametonuclei.[1]

A frequent, but certainly not universal, process in gametogeny is the reduction of the number of chromatomeres or

these eggs, destined to form the two polar bodies and to be fertilised by a spermatozoon, a portion of the nucleus is segmented off before maturity and remains in the egg, ultimately to fuse with one of the segmentation nuclei at the first or third segmentation. I confess myself unable to explain this fact, or bring it into line with other phenomena of gametogeny.

[1] Most of the details of this section are taken from Strasbürger, 'Kern-und Zelltheilung' (1888), and from R. Hertwig's paper cited above; and during the impression of this paper I have consulted Guignard's "Sur la Consti-

rods, into which the chromatin of the nucleus resolves itself in the early stages (prophases) of mitosis.

It appears to be the rule that, apart from gametes and their antecedent cells, all the nuclei of a given species have the same number of chromatomeres; each of these during mitosis splits into two, lengthwise, and one half goes to each daughter-nucleus.

In most flowering plants the normal number of chromato-meres in the vegetative cell is 16; at a certain stage, anterior to the gametogonium proper, the chromatin wreath (which had shown 16 rods at its formation) now segments in its pro-phases into a smaller number than were present in the meta-phases and anaphases of the previous mitosis, which number is perpetuated in the gametogonia and gametes.   Thus in Helleborus and most Liliaceæ the reduced number is 12, two thirds the original; in the Liliaceous genus Allium it is 8.   But in Convallaria (Lily-of-the-valley), also belonging to the same order, there is no reduction, and in Muscari (Grape Hyacinth) it is raised to 24.   In Orchids, also, no reduction has been observed.

The cell in which this reduction first takes place does not appear fully determined in all cases; but we know this much, that in the male (anther) it furnishes vegetative as well as reproductive offspring. For it occurs, according to Guignard, in the pollen mother-cell, which forms four pollen grains, each to produce a vegetative and a gametogenic nucleus.   All these perpetuate the reduced number of chromatomeres by normal mitosis.

In the female (ovule) this reduction is first shown by the original nucleus of the embryo-sac. In some cases, at least (Lilium Martagon), Guignard, whose recent account is slightly different from Strasbürger's and from his own previous statements, has shown[1] that the normal number of chromato-

tution des Noyaux cellulaires chez les Végétaux," 'Comptes Rendus,' May 11, 1891.   See also Boveri's " Zellen Studien : Verhalten der chromatischen Kern-substanz b. d. Bildung der Richtungskörper u. b. d. Befruchtung," in 'Jen. Zeit.,' 1890.

[1] " Nouvelles Recherches sur le Noyau cellulaire," in 'Ann. des Sci. Nat. Bot.,' ser. 6, xx, p. 334, and 'Const. des Noyaux.'

meres in the vegetative cells is 24;[1] that of the progametal and
gametal nuclei, 12; and the "basal nucleus" produced at the
first division of the nucleus of the embryo-sac reverts to 16
for itself and its offspring, so that of the two gametonuclei
that conjugate to form the endosperm nucleus the lower has
the normal, the upper the reduced, number of chromatomeres.
In the liverwort Riella Clausonii, O. Kruch has found the
number 8 constant for the inner cell of the antheridium and
its brood-cells, and the oosphere; the first two cells of the
embryo have each 16 chromatomeres; but whether the latter
number is characteristic of the tissue-cells, and the number
found in the spores, has not been made out.

In Metazoa the reduction appears usually to take place in
the gametogonium, and is much more uniform in most animals
than is demonstrated for plants,[2] the number of chromatomeres
being here usually one half the normal; consequently in
karyogamy or fertilisation as spermato- and oo-nucleus each
brings an equal number of chromatomeres, the zygote nucleus
and its offspring reverting to the number characterising the
species.[3]   This, however, is not always the case; for in Arion
empiricorum (the Cellar Slug) there are numerous chro-
matomeres in the oo-nucleus, but two only in the spermato-
nucleus.[4]

The schema of mitosis is sometimes modified in the animal
gametogonium, the longitudinal splitting of the chromatomeres
taking place earlier than usual, and even being doubled, so as
to produce four times the (reduced) number of chromatomeres.
In this case, which occurs notably in both the male and female
gametogonia of Ascaris megalocephala, the longitudinal
splitting does not, of course, occur again in the successive
gametogenic mitoses; but the chromatomeres, thus formed in

[1] Not 16 as given in his previous paper.

[2] In ' Malpighia,' vol. iv (1891).   Professor Strasbürger kindly directed my
attention to this paper.

[3] It must be remembered that the numbers have only been counted in very
few cases—at the outside twenty or thirty.

[4] See Platner, "Ueb. d. Befr. bei Arion empiricorum," in 'Arch. f.
mikr. Anat.,' vol. xxvii.

advance, are only evenly distributed between the daughter-cells. This obviously, as stated, is only another way of distributing the segments formed. If in the schema B, C, represent two of the chromatomeres of the gametogonial nucleus, B^1, C^1—B^2, C^2, those produced by their splitting, and the dotted lines the cell divisions, we see that the sole difference needed to change this into a schema of ordinary cell division would be to prolong the middle dotted line back to the level of B^1, C^1—B^1, C^1.

$$
\text{B, C}\begin{cases}
B^1, C^1\begin{cases} B^2, C^2 \\ \dots\dots\dots \text{ line of second fission (absent in} \\ B^2, C^2 \quad\text{female of Ascaris).} \end{cases} \\
\dots\dots\dots \text{ line of first fission (formation of} \\ \quad\quad\quad\quad\quad\text{first polar body in female).} \\
B^1, C^1\begin{cases} B^2, C^2 \\ \dots\dots\dots \text{ line of second fission (formation of} \\ B^2, C^2 \quad\text{second polar body in female).} \end{cases}
\end{cases}
$$
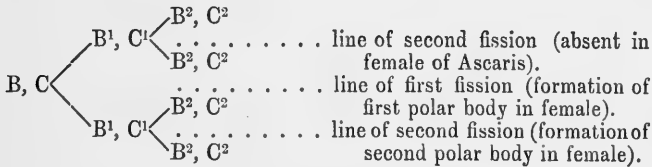
FIG. 10.—Schema of modified mitosis in gametogeny of Ascaris megalocephala bivalens.

A misinterpretation of these facts has led to a belief that the reduced number of chromatomeres in gametes was due to the removal of half of them, or that a true transverse division of chromatomeres replaced the ordinary longitudinal splitting.

A complete hypothesis to explain this reduction is certainly premature when we know practically nothing of the numerical relations of the chromatomeres in Protozoa and any (save one) Cryptogams. We may, perhaps, regard it as an adaptation to prevent an undue multiplication of chromatomeres in the zygote, and the cells produced therefrom. The halving cannot be essential, for the reduction is only by one third in many plants. The reduction itself cannot be essential, for it is absent in some plants, and an augmentation probably occurs in the cases where the gamete possesses a fusion nucleus, as well as in Muscari. Hence all theories of gametogeny and fertilisation based on the assumption that this reduction is universal or uniform must fall to the ground.

We must remember the fact that the reduction takes place in the pollen-mother-cells of Flowering Plants, which are themselves homologous with the mother-cells that form tetrads of

asexual spores in Archegoniate Cryptogams.   Hence we may
be allowed to conjecture that the reduction also takes place
in the latter group ; and, by parity, that it is not confined
to gametogonia, but will be found in all mother-
cells destined by multiple fission to give birth to a
brood of reproductive cells.   In that case the return to
the normal number of chromatomeres would necessarily be
effected by the slow process of nutrition in asexual spores
or their offspring, instead of by the direct process of
summation in the case of gametes.   This would give a clear
insight into the action of karyogamy in bringing about rapid
and complete rejuvenescence.

> The question of the individuality of the chromatomeres and their persist-
> ence, as maintained by Boveri and Rabl, should find its treatment here ; but
> I have not yet completed a research bearing on this point.   I therefore con-
> fine myself now to the statement that, with Hertwig, I believe the evidence
> very inadequate for the support of the theory that the chromatomeres have
> distinct and persistent individualities.   Professor Strasbürger has written me
> a letter to the same effect, though he had advocated Rabl's views in his
> ' Kern- und Zelltheilung.'

B. On the Reproductive Incapacity of Obligatory Gametes.

The reproductive incapacity of gametes is no exceptional
phenomenon among cells, nor is it brought about only in the
differentiation of gametes.   It occurs in other cases through-
out the Metaphytes and Metazoa, and we have instances of
its existence as low down as the Colonial Flagellates.   In the
genus Volvox all the numerous cells of the colony other than
the few " germinal cells " (parthenogonidia, oogonia, or sper-
matogonia), perfect flagellates equipped with eye-spot, con-
tractile vacuoles, and nucleus : all these, I say, are affected
by that very reproductive incapacity which is the character-
istic of the gametes, while they lack the potentiality of
karyogamic rejuvenescence possessed by the latter.   In
the majority of Metazoa and Metaphytes the tissue-cells as a
rule suffer from the same impotence in virtue of their differ-

entiation, so that the indefinite reproductive capabilities of the cells of Mosses and Begonias, Cœlenterates and Flat-worms are usually cited as exceptions to the rule; though the indefinite, if not unlimited, capacity for fissile reproduction must have been primitively inherent in every cell. The limitation of this power in most Metazoa occurs at a very early stage if the brilliant results of Chabry[1] prove to have a general application; for he has shown that in Tunicates the destruction of a single blastomere may determine the absence of the organ it should produce.

C. The Adaptation of Gametes to Different Fates.

Our next subject for parallel is the arrest or degradation of certain gametes of a brood, or, if we please, the favouring of single gametes of a brood at the expense of others. This is a common phenomenon of the struggle for existence between members of a colony, whether cells, organs, zooids, or even individuals, especially those destined for reproduction. Thus, in the ascus of the Truffle four to six spores are formed endogenously, but usually only one matures. In the Heterosporous Filicineæ sixty-four megaspores are formed (in sixteen tetrads) in each megasporange; but only one matures, the other sixty-three undergoing complete disorganisation. In Eleocharis and other Sedges the pollen mother-cell undergoes two unequal divisions, just like the Metazoan ovum, and produces a single fertile pollen grain and three abortive ones.

The ovarian ovum of Hydra attains its full size by devouring all the others in the ovary, and in many Arthropods the fertile ova develop at the expense of others. In Ascaris megalocephala some of the ova and spermatogonia are sacrificed and abort in the germ-tubes (O. Hertwig). In Vertebrates also many of the primitive ova are degraded to mere food material.

As for organs and individuals, in Gymnosperms several archegonia may be formed and fertilised in each embryo-sac;

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[1] "Embryologie Normale et Tératologique des Ascidiens Simples," in 'Robin's Journ. de l'Anat.,' 1887, p. 167.

nay, in some cases the oosperm or zygote in each archegonium by early fission produces four embryos; and yet only one embryo ripens in the seed. In Angiosperms the proportion of matured seed to ovules and carpels varies very greatly. In Anemone several ovular origins are formed, but only one ovule attains full size in each carpel; in Drupaceæ two ovules are present, but only one ripens into a seed, the exception constituting the well-known " Philippina " of stone-fruit. The one-seeded Acorn is the outcome of an ovary with three biovulate carpels.

The fate of the central procarpia or oogonia of Corallina, reduced to the position of mere agents for the transmission of the male substance, finds a parallel in the peculiar transformation undergone by the showy sterile peripheral flowers in the inflorescence of Viburnum, Hydrangea, and some Composites, into mere signboards, attracting the insects whose visits fertilise the less conspicuous central flowers. The petals of many flowers (e. g. Ranunculaceæ) are merely the degraded sterile outer stamens.

In the animal world severe competition exists between embryos in those Molluscs which have numerous eggs in a single capsule. Here the first larvæ to hatch out in the veliger stage eat up their more tardy brothers and sisters.

We may note that some of the nuclei produced by the zygote nucleus of the exconjugate Ciliate are rejection-nuclei, and fail to take any share in the life of its offspring.

D. Summary of Gametogenic Processes.

1. In many Protozoa the gametes are apparently ordinary individuals or swarmers, the product of normal fission or brood-formation.

2. In many Phytomastigopods and Protophytes the gametes differ from ordinary zoospores in being produced by more rapidly repeated acts of fission or segmentation, and in their smaller size.

3. When the gametes are unequal the males are usually the product of a more complex segmentation than the females.

4. The number of repeated fissions to form the oogametes from the oogonium varies, and the divisions may even remain in abeyance and the oogonium assume directly the character and functions of an oosphere (Volvox, Florideæ, Cycas).

5. Owing to adaptive modification only one gamete of a brood may be fertile, and the rest aborted, either arrested, or degraded into accessories of the sexual process. Thus in Metazoa out of a brood of four gametes three are arrested as polar bodies. In the Archegoniate Cryptogams, Conifers, and Gnetaceæ, a similar formation of a single fertile oosphere takes place, but the infertile gametes are degraded to form the channel for the transmission of the spermatozoon.

6. The gametogenic divisions may only affect the nuclei, cytoplastic fission remaining in abeyance. In such cases arrested gametonuclei may remain (a) in the periphery of the protoplasm of the fertile one (polar bodies of many Arthropods); (b) be digested, ejected, or excreted (certain Ciliata and Fucaceæ), or simply degenerate in situ (periplasm of Peronospora, Ophryocystis).

7. The number of such rejection-nuclei is determined by two factors : (a) the primitive number of gametes in the brood ; (b) the number of fertile gametes formed and requiring nuclei. Thus in Fucaceæ, where eight is the primitive number of gametes, four, six, or seven sterile nuclei are eliminated, according as the number of fertile oospheres produced in the oogonium is four, two, or one.

8. Hence it follows that the number of arrested gametes (or rejection-nuclei), being variable, can have no universal physiological significance.

9. While the specialisation of gametes by fission is the rule, gametogenetic fissions do not occur when a vegetative cell undergoes direct conversion into an oosphere.

10. In some Apocytial Plants the gametes are formed by the resolution of the apocytium into uninucleate cells,

either directly or after preliminary division of the nuclei (Cladophora).

11. In some Apocytial Plants the gametonucleus (whether of iso- or oo-gametes, but not of spermatogametes) is the product of the union of several nuclei, either the ordinary vegetative nuclei (Dasycladus), or the offspring of the vegetative nuclei by antecedent mitotic divisions (Peronospora). In both cases the gametes are obligatory.

12. Hence preliminary nuclear division is not a necessary antecedent to the differentiation of obligatory gametes.

13. Since the nuclei formed in heterogeneous gametogeny (§§ 5—7) are all similar, and contain (in every case when the point has been worked out) an equal number of chromatic elements, we conclude that the difference between an arrested and a functional gamete lies in their cytoplasm, not in their nuclei (O. Hertwig).

14. The alleged "processes of excretion" antecedent to fertilisation and incidental to gametogeny are neither universal nor uniform. Under this head we may group the following processes, which I have made to include all cases where part of the gamete takes no share in the formation of the zygote.

(a) Part of the oogamete is utilised in the formation of a receptive or transmitting organ for the male; of this character is the trichogyne in Coleochæteæ and Florideæ; the beak with its mucified cytoplasm in Oedogonieæ (and Vaucheria?).

(b) The formation of non-nucleated epiplasm by isogametes, originally referable to a formation of cell-walls, which remains in abeyance, in some apocytial plants (Cladophoreæ and Protomyces; found also in similar formation of the asexual zoospores).

(c) The cutting off of a wall of cytoplasm around the central vacuole of an apocytium or cell (Acetabularia, Botrydium, and Ulothrix).

(d) A similar excretion to (b) of non-nucleated protoplasm from the oospores of Saprolegnieæ, afterwards resumed by them; the same process occurs with the asexual zoospores.

(e) The non-utilisation of all the cytoplasm in the elaboration of the flagellate (or ciliate) spermatozoon from a tissue-like cell, leaving a non nucleated residuum (higher Cryptogams and some Animals).

(f) The leaving over of a central portion of cytoplasm, as a non-nucleate blastophore, in the modified sperm morula, where budding replaces true segmentation (Lumbricus, Metazoa, &c.).

(g) The differentiation of the basal cell of one of the earlier stages of the fission of an attached spermatogonium as a nucleated blastophore (Helix and some Vertebrates ; it is the first daughter-cell of the spermatogonium in Sponges).

(h) The abortion of some of the spermatogonial or oogonial cells (Ascaris).

(i) The abortion of some of the gametes of a brood as "polar bodies" in the Metazoa, owing to one appropriating the greater part of the cytoplasm.

(k) The abortion of some of the gametonuclei, with or without a minimal quantity of cytoplasm, as rejection-nuclei (Ciliate Infusoria and some Fucaceæ).

(l) The degradation of some of the oogametes or progametes of a brood to serve as transmitting media for the spermatozoon (canal-cells of Archegoniates and Gymnosperms).

(m) The retention of numerous gametonuclei in the peripheral part of the apocytial cytoplasm, which is destined to form an outer investment around a single central gamete when transformed into the zygote (Peronosporeæ and Ophryocystis?).

(n) The first formation of sterile cells in the pollen grain of Gymnosperms, and of a vegetative nucleus in those of Angiosperms representing the formation of a vegetative prothallus, and having no real connection with the subsequent gametogenic divisions of the other or sexual nucleus.

(o) The non-utilisation of some of the gametonuclei formed in the pollen-tube of the Siphonogamous Metaphytes, and the antheridium of Peronospora.[1]

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[1] This case finds a close parallel in the formation of the innumerable sper-

We see that these processes fall into several distinct classes, which cannot be at all homologised morphologically or physiologically. (a) is a type quite apart; (b) (c) and (d) are modifications of one and the same process; (e) (f) and (g) again may be grouped together, though a nucleus is lost in (g), but not in the two other types; (h) (i) and (k) again form a similar group united by homoplasy, to use the term that Lankester introduced to denote the similar products of similar physiological conditions, irrespective of common origin; (l) is distinct, showing a certain analogical relation to (a); (m) stands alone, and so does (n); (o) is only brought into the present relation because of the false homologies to which it has given rise.

15. From the above it follows that excretion of protoplasm is no essential condition of gametogeny.[1]

VII. The Causes of Protoplasmic Senescence and Ultimate Reproductive Incapacity.

Maupas has established an important fact which sheds a flood of light into a very dark corner of biology. In the Ciliata, the offspring of a long-continued series of fissions ultimately degenerate, and lose first the power of entering into the conjugation that would rejuvenate them, and finally that of further fission. This degeneration he terms SENESCENCE. We have evidence on all sides to show that asexual reproduction, colonial or cellular, is rarely continued indefinitely in those organisms which have a sexual process. After a certain continuance of asexual reproduction the strain deteriorates, as Andrew Knight showed a century

matozoa in many mammals, where only one fertilises the single ovum. Yet no one has suggested that the others are excretion products.

[1] From the above summary it is obvious that Waldeyer fails utterly in his contention that Bütschli's identification of the formation of polar bodies "must fall to the ground if it be established that sperm mother-cells also give rise to polar bodies," since the "polar bodies" or "excretions" in spermatogeny are neither universal nor fully homologous with one another, nor with the formation of polar bodies in the egg.

ago. The one case which occurs to me, writing in Ireland, is the Champion Potato, which proved the salvation of the country after the great famine by its resistance to the "blight" (Phytophthora vastatrix), but which after forty years has now completely lost this resisting power. Again, we have ample direct evidence for regarding the apparently "resting" nucleus in a cell as having the same sort of relation to the cytoplast as a nerve-centre has to an organism,[1] a view supported too by the fact that the nucleus approximates in chemical composition to nerve substance, being richer in lecithin and phosphorus generally than the cytoplasm. Now, in ordinary cell division, on the principle of continuity, there is no essential change in brood-cytoplast and brood-nucleus, and the result of repeated cell fission is merely a multiplication of these. But we know that a nerve-centre ceases to respond readily to a continued or repeated stimulus of the same kind. It would seem then probable that, after a prolonged association in life continued through a series of fissions, the nucleus would respond less readily to the stimuli received from the cytoplast; consequently its directive powers would be diminished; and conversely the protoplasm would do its work more imperfectly; the nucleus again would be less nourished; and a vicious circle of deterioration would set up in the cell, ending in senescence and death. Maupas has told us that in the senescent Ciliata the cell-body is dwarfed and deformed, the nuclear apparatus reduced and degenerated.[2]

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[1] Cf. Haberland's researches on the behaviour of the nucleus in the activity of the vegetable cell; Grüber's on artificial division of Ciliata; Eimer has even adduced evidence to show that in nerve-centres themselves the nuclei of the ganglion-cells play the part of primary centres ("The Cell-nucleus as Central Nervous Organ," in 'Organic Evolution' [Eng. Trans.], p. 349).

[2] The recent researches of Fol ('Comptes Rendus,' April 20, 1891), Guignard ('Comptes Rendus,' March 9 and May 11, 1891), and Flemming ('Arch. f. mikr. Anat.,' t. xxxvii, pt. 2) complete the evidence that the "centrosome" of Boveri plays an essential part in mitosis and karyogamy; and the phrase "nucleus and centrosome" should in this section be used to replace "nucleus" wherever it is used in antithesis to cytoplast in the present discussion.

Parallel cases of failure by continued association are nume-
rous, both in organic life and in human affairs. Seed raised on
the same soil for several generations yields stronger plants
when transferred to a different soil or another climate. In a
great business the disadvantages of too unchanged a manage-
ment or a staff are recognised.

As cell multiplication is essentially an exhaustive process,
requiring nutrition to compensate for the losses incurred by
the active metabolism involved, it is obvious that any accel-
eration of the rapidity of the fissions and reduction of the
interval of recovery of the cell must necessarily weaken the
organism in a sort of multiple ratio, and so precipitate
degeneration.

In this way we can see how the reproductive incapacity of
obligatory gametes may be frequently effected merely by the
rapidly succeeding fissions that differentiate them. The re-
placement theory of Balfour is true in its main proposition,
that the formation of polar bodies is a process whose object
is to prevent parthenogenesis ; though not by the mechanism
he implies, the removal of a male element. The rapid cell
divisions, uninterrupted by any interval for nuclear rest and
reconstitution, must precipitate and accentuate reproductive
incapacity—a view which is essentially O. Hertwig's.[1] It is
probably due to this physiological gain to the race that the
page of morphological history, revealing that the oogamete
was primitively one of a brood of at least four, has not been
obliterated from the ontogenetic records of the Metazoa.

From the standpoint that a well-constituted cell should be
capable of doing anything that any cell can do—feeding,
moving, growing, dividing, and so on—our specialised gametes
are indeed stricken with utter degeneration; for they can
neither feed, move (as far as the oogamete is concerned),
grow, nor reproduce their kind, but as individuals are doomed
to death or to the extinction of their individuality in a zygote.

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[1] But it does not seem made out or even probable that in gametogeny the
nuclear divisions proceed, as a rule, in the same breathless hurry as in Ascaris
megalocephala ; they certainly do not in the Angiospermous embryo-sac.

VIII. PROTOPLASMIC REJUVENESCENCE, ITS NATURE AND MODES.

From the degeneration and loss of constitutional vigour produced by the over-prolonged association of nucleus and cytoplast, unchanged through a long chain of fissions, the escape lies through a REJUVENESCENCE of the " firm," as we may term them. And this is effected in various ways.

A. THE MODES OF REJUVENESCENCE.

1. REST from a given stimulus is sufficient to rouse again the irritability of a nerve-centre when not unduly fatigued. Even the operative weaver or working engineer, who from constant habit is barely conscious of the unceasing din of the machinery, would feel it afresh after a few weeks' absence. And in the resting cell the nucleus has, moreover, the opportunity of complete nutritive restoration. In the agamous Monadineæ, resting states, more or less prolonged and accentuated, separate the stages of active growth and fission. Here, too, as so often occurs in plants of higher organisation, the more marked resting state usually precedes a recrudes-cence of active cell division.

2. CHANGE OF THE MODE OF LIFE is another mode of bringing about an harmonious readjustment of the relations of nucleus and cytoplast. It may be accomplished by mere POLYMORPHISM, or by HETERŒCISM, the change of host, so frequent in the life cycles of parasitic organisms. Marshall Ward has drawn attention to this in the case of the higher Fungi which are so frequently apogamous.[1]

3. NUCLEAR MIGRATION, i. e. the transference of a nucleus to a portion of cytoplasm with which it has not been asso-

[1] " On the Sexuality of the Fungi," in ' Quart. Journ. Micr. Sci.,' 1884 ; see pp. 59, 60 (of reprint) especially, where Ward compares the sojourn in a new host " to a trip to the sea-side, where the weary and enfeebled organism enjoys fresh diet and associations for a time, which in their turn pall and prepare their recipients to renew old modes of life."

ciated, may occur in apocytial plants, as through the clamp connections[1] and anastomoses of the Fungi with septate hyphæ.

4. PLASMODIUM FORMATION, that is the cytoplastic union of cells without nuclear fusion. This, of course, brings about complete mixture of the cytoplasts, comparable to that of the nuclei in karyogamy, and which we have termed plastogamy. The nuclei are thus furnished with totally new cytoplasts on the resolution of the plasmodium into cells. This is a more thorough-going process than the preceding, and occurs in agamous plants only. It is generally held that karyogamy arose as an advance on this process.

5. KARYOGAMY, or the fusion of two or more nuclei as well as of their cytoplasts into a uninucleate cell, the zygote. In binary union the cytoplast of one of the gametes may be practically nil.

6. THE FUSION OF APOCYTIAL GAMETOIDS. We distinguish this for convenience' sake, as we know nothing of the cytology of this process ; but it must ultimately fall under the head of plasmodial formation or karyogamy ; possibly the cytological details may vary even from species to species.

B. THE ADVANTAGES OF KARYOGAMY AS COMPARED WITH AGAMY AND APOGAMY.

If the rejuvenescence due to karyogamy be of the nature I describe, that is, the formation of a nucleus new to the cytoplast with which it is associated, a change in the constitution of the "firm" and "staff," to speak metaphorically, the consequence should follow that, by introducing a suitable nucleus into an empty cytoplast, we ought to obtain the same rejuvenescence

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[1] These are formed by lateral outgrowths above and below a septum, which meet and anastomose to form an open loop round the barrier.

[2] Ward, op. cit., p. 58, regards the sexual process as "consisting essentially in the invigoration of the protoplasm ;" but in the preceding pages he clearly shows a belief in replacement theories. But his statement "that the sexuality of the higher fungi has disappeared, because its purpose has been equally well or better attained otherwise than by means of sexual organs," is fully in the spirit of the views advocated here.

as in karyogamy. And this very feat has been accomplished ; for the Hertwigs some years ago showed that Echinoderm eggs when shaken up in sea water break into fragments ; and observed a spermatozoon entering such a non-nucleated frag-ment and segmenting therein, like the zygote segmentation-nucleus. Since then, as O. Hertwig recalls in the above-cited paper,[1] Boveri has repeated the observations, and proved that the normal morula was formed and developed into a larva. I do not see how this is consistent with any theory of karyogamy but Bütschli's—that it is a process of rejuvenescence, to which term we are now endeavouring to attach a definite connotation.

Our definition of rejuvenescence, karyogamic or other, is that it is essentially a process of constitutional invigoration,[2] as its converse, senescence, is one of consti-tutional enfeeblement. We can now, grasping this idea, under-stand the continued existence of agamous and apogamous forms side by side with those where not merely karyogamy, but allo-gamous sexual reproduction is essential. Every arrangement that makes for protection and comfort tends to become by habit indispensable, and the privation of such an "acquired need" may produce effects none the less disastrous because it was ac-quired, and not primitive. A couple of examples from human life will illustrate this. The Maoris found scant clothing neces-sary in their cool but not extreme climate until the Europeans introduced blankets ; but now their occasional reversion to the practice of going unclothed is said to lead to disastrous results. Civilised nations who cook their food largely escape the attacks of entozoa : but, on the other hand, when they do occur, these attacks disturb and disorder the system the more seriously for their rarity ; while the Abyssinian, who feeds daily on raw beef, thinks it positively unlucky to be without a tapeworm in his intestines. The coexistence of agamous, karyogamous, and apogamous types proves that the need for karyogamy belongs to the class of acquired needs or necessary

[1] 'Vergleich der Ei,' &c., p. 85. I have not been able to consult Boveri's original paper in the libraries of our scientific societies.

[2] Of course I use "constitutional" in the medical sense.

superfluities—all but indispensable to those that can obtain them, not missed by those that have never known them, and capable of being laid aside in time by a few of the former class. Conversely, referring karyogamic rejuvenescence to mere constitutional invigoration, we can see that agamy and apogamy are harmless conditions where other modes of rejuvenescence prevail.[1]

C. Allogamy and Sex.

Again, if the invigorating effects of karyogamy be due to the mere infusion of new blood into the firm " Cytoplast, Nucleus, and Co.," it is, of course, an advantage that the new blood should be as new as possible within the limits of possible harmonious co-operation—that is, usually, within the bounds of the race or species. And this must have been the cause which determined exogamy in the lowest organisms. In this case the members of a brood of gametes are incapable of entering into fertile karyogamic union with one another, being affected, as we may say, by the disqualification of consanguinity. This disqualification has been attributed to a latent sexual differentiation ; but to admit this view is, as we shall see at once, to deprive the word "sex" of the connotation of a differentiation of organisms into two complementary categories.

For if exogamy implied any sexual differentiation in the ordinary sense, and we considered any twenty-six broods of gametes, say of Botrydium, they should fall into two categories, which we will term A—M, N—Z respectively ; any of the gametes of the former category would be incapable of forming fertile union with any other of its own category, but would pair freely with any of the other, and vice versâ. But

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[1] It is interesting to note that Ferns, which are dimorphic, have a resting state (spore) interposed between the terrestrial Fern-plant and the palustrine prothallus, a sexual karyogamic union between the palustrine and the terrestrial states. In some exceptional cases the one or other transitional form of rejuvenescence is omitted : in apospory the palustrine form supervenes without the resting state of spores ; and in apogamy the terrestrial state supervenes without karyogamic rejuvenescence : but a combination of these two phenomena is not known in the same species, or I think I may say the same group.

no two such categories exist; on the contrary, all the evidence goes to prove that a gamete of the A brood will pair with one of any other brood from B to Z, and so on right through the alphabet. Maupas has told us that for two Ciliates in the eugamic state to pair successfully they must belong to two different cycles of descent—that is, they must be descended from two different exconjugates. If we use the term sex for such cases as these we must admit the existence of as many sexes as there are broods or cycles of the species in existence, and that difference of sex means not a binary antithesis of characters, but a mere question of kinship, which is a reductio ad absurdum.

We see, then, that exogamy is merely the expression of consanguineous incompatibility, or allogamy, as it has been long termed. So far from indicating latent sex, allogamy may or may not coexist with very high binary sexual differentiation. In Orchids, for instance, side by side with the majority of flowers adapted for cross-fertilisation exclusively, we find one or two species that are " autogamous" or self-pollinating. If we call allogamy by the name of " sex," it is a sex superimposed on ordinary binary sex, and distinct from it; and the question occurs here, in an allogamous species, How many sexes are we to ascribe to the innumerable individuals, each incapable of self-pollination, but capable of fertilising the flower of any other individual?

We must remember, too, that in many isogamous forms, even those which are exogamous, like Acetabularia, conjugation may be multiple, as many as five gametes uniting into the single zygote. Admitting the supposition that exogamy involved latent binary sex, what would be the several functions of each of these five gametes? The only conclusion left us is the one we have stated, that exogamy expresses not an early form of sexuality, but a growing sensibility of the organism to the fact that the advantages of karyogamy are not fully gained by the union of closely allied gametes; and this fastidiousness we find an increasing factor as we ascend the scale of karyogamic unions.

D. The Origin of Sex.

If we seek for the origin of binary sex we may find a clue in the history of Ulothrix, or even better the Volvocine Pandorina, referred to above (p. 9).[1] The gametes of this last species are of three sizes, micro-, meso-, and mega-gametes, which we may letter a, b, C respectively. These are in the first place strictly exogamous, but subject to this con-dition the following unions are said to be possible—a+a and b+b (isogamous), as well as a+b, a+C, b+C (anisogamous) : but the other conceivable pairing, C+C, does not occur; as if, con-current with its enlargement, the form C had become too inert to form isogamous unions. We might say that a and b are sexually differentiated with respect to C, but not between them-selves or with one another. We may conceive that the game-togenic divisions in a species being inconstant, broods of gametes would be formed whose size was inversely propor-tional to the number of the brood ;[2] the extreme forms would be small active gametes and large sluggish ones respectively. As the latter are ill fitted to conjugate among one another, in the struggle for pairing the small numerous active ones would be most likely to find pair with these large ones, and the rejuvenescence of such unions would be the more efficacious because of the difference of temperament between the parent gametes. The middle forms being produced in smaller numbers than the little gametes, and less useful either way, would tend to disappear. The difference of size between the micro- and mega-gametes would tend to increase and a division of labour take place, the megagamete tending to accumulate nourishment to give the zygote a good start, the microgamete gaining activity

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[1] The following account is based on the abstract in Bütschli, op. cit., p. 788.

[2] In Pandorina, however, each gametogonium forms eight gametes, large, medium, or small as the case may be. In Ulothrix the number is inversely as the size : the smallest are capable of isogamous union, which is the rule ; but they are also capable of anisogamous unions with larger, more sluggish zoospores.

and delicate sensibility ;[1] and by this differentiation of temperament the zygote would be the gainer. This I take to be the ORIGIN OF SEX. Once started in some such way, the difference of temperament between the gametes would tend to be more and more accentuated and, so to say, crystallised; and this would be as it were anticipated, first in the organs and then in the individuals producing the gametes. I accept then one main thesis of the "Evolution of Sex," that male and female are distinguished by their respective temperaments; though it is obvious that I reject utterly its theory of sexual karyogamy that the male brings "katastates," the female "anastates," which combine to make the zygote a perfect organism equipped for any event.

I have stated that I consider the difference of temperament to be the advantage brought by bisexuality; and in allogamic bisexuality this advantage is doubled : hence the many indications on which has been based the old adage that "nature abhors perpetual self-fertilisation." But, on the other hand, if we admit that allogamy is, like karyogamy itself, a mere "acquired need" or "necessary superfluity," we have no difficulty in understanding the continuance and hardiness of many self-impregnating flowers, and of that sturdy group of self-fertilising animals, the parasitic Flat-worms.[2]

E. PARAGENETIC PROCESSES, USUALLY COMPRISED UNDER THE TERM "PARTHENOGENESIS."

By PARAGENESIS I designate all modes of reproduction in which a body, not the zygote, simulates the behaviour of the

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[1] The way many Arthropods find their mates by smell is well known; spermatozoa of plants find the oosphere owing to their very delicate sensibility to chemical stimuli.

[2] The variation in individual susceptibility to harm from close breeding is extreme. The human race is usually believed to suffer greatly from close breeding; and yet some of its hardiest and finest specimens are the members of fishing communities, isolated by position or by custom, and bound together by the closest and most complex ties of blood. Similar facts as regards the vigour of cleistogamous and other self-pollinating types of plants have led to many attacks on the adage cited above, made notably by A. W. Bennett, G. Henslow, Neehan, &c.

zygote in the same or allied forms. Logically, of course, all these being processes of rejuvenescence should have been treated earlier, but the foregoing discussion was necessary to the full understanding of the analysis of paragenetic phenomena which I now proceed to give.

1. TRUE PARTHENOGENESIS we define as the development of a single unfertilised (facultative) gamete. It occurs in the following cases :

a. ISOGAMETES.—Not infrequently facultative.

b. MICROGAMETES.—Only known to be facultative in Ecto-carpus; the reduction of cytoplasm is too heavy a disadvantage for the resumption of active cellular life.

c. MEGAGAMETES are more frequently facultative in the lower Algæ and in Chara crinita. In other Metaphytes parthenogenesis is unknown. The only cases of true parthenogenesis of the Metazoan oosphere, differentiated as such by the formation of both polar bodies, are the following —Liparis dispar and some other Lepidoptera, and Apis (Drone eggs).

2. SIMULATED CELLULAR PARTHENOGENESIS occurs when a vegetative cell that might otherwise have formed a gamete assumes directly the behaviour of a zygote ("azygospores" of Conjugatæ, "auxospores" of certain Diatoms).

3. SIMULATED APOCYTIAL PARTHENOGENESIS occurs when an apocytial gametoid assumes the behaviour of a zygotoid ("azygospores" of Mucorini).

4. PROGAMETAL REJUVENESCENCE occurs when a progamete assumes the behaviour of a zygote. This is stated to be the case in many Arthropods where the ovum, after the expulsion of one polar body only, develops without fertilisation. According to the recent discoveries of O. Hertwig and Boveri, many cases referred to this (if not all) should be placed under the following heading.

5. METAGAMETAL REJUVENESCENCE is the best term I can find to fit the case of certain flowering plants (Cœlebogyne, Citrus, Funkia, Nothoscordum), where the tissue-cells adjoining the apex of the embryo-sac grow into it, and

assume the behaviour of zygotes by developing as normal embryos.[1]

6. PARAGAMY comprises those cases where the fusion of sister-nuclei replaces the advent of a male nucleus.

a. OOPARAGAMY occurs in the Metazoa in this wise: after the first polar body is formed a second polar spindle is formed in the egg, as if to form a second polar body; but the nucleus corresponding with the second polar body moves back again to fuse with the nascent oosphere nucleus. This has been observed in Ascaris and Pterotrachea by Boveri, and in Asteracanthion by O. Hertwig. The fusion nucleus thus formed has the same number of chromatic elements as the normal zygote nucleus, double that of the gametogonium; and it is essentially different from the nuclei produced by mere fission during the whole cellular cycle since the last rejuvenescence.

b. APOCYTIAL PARAGAMY occurs when the fusion of the nuclei of an apocytium wholly replaces the formation and union of gametes. This occurs in Saprolegnieæ and Derbesia.

IX. GENERAL CONCLUSIONS.

The following theses state concisely the results of our inquiry:

1. Absolutely agamous forms exist in the group Monadineæ; in these REST is the only agent of rejuvenescence.

2. CHANGE OF THE MODE OF LIFE is a frequent mode of rejuvenescence in apogamous and self-fertilising organisms.

3. In the higher Monadineæ and the Myxomycetes a plasmodium formation occurs, so that the cytoplasm is renewed by PLASTOGAMY, and the nuclei wander from their original cytoplasts.

[1] The apogamic development of the plant of Pteris cretica from a group of cells in the prothallus, instead of from a fertilised oosphere, comes very close to this group of processes. I should note that apogamy is a purely negative word, implying solely the excision or loss of a sexual process from the life-history of an organism; but this may be effected and compensated in most divers ways, which might be classified if it were not rather outside the scope of the present essay to do so.

4. Isogamy, plural or binary, is a step in advance of plasmodium formation, involving, as well as plastogamy, KARYOGAMY, or the reconstitution of a nucleus by the fusion of old ones.

5. The rejuvenescence of karyogamy is due to the fact that the zygote nucleus and cytoplast form a new cell association.

6. A similar rejuvenescence may take place by the mere migration of a nucleus into a vacant foreign cytoplast, as in the union of a spermatozoon with the non-nucleated fragment of the egg of an Echinoderm.

7. Many cases of so-called "parthenogenesis" involve really the fusion of nuclei, the resulting nucleus being essentially different from the fission nuclei of the previous cell-cycle.

8. Other modes of rejuvenescence may replace the karyogamy of gametes (e. g. a prolonged rest of the gametogonial cell of Botrydium gives its brood-cells a power of independent development instead of the tendency to unite as gametes).

9. Those organisms that have attained the capability of karyogamic rejuvenescence may, by prolonged fissile reproduction without karyogamy, pass into a senile condition marked by reproductive incapacity. In these, therefore, karyogamic rejuvenescence has become essential to the preservation of the race.

10. Rapidly repeated nuclear fissions, without sufficient interval for nutrition and recovery, may lower the vital energy or constitution of the cell, and accelerate this reproductive incapacity ; and this may be the physiological import of the fissions that so frequently differentiate the gamete, and determine its obligatory character.

11. The reproductive incapacity of most microgametes finds, however, a sufficient explanation in the extreme reduction of their cytoplasm.

12. The reproductive incapacity due to long or rapidly repeated acts of fission uninterrupted by karyogamy is a matter

of constitutional temperament or vigour characteristic
only of the race, for—

(a) It is absent in primitive, agamous types.

(b) It is slight in groups where parthenogenesis occurs,
though often absolute in closely-allied forms.

(c) It has been lost in apogamous groups.

13. A further evolution of this constitutional weakness takes
place in forms which are either (a) exogamous or (b) sexually
differentiated.   Here the nuclei that fuse to remove this re-
productive incapacity by rejuvenescence must be of distinct
origin.

14. Exogamy of isogametes cannot be taken as indicating
latent sex ; it is merely the expression of karyogamic incom-
patibility of close blood-relations ; this, under the name of
Allogamy, has been long since recognised when associated
with, and superadded to, bisexuality.

15. The constitutional weakness reaches its highest degree
in those organisms where allogamy is most marked ; the evil
effects of close-breeding are commensurate with the habitual
advantages of cross-breeding which has here become an
" acquired need."

16. Here again we find, from the occasional existence of
types, strains, or even couples, whose offspring does not
degenerate from close breeding, that the need for allogamy is
not absolute, but a question of constitutional weakness or
vigour.

17. Since, in all cases of plasmodial and karyogamic reju-
venescence, we find the migration of the nucleus to foreign
cytoplasm, or the reconstitution of the cytoplasm or of the
nucleus, or a combination of these to be the sole necessary
factors : we infer that the constitutional weakness of the
later terms of a cycle of fission is largely due to the
continuance of the association of nucleus and cytoplast
unchanged.

18. From considerations of (a) the known functions of the
nucleus ; (b) its chemical composition ; (c) the effects of rest,
change of form, or change of habit (polymorphism and

heterœcism) in effecting rejuvenescence, and often replacing
karyogamy: it is suggested that the evil effects of the pro-
longed association of cell and nucleus are due (a) to the
nucleus responding less actively to the stimuli from the cyto-
plasm ; (b) its consequently inadequate directive power; (c) to
the resulting bad performance of its work by the cytoplast;
(d) to the imperfect nutrition of the nucleus; (e) the failure
of the cell as an organic whole.

19. The process of nuclear reduction in progametal cells
and gametes is, though general, neither uniform nor universal.
Its occurrence in the pollen-mother-cells of Flowering Plants
leads us to anticipate its occurrence in the mother-cells of
broods of reproductive cells generally, sexual or asexual.
Pending the settlement of this point, explanation is premature.

20. Replacement theories of fertilisation are inadmissible,
since all fail to account for one or more of the following facts :

(a) Multiple isogamy.

(b) The non-discrimination of the broods of exo-isogametes
into two categories, of which the members of either would pair
with those of the other category, but not of their own.

(c) The absence of " excretion phenomena " of any kind in
so many cases of gametogeny.

(d) The existence of true parthenogenesis of male as
well as female gametes.

(e) The formation of a male individual from the exclusively
female oosphere of the Hive-bee.

We have now finished our task ; the theory (theses 1—17)
and the hypothesis (18) set forth are based only on facts lying
in the fields of biological observation and experiment; an
undertaking which should be more profitable than castle-
building in the shadowy dreamland of à priori speculation.

CORK, March 3, 1891.[1]

[1] Section VI, A was written after the completion of the rest of the MS.,
and again modified during its passage through the press.   Slight alterations
and additions have been made at the same time in the rest of the study
(July 30th, 1891).

Postscript.—In writing the foregoing essay, and even in seeing it through the press, I omitted to state one cardinal point, really underlying the whole theory of gametogeny now proposed; but the necessity of expressing it in set terms appeared as soon as I had to consider the best mode of presenting a concise account of my views to an audience of the British Association in Cardiff (August 22nd), 1891. Two distinct modes of fission occur in relation to the growth of the organism in Protozoa and Protophytes : in the first, after each division the daughter-cells grow to the size of the parent (more or less) before dividing in turn; in the second, the intervals of growth are suppressed, and a series of successive fissions takes place, resulting in a brood of small individuals ("swarmers," "zoospores," &c.). We call this second type of fission "brood-formation," the resulting individuals "brood-cells." Necessary, like facultative, gametes are essentially, in origin at least, modified brood-cells. Hence, when the ancestral development is not lost, gametes will always be produced by brood-formation, while tissue-cells (except in the earlier embryonic state) are formed by the first mode of fission.

September, 1891.

On Wandering Cells in Echinoderms, &c., more especially with Regard to Excretory Functions.

By

Herbert E. Durham, M.A.,
King's College, Cambridge, and Guy's Hospital, London.

With Plate I.

INTRODUCTORY.

THE following observations have been made partly during two visits to the Marine Laboratory at St. Andrews (July and August, 1887, and March and April, 1888)—for the facilities there offered and for every kindness I have to thank Professor M'Intosh; partly in the Morphological Laboratory at Cambridge, thanks to the indulgence of Mr. Sedgwick, during the Michaelmas Term, 1888, when fresh material obtained from Jersey and that preserved at St. Andrews were examined; partly also at home, living material being obtained from the Laboratory of the Marine Biological Association at Plymouth, for sending which I must here tender my thanks to the director and his assistants.

Lastly, I must thank the late Dr. P. H. Carpenter for his kindness and ever ready advice, as well as Mr. S. F. Harmer for criticism and for references.

The following forms have been used during the investigation :

Asteroidæ	Asterias rubens (young and adult).
	„ glacialis.
	Cribrella ocellata (vel sanguinolenta).
	Solaster papposus.
	Asterina gibbosa.
Ophiuridæ	Ophioglypha lacertosa.
	Ophiocoma rosacea.
Echinoidæ	Echinus sphæra.
	Spatangus purpureus.
	Amphidotus cordatus.
Insecta	Dytiscus marginalis.
Mollusca	Unio and Anodonta, sp.

I. On the Fate of Insoluble Foreign Particles.

In the 'Proceedings of the Royal Society' (26) I have already shown that insoluble granules introduced into the body-cavity of the common starfish (Asterias rubens) are ingested by leucocytes; and that these particle-holding leucocytes become first adherent to the cœlomic epithelium of the dermal branchiæ, and then by their own amœboid power make their way through the wall of the branchia to the exterior; arrived at the exterior these leucocytes disintegrate. If the migration is going on actively, small clumps of colour visible to the naked eye may be formed at the summits of the branchiæ; such clumps consist of granule-holding leucocytes on their outward journey.

The same result was obtained when the experiments were repeated in the spring of 1888.

It seemed of interest to see what became of insoluble particles introduced into other animals; for though it is well known that such material is ingested by amœboid cells, yet the subsequent fate of these pigment-containing corpuscles has not been traced in many instances.

The only animal I have so far experimented with has been Dytiscus marginalis; one set of experiments was made with Indian ink rubbed from the solid stick in normal physiological salt solution (sodic chloride, ·75 per cent.). (Another set

was attempted with defibrinated rat's blood, but these latter did not give any results worth recording here.) The Indian ink was injected with a hypodermic syringe into the abdomen ; an examination of the blood fluid soon after showed that many of the amœboid blood-corpuscles had taken up one or more particles of the Indian ink, which gave the fluid a dusky, dirty appearance to the naked eye. Even after the lapse of a month (thirty days) there were found to be still some free granule-containing leucocytes, though not so abundant as at earlier periods.

What interests us here is that, in specimens which have been kept for some time after the injection (e. g. ten days to thirty days), there were seen to be small black specks, visible to the naked eye, which were scattered about in different regions of the body, especially about the heart and the more dependent parts of the abdomen. When examined under a low power each dot was found to consist of a central mass of black carbon particles surrounded by a clear zone formed of nucleated cells, some of which also contained granules of carbon.

It might be said that these collections of pigment and cells were usually close to or attached to the tracheal tubes, were it not that these structures are so ubiquitous in their distribution ; they are mostly of spherical shape after ten days or thereabouts, but after longer periods they may become larger and more elongated, losing the spherical shape by the addition of fresh cells with carbon particles (fig. 1). These small masses originate about a centre which may begin as an accidental collection of carbon particles which have become stranded close together, and become afterwards invaded more or less by leucocytes ; or the carbon particles may be first ingested by leucocytes which subsequently come together ; in both cases other leucocytes, either with or without carbon granules, become adherent to the mass and add to its size. After getting attached to the mass they become flattened and appear crescentic in section, the nucleus appearing elongated ; thus a capsule is formed round the mass.

It is usual for each mass to be multinuclear—that is, there are several foci in close approximation.

Whether or no the cells of the neighbouring trachea and connective tissue take a share in the capsule is not of so much interest as it is in the inflammatory processes of Vertebrates, because there is no formation of definite fibrous tissue, so far as I have been able to observe. It is possible that some assistance may be afforded by the connective tissues in the neighbourhood; but anyhow all stages can be seen between the rounded leucocyte with its spheroidal nucleus and the thinned-out cell with its altered nucleus (cf. fig. 1).

By such a process of encapsulation the particles are removed from the circulation, and thus prevented from causing injury or irritation to the tissues with which they might come in contact.

In Section III this aspect of the matter will be more fully dealt with.

II. On Diapedesis of Leucocytes containing Products of Normal Metabolism.

In the above-cited paper (No. 26) I also called attention to the emigration of amœboid cells from the body of individuals of Asterias rubens; which amœboid corpuscles contained refringent spheres, and were called spheruliferous corpuscles, and led to the formation, with assistance of mucous secretions, of a brownish slime on the surface of the animal: this slime was found to contain such corpuscles in various stages of dis-integration (26, pl. iii, fig. 4).

Vogt and Jung, in describing the dermal branchiæ of Astropecten aurantiacus, say, "Auf dem Gipfel des Röhrchens [= branchia] fliessen die Zellenreihe zu einer Art Haube zusammen. Aber auf allen Schnitten haben wir stets in der Spitze des Röhrchens einen rundlichen Propf von kleinen Zellen gesehen die sich nicht wie die andren Gewebe färbten und eine gelbe Farbe und wachsartiges Aussehen hatten. Diese Pröpfe könnten von der Coagulation der in

den Röhrchen enthaltenen Flüssigkeit durch die Reagentien herrühren : aber ihre Constanz und ihre Zellenstructur spricht aber gegen diese Auffassung : wahrscheinlich gehen sie aus einer bedeutenden Verdickung des Endotheliums des Röhrchens hervor " (No. **6**, p. 589).

I have not examined A. aurantiacus, and cannot quite understand what is here referred to ; probably it is a patch of pigmented cœlomic epithelium, such as occurs also in Asterina gibbosa. However, from the curious woodcut in illustration of the description one might suppose that the cells described were in reality "spheruliferous" leucocytes forming a plug at the apex of the branchia previous to their diadermal wander.

Cuénot (No. **4**) says, "La couche épidermique extérieure renferme beaucoup de cellules glandulaires comme celles que nous avons décrites à propos des téguments:" it seems not unlikely that some of these "cellules glandulaires" may only be spheruliferous corpuscles : vide also Prouho (**11**), and cf. the corpuscles of Holothurians. The process of emigration is far better marked in the ambulacral branchiæ of the Urchin. Hamann (No. **1**, Echiniden) has already both described it and hinted at its meaning. In his description of the gills he says, "Sie sind Ausstülpungen der gesammten Körperwand anzusehen und morphologisch wie physiologisch gleich zu setzen den Kiemenbläschen auf dem Rücken der Asteriden." This is more true than Hamann thought, for by means of these processes spheruliferous corpuscles are got rid of in both groups : when talking of these spheruliferous cells he remarks "da die Bindesubstanzschicht über und über von ihnen[1] erfüllt wird, sowandern sie wahrschemlich aus dieser in das äussere Epithel, um vielleicht von hier nach aussen zu gelangen " (cf. taf. vi, fig. 12).

I have observed the same process in Echinus sphæra ; when a portion of a branchia is removed and examined it will be seen that the apices are occupied by a dusky brown mass, and that scattered about in the wall are smaller masses of

[1] "Eiförmige, stark lichtbrechenden Körnchen erfüllten Zellen."

similar colour. These are collections of large cells containing refringent spheres; they break up very readily with slight pressure; even in the fresh living condition they can be seen at various levels in the wall of the branchia as well as actually outside it.

Much trouble was experienced in preparing sections where the epithelium was extensively infiltrated with spheruliferous cells, as appears to be frequently the case, because the cohesion of the epithelium is much diminished, and therefore it easily breaks up and disintegrates in the hardening and other preparatory processes.

The spheruliferous cells found here differ from those which are found so abundantly in the dorsal organ, &c.—(1) in having a more dusky brown appearance, the majority of those of the dorsal organ, &c., being of a faint yellowish colour (corpuscules mûriformes), and (2) in disintegrating very readily. So that there appears to be two forms of sphere-holding corpuscles; in the dorsal organ one sees corpuscles containing some of the clearer and some of the more dusky spherules as well as some containing only dusky ones, besides the more abundant cells with the clearer cells; now as the dusky ones are found leaving the body through the branchiæ it seems possible that they have performed their functions, whatever they may be, and are no longer of use in the economy of the animal: whether the bright orange-red pigment-corpuscles have any share also in the process of transformation I have not been able to determine.

The spherules of the corpuscles found in the branchiæ stain very deeply with acid hæmatoxylin, as well as with aniline colours. Hamann pointed out that they stain with anilines but not with carmine, which my sections confirm.

In the irregular Echinids (Spatangus purpureus and Amphidotus cordatus) the process of removal of products from the body by means of amœboid cells is seen to be more definitely associated with pigment. In these forms there are some cells with pigments of a brighter colour which readily dissolve in alcohol, &c., and are not easily recognised in pre-

pared specimens; and others with brown or blackish pigment which remains undissolved, and is therefore readily seen in sections from hardened specimens. These latter, with the insoluble pigment, take part in a process of wandering out from the body; this may happen—

1. At any point on the free surface of the body.

2. In the neighbourhood of the circumoral rosette feet (Rosetten füsschen) and in the feet themselves.

3. Into the tubes of the madreporite.

Outwandering is seen occurring through the ordinary epidermis in fig. 3. In the oral feet Hamann has figured and described the presence of pigment-cells (1, taf. ii, fig./7), but he gives no suggestion as to the meaning of their presence. In young specimens only a few scattered pigment-cells leave the body by the madreporite—in older specimens the process goes on more actively; in some cases, e. g. the specimen from which fig. 2 is taken, it is going on exceedingly vigorously. In this specimen pigment-holding cells are seen abundantly in the ossicular tissue of the madreporic plate and in the connective tissue surrounding the canals into which the water tube (" stone canal ") has broken up. They are also seen amongst the epithelium-cells which line these tubes and the pore canals of the madreporite itself, whence they pass into the lumen of the tubes.

From here I considered that they were carried to the exterior by the outward ciliary current described by Hartog (No. 17); but since this was originally written Ludwig (18) has denied that the current is outward, and he states that it has an inward direction. Upon this point Cuénot (4, p. 85), speaking of Asterids, says as follows:—

" L'observation des animaux vivants nous montre que le madreporite n'est le siège d'aucun courant d'eau, ni pour l'entrée (Jourdain, Perrier, &c.) ni pour la sortie (Williams, Hamann). . . . Enfin si l'on met une Astérie dans un bac rempli d'eau colorée, on voit qu'il n'en pénètre pas du tout par le canal de sable." Unfortunately, from not having been near the sea, I have been unable to work at the point; but I think

that, taking into consideration the undoubted outwandering of similar pigment-cells from the free surface (fig. 3), we must believe that those seen in the madreporic tubes are likewise travelling to the exterior. It is noteworthy that free wander-cells are usually present in the madreporic tubules both in Asterids and in Echinus sphæra. Sometimes the pigment, probably owing to the death and decay of the cells containing it, never reaches the exterior, and masses become formed in various regions and organs of the body : such masses have been frequently described (e. g. Hamann). They consist of pigment-granules, some free with nuclei scattered here and there, others definitely located within corpuscles. It would seem as if the protoplasm of the cells became exhausted before its duties had been entirely fulfilled. It appears that the older the specimen the more abundant the pigment; it is possible that other observers have chiefly confined their sections to young individuals in which the process is not so marked, and therefore has been overlooked.

III. Considerations on the above.

We have now to consider the importance of these two processes :—

    A. The reaction to minute foreign bodies.

    B. The use of wander-cells in getting rid of effete material from the system.

A. In a very large number of animals, of one kind and of another, it has been frequently shown that small foreign particles are ingested by amœboid cells (vide Metschnikoff, 49, &c.) ; but as regards the ultimate fate of these particles, except in the case of mammals, our knowledge is extremely limited : in the known cases most of our information on the matter has been obtained from observations upon man. For instance, it is now very well known that in persons who are exposed to a dusty atmosphere (townspeople, miners, stone-masons, &c.) the lungs are found to be deeply pigmented, the

pigmentation being largely due to a deposit of the particles inhaled : besides the lungs, the bronchial lymphatic glands are found to contain collections of similar material; and this has been brought there by the industry of leucocytes which have ingested the particles, gained the lymph-stream, and so arrived at the glands (vide Ziegler, No. **62**, ii, p. 673; Klein, No. **38**).

Many of the particles, however, remain permanently in the walls of the lymph-vessels, and of the alveoli of the lung, and cause the pigmentation seen after death: some, again, may be got rid of by expectoration.

Again, in the case of tattooing (Ziegler, i, p. 128), the lymph-glands corresponding to the parts operated on are found to contain particles of the material used. In such pigment-containing lymph-glands the pigment remains permanently, the tissue often undergoing some fibrous change. In blood effusions cells filled with blood-corpuscles occur in the lymph-glands, the hæmoglobin becomes altered, and eventually the resulting pigment may be absorbed and disappear (Cohnheim, ' Allgem. Path.,' New Syd. Soc., i, p. 408). In such cases the pigment remains in the system—that is, it is not carried to the exterior directly. In some instances, however, insoluble granules seem to have been entirely got rid of, for in dogs and frogs (Ziegler, **62**, i, p. 40), when particles were injected into the blood circulation, some were actually got rid of through the pulmonary alveoli and sputum, some were found in the bile, the path by which they got there not being quite clear; and further in dogs some were found in leucocytes in the tonsils, by which they were being carried to the free surface through the epithelium. We shall refer again to the tonsil, but it is right to mention here that Armand Ruffer (No. **53**) describes the presence of carbon particles within leucocytic cells in the dog's tonsil, and he regards these as coming from the free surface into the organ. Which end of the balance contains the true and which the false weight is as yet doubtful. Hence it would be inadvisable, perhaps, to make any comparison with the process above described to occur in Asterias rubens, whereby

there is an actual transference of irritating insoluble material to the exterior.

In connection with this we might cite the bursting of a sub-cutaneous abscess (e. g. caused by and containing pathogenic schizomycetes) as an indirect attainment of the same result by means of leucocytic cells. With regard to the encapsulation of foreign particles, such as is above described in Dytiscus, we have many parallels in other animals, both with organic and with inorganic noxæ.

Reference has already been made to the fibrous hyperplasia which is caused in lymphatic glands by the irritative presence of pigment particles, and which prevent these particles from being distributed about the system.

Are not the thick fibrous walls of chronic abscesses to be regarded in the same light? as also in the case of gummata and the well-known healed phthisical foci, in which latter the tough fibrous surrounding may be often seen to have entirely prevented a general infection of the system as well as local spreading of the disease.

It seems not unlikely that in the case of carcinomata a similar endeavour is made by the tissues, or more exactly by the connective tissues, to prevent the spread of those most baneful growths. In those individuals whose tissues have the power of reacting with vigour to the stimulus, and of forming firm fibrous tissue readily, it is the hard scirrhus type that occurs ; indeed, by means of the contracting power of the new-formed (reactive) tissue, in rare cases the nutritive supply of the tumour may be so diminished that the tumour, as it were, cures itself (Bryant, 'Diseases of the Breast,' 1887, p. 142), or at any rate allows the patient to survive for considerable periods.

On the other hand, in the softer varieties or in secondary growths in the liver there is little or no check upon the growth of the tumour, which consequently can and does spread rapidly.

Such a view—viz. that the fibrous tissue present in a car-cinoma is formed owing to the irritation and stimulation by

the "cancer-cells," or possibly by some as yet unknown virus casually associated with these cells—is contradictory to the view set forth by Cornil and Ranvier (**23**, p. 199, et seq.) that the mesoblastic stroma forms the prime part of the tumour growth—a view, however, which has not found favour with other writers.

If the stroma of the growth is regarded as above suggested, the process of formation is quite in accordance with the deposition of fibrous material around other irritative objects.

Indeed, without necessarily upholding "vitalistic properties" on the part of the tissues, a matter so vehemently fought against by Haidenhain (No. **35**), there are two phenomena, related to the process of inflammation, which are especially beneficial to the body at large owing to the shelter they afford:

1. The exudation of fluid from the blood-vessels, whereby the lymphatic vessels are flushed, and any poisonous substance present is diluted before it is submitted to the purifying action of the lymphatic glands.

2. The formation of fibrous tissue round an irritant, which tissue of itself and by its subsequent contraction tends to prevent the spread of the local morbid condition.

B. We now come to the consideration of the part played by wander-cells in the normal economy of animals. This is so vast a subject—a subject with such numberless and crooked bypaths—that we cannot pretend to go at all fully into it, and we shall confine our attention particularly to the power of ridding the system of effete matters by means of the intervention of wander-cells.

It has been shown above that in many Echinoderms some substances are actually removed by the activity of such cells. Is this to be regarded as a process of ordinary excretion (urates, &c.)? All that can be answered so far is that both the slime collected from Asterias rubens and an extract of some thirty dorsal organs taken from the same species gave no results with the murexide reaction for uric acid.

Griffiths (No. **31**) has found uric acid as a constituent of the clear fluid contained in the "stomach-sacs" in starfish; but

he does not show thereby that the uric acid he found there was excreted by the animal; it is possible that it arrived into the starfish's stomach in the interior of small mussels, &c., whose excretory organs contain that body, and of which as food the starfish appears to be very fond; anyhow, such a source of the uric acid must be eliminated before Griffiths' conclusion can be accepted; for obviously, if the presence of urates were demonstrated in the gastric contents of an individual who had recently supped off oysters, it would by no means follow that the stomach was the organ whereby the individual excreted his urates.

Now, uric acid and its allies are by no means the only effete products excreted by animals which are known to get rid of these bodies; in reference to them, however, I feel inclined to agree with most authors in regarding the abundant osmosis that goes on in the animal sufficient to account for the excretion of the more soluble substances.

Kowalevsky (No. 40) has shown that in Echinids the dorsal organ takes up carmine, whereby it differs from urate-excreting organs, which take up indigo-carmine. It will now be advisable to notice what evidence there is of an excretory process by means of wander-cells in other animals.

Kölliker (No. 43) has shown that the pigment present in hairs, epithelium, and nails is derived from pigment-cells, which pass outwards from the cutis. He has investigated the point in many animals—man, anthropoid apes, cetaceans, &c.

Karg (No. 37) has demonstrated, by grafting epidermis upon ulcers in white men and negroes, that pigment-cells wander up into the epidermis and cause the pigmentation.

Riehl (No. 55) shows that in hairs the pigmentation is due to the intrusion of pigment-cells from the cutis into the epidermal layer in man; in a short paragraph he states that after cutaneous inflammation wander-cells containing yellow or dark brown pigment are seen in the cutis, whence they get into the stratum Malpighii.

Ehrmann (No. 28) gives an account of the process in Amphibia and mammals.

Gaskell (No. 32, p. 39) regards it likely that pigment is got rid of in Ammocœtes, &c., by " excretion into the skin."

J. H. List (No. 48) describes the formation of pigment granules in red blood-corpuscles, and their travel to the surface within wander-cells; moreover he definitely considers that the pigment is to be regarded as a " Zerfallsproduct" or an "Exkretionsproduct;" he also gives many references.

Leydig (No. 45), in a most interesting paper, calls attention to the different methods of external pigmentation; in snakes, &c., he shows that white and whity-yellow patches owe their colour to the presence of stellate cells containing granular concretions, which concretions consist chiefly of a uric acid holding substance (guanin), probably crystalline in form, with a proteid base. Such cells occur both in the cutis and amongst the epidermal cells, similarly to the cells containing dark pig-ment-granules. The following (from Section IX, p. 260) is worth quoting :—" Waren in den Vorausgegangenen Hautfär-bungen, so bald sich um wirkliche Farbkörper handelt, diese stets innerhalb der Gewebe des Integuments abgelagert, so gibt es endlich eine ganze Anzahl von Färbungen, welche Erzeugnisse von Hautsekreten sind, nach aussen gelangen und daher abwischbar werden."

Besides amphibians and reptiles he has demonstrated the presence of urate compounds in the skin of a mammal (Chry-sochloris), and remarks that it would be well worth while look-ing for such chemical bodies in feathers, hair, &c., of higher Vertebrates. In Vertebrates there is another organ—the iris—where guanin compounds (" guaninhaltige pigment") exist. In Invertebrates he has shown pigmentation to be due to a uric acid holding body in Asellus, Syrphus, certain slugs, and (quoting Eisig) in Capitellidæ. It is interesting to note, in relation to Dr. Gaskell's ideas on the meaning of the pigment about the brain, that Leydig states (p. 256) that he has not observed any contractile power in the pigment-cells of the dura mater in frogs.

Recently Kodis (No. 42) has opposed the view that the leucocytes in the epithelium have arrived there from subjacent

tissue, and he contends that they arise endogenously in the outermost layer of epithelial cells and travel inwards; so also with the pigment-cells. The evidence he brings forward, based largely on the relative numbers of cells in the different layers, is far from conclusive. His descriptions and figures are complicated by spherules of various staining powers, which in many cases seem to be food-yolk granules present, owing to the larval condition of the material he worked with—a condition which may account for the similarity he noticed between some of his "leucocytoid" cells and epithelial cells which had not yet attained to a complete individuality.

It is rather odd that the outermost layer of epithelial cells should be the active ones which give rise to the wander-cells ("Leucocytoiden und Perigenzellen"), as he maintains, his reason being that it is only in the outermost layer that "endogenous formation" is seen to occur; is not this appearance more probably accounted for by the decreased vital activity of the cells of the outermost layer having become unable to resist the invasion of the outwandering cells, which have consequently gained admittance to their interior? Moreover the guanin compound, as described by Leydig, is undoubtedly an excretory product, and there is considerable evidence[1] that the "melanin" granules are a product arising in the disin-

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[1] Sheridan Delépine (25), in a preliminary report of his work, considers that "melanin is elaborated in certain epithelial cells like other products of glandular activity out of plasma, and is not a derivative of hæmoglobin;" and that "an antecedent, a variety, or a derivative of melanin" passes to the lymphatics of the skin, and probably has something to do with the production of hæmoglobin. It is certain that pigment-cells exist in the cutis and amongst the epidermal cells, and pigment-granules within the latter; it is, therefore, a matter of interpretation as to which way the pigment is passing, for Delépine states that the process of solution of the melanin, and its absorption by the lymphatics of the true skin, are not visible. In frogs and newts there can be no doubt that "melanin" is got rid of through the medium of the skin, and is therefore to be considered as effete material; following List and others, it appears to be formed from hæmoglobin, the ferruginous portion of which may be used again for the formation of new hæmoglobin in the tissues which produce that substance, as Delépine supposes.

tegration of hæmoglobin, a process which Gaskell (No. **32,** p. 33) and J. H. List, among others, have followed histologically; so that I think we may conclude that such effete products would not be reabsorbed into the system, and that we may fairly adhere to the view which is advocated by Kölliker, &c.

It seems exceedingly probable that this process of excretion by means of wander-cells occurs in many other animals, and that many of the " mucous " and pigment-cells, &c., described in epidermis may really be of the nature of wander-cells, whose onward progress has been stopped for ever by the re-agents of the histologist. For instance, many of the leeches must have a very active "pigment metabolism," judging by their botryoidal tissue and branched pigment-cells. The following quotation from A. G. Bourne (No. **20,** p. 429) is not without interest :

" Two varieties of connective tissue may intrude upon the series of epidermic cells, and actually force their way up to the cuticle, pigmented connective-tissue cells and capillaries of the vascular system. No pigment is ever developed in the epidermic cells themselves." The matter requires further investigation, but the one or two specimens in my possession make me hopeful that a positive result might be obtained. Shipley (No. **57,** p. 19) considers that possibly in these animals wander-cells may collect effete products and carry them to the nephridial sacs, and there undergo degeneration, whence they are voided through the nephridium. This conclusion runs hand in hand with Vejdovsky's (No. **58,** pp. 111, 112, and 127) observations concerning the granules resulting from the disintegration of the chloragogen cells of Kükenthal (No. **41**), which get carried to the exterior through the nephridia.

In Oligochætes, Ude (No. **59**) considers that chloragogen cells are bodily carried to the exterior through the dorsal pores. I must own that his observations do not appear altogether satisfactory.

Eisig (**27**) gives a very full account of the utilisation of

" pigment-holding guanin " amongst the Capitellidæ. Though he does not describe any actual transference of pigment by wander-cells, he notes that blood-corpuscles (Blutscheiben) containing pigment concretions may become surrounded and encapsuled by a layer of leucocytes (p. 752), and that certain peritoneal epithelium-cells ingest concretions, and are to be considered phagocytic (pp. 753, 754) ; the figure 15 on pl. xxxv especially is suggestive of a transportation of concretions by wander-cells to different regions, e. g. skin, gut-wall, &c.; and if further observation shows this to be the case Eisig's conclusions on the specific excretory powers of such parts will require modification.

Amongst the Mollusca we have some evidence that wander-cells may take part in the excretory process. First of all the well-known observation that cells loaded with uratic concretions actually break off from the renal epithelium is not without interest in this connection (vide, e. g., Vogt and Jung, No. 6, p. 811).

Grobben (No. 33) has shown that the part played by the cells of the pericardial gland in Lamellibranchs is in more perfect parallel with the process we are considering. He describes how cells laden with pigment concretions are liberated from the pericardial gland and pass into the nephridium from the pericardium, and so gain the exterior. It seems possible that some of the cells described formerly as being free renal epithelium-cells may be really of this nature, but it is not necessary to press this further here.

I have made some preliminary investigations on the siphonal portion of the mantle in specimens of Anodonta and Unio ; this region is black in colour as seen by the naked eye. In sections (vide figs. 6 and 7) the epithelial cells are seen to contain numerous small dark pigment-granules, which are located for the most part external to the nuclei. Here and there pigment is also present in the basal parts of the cells, and strands consisting of rows of pigment-granules may sometimes be seen running up through the basement layer to the bases of the epithelium-cells. In the tissue below the epi-

dermis are seen scattered small rounded and irregularly shaped cells containing pigment-granules; these pigment-granules are mostly of small size, and have the same appearance as those seen within the epidermal cells. Occasionally one can trace fine processes from these cells up into the epidermal layer, and here and there one seees a rounded cell apparently identical with these pigment-cells between the bases of the epidermal cells (fig. 7). The pigment-granules either give a dark brownish or a brighter orange-brown colour when observed en masse under the microscope; these two forms are seen both in wander-cells about the tissues in the mantle, &c., and in the epithelial cells themselves. Whence these pigmented wander-cells come from I have not yet determined, either from the literature or actual investigation of the subject, but the description Grobben (**33**) gives of certain pigment-cells occurring in the pericardial gland of Arca Noœ is suggestive. Kowalevsky (**40**) has shown that this organ has some value as an excretory organ.

In some cases these pigment-granules are thrown off from the epithelial-cells by traversing their outer hyaline portion (vide fig. 6), in other cases by actual migration of the wander-cells; the former seems to be the general rule, that is the epithelium-cells act as middle men, a part which they also play to some extent in Vertebrates, and is represented in fig. 6, where also the second process is illustrated by the two pigment agglomerations marked Z_1 and Z_2 and Z.

In some of my sections some granules are seen in the thin attached edge of the shell, as well as in the epidermal cells of that region, and in wander-cells in the subjacent tissue; it seems likely that pigmentation of the shell takes place in this manner. F. Müller (No. **50**), however, makes no reference to pigment, nor does Leydig (**46**) describe such a process in his 'Histology,' but the above quotation (vide, p. 23, ante) from his more recent paper (**45**) shows that he has recognised it. In an intermediate paper on Gastropods (No. **46**) he does not actually state that the pigment-cells travel outwards through the epidermis, but indicates that they may find their way to

the kidneys: "Mitunter hatte ich auch den Eindruck, alsob die bekannten grossen Nierenzellen mit den Harnsäuren Concrementen, gar nicht das Eigentliche Epithel der Niere seien, sondern eher Bindesubstanzzellen, und dass die Stäbchenartigen Lagen das wirklich Epithel vorstellten."

Flemming (29) makes no mention of the wandering pigment-cells in his paper on the sensory cells.

We will now leave the Invertebrates and return to Vertebrates. P. H. Stöhr (No. 56) has called attention to the fact that the tonsils and Peyer's patches are a site for the constant out-wandering of leucocytes; for instance, he says, " Ueberall wo Anhäufungen von Leucocyten dicht unter dem Epithel liegen, wandern von ihnen aus zahllose Leucocyten durch das Epithel, und stellen, auf dessen Oberfläche gelangt die Schleim- und Speichel-Körperchen dar." And he remarks that though numerous observers have noticed the occurrence of the leuco-cytes in the epithelium, &c., he was the first to interpret the meaning of their presence.

Hodenpyl (36), by applying carmine, melted lard, atropine and other substances to the surface of the tonsils in dogs, comes to the conclusion that soluble and insoluble bodies•are not readily and immediately taken up through the tonsils; the longest time he allowed before killing the animal was one hour, so that his results with intra-tonsillar injections, which were negative as regards transportation by leucocytes, cannot be held to be final.

Armand Ruffer (53) admits that large numbers of leucocytes escape into the alimentary canal; but he goes further, and says that some return laden it may be with carbon particles (tonsil) or with micro-organisms; moreover he states that the latter are never seen within the epithelial cells themselves.

When I read Stöhr's memoir I made note that it would be of supreme interest to know whether the leucocytes he described were removing any deleterious matters from the animal,— whether, indeed, they were acting the part of scavengers in a manner similar to that which is described above to occur in the starfish, their prey, however, being micro-organisms; and

Ruffer (**54,** p. 108) notes that some emigrated leucocytes actually contain more or less digested microbes which have wandered out. Attention has been already called to the question of the presence of dust particles in the dog's tonsil, which must be regarded to be still somewhat open, though naturally we must rely on the more recent observations, carried on with more perfect methods than were those of the older observers.

These references show that there is considerable evidence that wander-cells are of service in getting rid of effete products; in view of them we may return to the Echinoderms.

MacMunn (**12**) has shown spectroscopically the presence of respiratory pigment in Strongylocentrotus lividus : and from his description it seems probable that this pigment, which he terms echinochrome,[1] is that readily soluble, bright-coloured (brun d'acajou) pigment which is located in a form of amœboid cell mentioned and described by many authors. What relation this or some closely allied pigment has to the pigment carried to the exterior (e. g. as is above described in Spatangus) is not quite clear. The granules are much smaller and of uniform size in the former ; in the case of Echinus sphæra the spheruliferous cells excreted and those (cellules mûriformes) of the tissues are, as has already been pointed out, very similar in the size of the spherules they contain as well as in their actual size, the only difference lying in the colour of the spherules. In the fresh dorsal organ I have occasionally seen cells containing both clear and dingy spherules, which are probably what some authors have described as intermediate forms. Prouho (**11**) considers that there is a close relation between the " cellule mûriforme " and the pigment (p. 300) " granulations brunâtres, sphæroïdales, de différents grosseurs, tantôt éparses, tantôt agglomérées" which occur in Dorocidaris. He says, " Ces sphærules proviennent probablement des globules mûriformes qui absorbent peu à peu les matières excretées par les tissus dans lesquels ils séjournent et finissent

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[1] Cuénot describes a yellow pigment which he calls " hæmoxanthin," and which he asserts without experimental evidence to be non-respiratory (**3,** p. 49).

par se désagréger." In the Spatangid where the effete pigment is more insoluble its porterage to the exterior is readily followed.

The best known respiratory pigment is hæmoglobin, and we know that it is constantly being destroyed and as constantly being manufactured; moreover, that some of the products of its destruction are themselves pigmented. A priori, therefore, we should expect that further researches will show that the respiratory pigment (echinochrome) is always being destroyed and replaced by new; and it is probable that the pigment which is got rid of or deposited in the tissues is a product resulting from its disintegration; the process in the Spatangid then would form a fairly complete parallel to the above-quoted phenomenon, which occurs, e.g., in the frog, where respiratory hæmoglobin gives rise to the dark effete " melanin " (Gamgee, No. 30; J. H. List, No. 48; Latschenberger, No. 47; and Gaskell, No. 32) which is then got rid of through the skin, being of service as a colouring agent whilst en passage.

Whereabouts in the Echinoderm does the pigmental change take place? Some authors (e.g. Hamann, No. 1) consider that the dorsal organ (chromatogen organ) is the seat of this change, others deny that it is—for instance, Prouho (11), on the ground that more effete pigment is found in the mesentery, &c., than in the dorsal organ. But the presence of inter-mediate forms of corpuscles show that some transformation occurs in the organ itself, though probably the change is not limited to the precincts of the organ.[1] Its structure offers some facilities for the distribution and consequent non-accumulation of the pigment within it. If we could accept it as the place where these pigmentary and other waste substances are worked up, the organ could be closely compared to the pericardial gland of Lamellibranchs, &c. (Grobben, 33), and to the chloragogen cells of Chætopods (Véjdovsky, 58); moreover it bears a very similar position to these structures as regards the possibility of actual ejection of the effete materials: with the

[1] Cf. Eisig, 27, 'On Deposit of Concretions in other Organs than the Nephridia.'

organ itself the strands of similar tissue in continuity with it
must also be included (hæmal system).   It is obvious that if
pigment is brought up to the skin faster than it is extruded
through that structure the surface of the animal will become
coloured; but before entering into this more fully it might be
as well to point out the various means by which pigment is
eliminated in animals generally, for of course the method by
the interference of amœboid cells is not the only one which
occurs in the animal kingdom.

Pigment (i) may be got rid of in soluble form, e.g. bilirubin,
biliverdin, &c.; (ii) it may be extruded in solid form through
the special excretory organs; thus in a large proportion of
animals the excretory organs contain considerable quantities of
pigment—cf. the nephridia of molluscs, of Capitellidæ (Eisig,
27), &c.   Here it is interesting to note that in man there is
some close association between certain pigmentary bodies and
uric acid ("cayenne pepper coloured crystals").   Or, again (iii),
it may accumulate in organs which have no connection with
the exterior, where by being deposited it is removed from the
circulation, e.g. the uratic tracts in Tanais (Claus, No. 24),
the coxal glands (red brick glands) of Limulus and Scorpio;
moreover (iv) it may accumulate as a result of the degeneration
of an organ, ontogenetically, and remain in the remnants
thereof, at any rate for a time (Gaskell, No. 32); or lastly (v),
and this is the method that concerns us here, it gets excreted
through the free surface by the intervention of amœboid cells.

It has already been pointed out that pigmentation will ensue
if pigment-granules are brought to the epidermis more rapidly
than they can be got rid of, and many instances (Vertebrates,
Spatangids, &c.) have been cited where this does take place.
That pigmentation may and does arise through the use of effete
matters is an excellent illustration of the economical way in
which the material at the disposal of an animal is used.   After
pigment has done its work in respiration, some part of it be-
comes used again to colour the individual, and thus help it in
the struggle for existence; but cutaneous pigmentation is by
no means always due to effete respiratory pigment.   Thus

Leydig (45) shows that calcareous concretions and bodies of the nature of urates may give rise to coloration; now the latter class of substances are certainly waste products. Also F. G. Hopkins (No. 34) has shown that the yellow colour of certain butterflies' wings is due to the presence of a salt of "lepidotic acid," a substance very closely related to uric acid.

If a comparison may be allowed, we might depart from the classical comparison of an animal to a coal-burning steam-engine, and liken it broadly to the present coal-gas industry. Here we find great economy exhibited, for though we obtain motion (by the gas-engine), light, and heat directly from the main product—coal gas—we also meet with by-products which arise in the course of manufacture; strictly speaking they are "waste products," yet nowadays they are of such value that they are said to be as profitable and lucrative as the coal gas itself. We may take the coal-tar colours, or "aniline dyes," as the instance most allied to the matter in hand, for they are used to some extent to make individuals attractive to one another, or more suited to their environment (cf. the "invisible colours" of soldiers' uniforms during active service).

Eisig (27) discusses the relation of pigmentation by effete matters to natural selection.

The fact that such processes of cutaneous excretion occur, gives a tangible appreciation of the differences which are asserted to exist between human beings of different complexions; thus it is known that the exhibition of large doses of mercury (Hutchinson) is invariably well borne by dark-haired persons; now in them the pigment excretion through the hairs is far more active than it is amongst the blond, and this indicates that at any rate one particular form of excretory activity is greater.

This leads us to a subject which has not yet been mentioned —the pathological formation and deposition of pigment.

Pathologically, pigment may be formed as, for instance, in the melanotic tumours, in which the production appears to be local (List, 48); in Addison's disease, where the cutaneous

pigmentation is caused by wander-cells ("carrier-cells") passing their pigment on to the stratum Malpighii (vide **51**). H. v. Planner (**52**) describes a case of congenital nævus in which there was considerable tumour formation as well as pigmentation. He figures pigment-bearing cells working their way up into and through the epidermis. Here also, then, there is evidence that a process of an excretory nature may take place.

Why should pigment-cells travel towards the free surface? It is difficult to fix upon any one determining cause, but it seems most probable that light has some guiding influence upon them. The movements which can be elicited in the pigment-cells of, e.g., frogs, and the chromatophores of Sepia, &c., show that light has some effect upon these structures; moreover it is usual to find the dorsal surface of animals more deeply pigmented than are the ventral and more shaded ones. On account of the pigmentation of internal organs in some animals, and of animals living at great and lightless depths, Eisig (**27**) denies that light is to be considered as a factor determining the production of pigment, though it may subsequently act upon or otherwise modify it.

Leydig (**45**) considers that there is some connection between nerve-filaments and the processes of the pigment-cells in the skin; and, notwithstanding the opposition which has been raised to this view, it is difficult to understand how symmetrical patterns could ever be produced without a bilaterally acting guide, such as the nervous system, although it is by no means clear how nervous interference could act.

There seems to be some selective capacity on the part of the cells themselves, which causes them to steer for certain havens; for instance, although pigment-cells occur abundantly in the somatic peritoneum and about the renal tubules in the newt, yet they do not wander through the epithelium of those tubules. In some cases the mechanical resistance offered by the tissues may be the real factor which prevents intrusion of wander-cells; for instance, sections through the pineal eye and neighbouring structures in Lacerta show that, whilst there is

abundant pigment in cutis and epidermis all round the region of the eye, there is none actually over it.

There is another factor which may be of importance ; this is the stimulus afforded by the contents of the cell. Inert granules, micro-organisms, pigment concretions, &c., may cause the cell to wander further than it ordinarily would. It is well known that the coarsely granular leucocytes of Vertebrates are the most rapid in their locomotion (vide, e.g., Lawdowsky, **44**, p. 196, "Die grobkörnige raschkriechenden Leucocyten ").

IV. Notes on Echinoderm Histology.

So much and such careful work has been done in the past few years on the histology of Echinoderms, that it would be superfluous to attempt to give anything like an exhaustive account of this large subject. I shall confine my attention to some points in which my observations do not entirely tally with those of other observers.

This is especially the case with the "dorsal organ" of Asteroidæ: no author I have hitherto read has given what seems to me to be a satisfactory account of this enigmatical organ, and of the tracts of similar tissue which are connected with it; the published figures give little or no conception of the structure of the organ. My chief investigations have been made on Asterias rubens; the other forms mentioned in the introduction have also been used: unless otherwise stated the descriptions apply to A. rubens.

As regards preparation, all that need be said here is that the dorsal organ is best fixed in situ after exposing it so that the preservative agent can act on it well: when fixed it can be completely hardened in alcohol or removed from the various skeletal portions before that process: decalcification is deleterious to its finer structure.

Next, it will be advisable to note the nomenclature to be used hereafter, on account of the large number of names which different authors have used.

Hæmal system is a less clumsy term than, e. g., "système lacunaire viscéral" (Prouho), and does not imply that true vessels are present, as do "blood vascular system," "blutgefäss system," &c.; moreover, it is merited because the system seems to contain a fluid rich in nutrient substances, an important property of the blood in other animals.

The term Dorsal organ, recommended by Dr. P. H. Carpenter, will be applied to the organ variously called Heart-Herzgeflecht (Ludwig), Chromatogen organ (Hamann), Glande ovoïde (French authors), &c.: the term is a neutral one, not implying any particular function. "Glande ovoïde" is unfortunate in not being apt to the form of the organ in all Echinoderms.

In so far as it is the "central organ of the blood system" (which was Ludwig's reason for using the term "Herz" in connection with it), it might be still termed as Ludwig suggested but there is not evidence to show that it is the propulsive organ for the contained fluid more than are the tracts proceeding from it. Hæmal strands is applied to these tracts; it is short and expressive, it does not assume the presence of true vessels as does "Blutgefässe," and it is more inclusive than the term "Blutlakunen."

The different hæmal strands are distinguished as circumoral, radial, genital, and gastric.

In Asterias rubens and other forms the gastric hæmal strands are enlarged compared to the other strands or tracts: they have been called "die frei in der körperhöhle hineinragende körperchen" by Hoffmann, and "glandes lymphatiques de la cavité générale" by Cuénot: neither of these can be said to be other than long-winded expressions. The term strand or tract is more applicable than vessel; for, as has been already pointed out, they do not conform to the vessels of Vertebrates, &c., in structure, and also each is not necessarily a single tube; it may consist of a number of anastomosing tubular spaces.

The whole hæmal system—with the exception of the gastric strands—is contained in canalicular spaces, to whose wall its

parts are slung by laminæ of connective tissue : for those spaces which surround the peripheral parts of the system Ludwig's term perihæmal canals will be retained. The canal in which the dorsal organ is contained may be called the axial or dorsal (perihæmal) sinus, the " schlauchförmiger Kanal " of German authors. Water-tube, as recommended by Dr. P. H. Carpenter, will be used for the " stone canal " or " canal de sable ; " and madreporic tubules for the appropriate portion of the water vascular apparatus.

Of the naked eye appearance of the dorsal organ in A. rubens it would be superfluous to say more than that it tapers towards its oral end, and is of a pale colour which varies in different specimens (brownish to purplish). Examined with a low power, in the living state, it has a lobulated appearance, and is seen to consist of a number of strands of tissue, upon which there may be variously sized clear transparent swellings (fig. 4). Under a high power the following can be seen :—1. Fibrils running longitudinally. Whether any of these are contractile, authors are disagreed ; anyhow, the organ becomes shorter when removed, and exhibits contractions when irritated with a needle. Kowalewsky (No. 40) says, " Bei den Echiniden habe ich deutliche Kontraktionen der ovoiden Drüse gesehen : wenn es noch keine regelmässigen Pulsationen waren so waren es doch wiederholte Zusammenziehungen des ganzen Organs."

2. Large numbers of cells similar to the leucocytes seen in the cœlomic fluid, &c., some containing small granules, and sometimes with pseudopodial processes projecting from the surface of the strands of the organ, exactly like those of certain free corpuscles ; others contain larger spherules, and have been called spheruliferous corpuscles.

It would be supposed from most descriptions that the surface was freely ciliated ; this I have not found to be the case, there is very slight movement amongst particles floating in the neighbourhood. At first I thought the pseudopodial processes were cilia, but careful observation showed that this was not the case ; the individual cilia are few and scattered, and are

very hard to see : the surface of the gastric hæmal strands is
more thoroughly ciliated than that of the dorsal organ, but
even here the difference is very great between its surface and
that of the cœlomic epithelium, for instance.   Cuénot (4)
mentions that there is slight movement about the dorsal organ,
but goes no further into the question.   By dissection (fig. 4) or
by sections the different strands are seen to anastomose with
one another: each is tubular in structure, the walls of the
tube being constituted of a fine extensible and elastic mem-
brane with the longitudinal fibrils mentioned above ; in parts
this tubular nature is shown clearly where the contained fluid
is more abundant.   These have been already referred to as
transparent swellings ; their surface may be perfectly free from
cells, or there may be a few cells forming a reticulum over the
surface by means of their processes (fig. 5), or the cells may be
flattened out and form a complete covering.

    The fluid gives a granular coagulum with reagents which
precipitate proteids (alcohol, picric acid, &c.), showing that it
contains a considerable amount of these substances.   The dila-
tations seem to be parts where this nutrient fluid is tempo-
rarily more abundant ; I have always seen them even when the
greatest care has been taken in exposing the organ to prevent
injury.   In sections similar coagulum is seen in varying
amounts in non-dilated tubes.   The highly albuminous nature
of the fluid no doubt led Ludwig, &c., to regard it as the
nutrient fluid, and therefore worthy of the appellation " blood."
Cuénot, on the other hand, considers that the perihæmal canals
contain the true blood-fluid.   My sections show a very slight
amount of coagulum in these spaces, often none at all.   He
regards the dilatations (p. 90) as " places predisposed for the
exit of lymph-cells ;" my observations lead me to think that at
these points the migration of cells from the gland is least
active.   Speaking of the " epithelium," he says, " Mais il
manque dans tous les endroits de sortie," a matter which
we have mentioned above.   Vogt and Jung (No. 6, p. 610)
describe these dilated tubes as " grosse vollkommen homogene
und durchsichtige Bläschen welche sich mit Beale's Carmin

nicht färbten. Vielleicht waren es Aufblähungen der Cuti-
cula." They say nothing further about them.

Similar dilatations are seen upon the gastric and other
hæmal strands, as well as coagulum in non-dilated parts. It
seems likely that there must be some slight circulatory onward
movement of the fluid, probably from the gut·wall where
absorption is going on, along the gastric hæmal strands to the
dorsal organ, and then to the genital and other tracts. With a
view to observing whether any onward movement could be
discerned with the dilatations, the whole madreporic interradius
was rapidly removed from specimens, and then the wall of the
dorsal perihæmal sinus and the water·tube were carefully
removed with fine scissors; the resulting preparation, consist-
ing of the dorsal organ slung by the skeletal tissues, was then
put in a watch-glass with fluid under a low power. Such
preparations were made in the morning and observed from
time to time throughout the day. I was never able to discern
any change in the position or appearance of the dilatations.
Prouho (No. 11) very properly insists on the probability that
circulation is carried on (in Echinids) chiefly by a vis a tergo
through absorption from the intestine. The preparation of my
specimens entirely precluded any such factor, and therefore they
do not seem to be of importance in their negative result.

When the size of the individual tubes is considered it is not
surprising that attempts to inject the system fail, and the
dorsal perihæmal sinus only becomes filled (vide Vogt and
Jung, 6, p. 610), or that Cuénot's endeavour to inject the
gastric hæmal tracts did not succeed (No. 4, p. 92).

As far as I have been able to make out, all the cells of the
organ are of a leucocytic nature; and as such they are irre-
gular in form and place. Hamann (1) figures and describes a
regular epithelium within and without the extensile mem-
brane; I have not seen a condition so regularly arranged as his
figure represents, even in specimens of A. rubens and Cri-
brella ocellata (vel sanguinolenta) of 1—1·5 mm. in dia-
meter (vide Hamann, woodcut, p. 54, ' Die Asteriden '). From
the irregularity in the disposition of the cells, as already

described, and their amœboid nature, it seems hardly correct to speak of an epithelium; they merit the expression used by Grobben (No. **33**) in describing the cells of the pericardial gland—" Die Zellen bilden kein schlossenes Epithel." As regards the cells inside the membrane, they are even less regularly arranged.

The wall of the tubules consists of a thin structureless membrane which has great extensibility, and also of fibrillæ. In some places it seems to be split into two or more laminæ by the intrusion of the amœboid cells; in other places it is plicated, and in some cases it can be seen that plications are due to the presence of a moderately projecting cell which has pushed it up; often, as above stated, it forms the sole covering of the tube where there is dilatation.

It is generally believed that the hæmal system is the source of the amœboid cells, cells which are being lost constantly (by emigration, possibly also by disintegration, as Cuénot says [1]). With a view to seeing if this is truly the manufacturing place for new wander-cells, I have searched for nuclear divisions in it as well as in " Tiedemann's bodies." Specimens were carefully preserved in chromic, osmic, and acetic acids, stained with saffranin or hæmatoxylin, and examined under a Powell and Lealand $\frac{1}{20}$ inch apochromatic. The nuclei are rounded or oval ; most of them have a single mass of chromatin in the centre, from which sometimes bridles can be traced connecting it with the peripheral chromatin, which seems to form a sort of capsule for the nucleus ; in some, especially the round ones, a nuclear network can be more definitely made out ; here and there one sees a more elongated and larger oval nucleus with two central chromatin masses ; occasionally in these and others there is a constriction in the middle of the length of the nucleus. These may be stages in the nuclear division ; often one can see two closely approximated nuclei in the same protoplasmic mass. I have not been able to make out any regular

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[1] I have not observed disintegration of amœboid cells to occur within the body of starfish ; he considers that such is the source of the proteid constituents of the " blood-fluid " (= fluid contained in perihæmal spaces).

karyomitosis; the nuclei are small, which makes the matter a difficult one to investigate.

Prouho considers that the dorsal organ is the seat of "une production constante des éléments figurés, destinés à remplacer ceux qui ont cessé de vivre dans les fluides nourriciers de l'individu" ('Compt. Rend.,' cii, 1886, pp. 1403–6).

The remaining parts of the hæmal system are similar in structure, but with the exception of the gastric tracts they do not consist of actually separate anastomosing tubes.

Sections of the gastric hæmal strands are very similar to those of the dorsal organ; the spheruliferous corpuscles are much more abundant, however, than they are in that structure. They are not necessarily identical with those that leave the body through the dermal branchiæ (26) (cf. the two similar forms of corpuscle in Echinus sphæra), but the pigmentation in the forms I have investigated is too slight to make any distinction from their appearance. Their presence suggests that they may play some part in the absorption of food material (cf. the fat absorption question in Vertebrates), and possibly also its subsequent distribution to the tissues. Sections of young specimens of A. rubens (2 mm.) prepared with osmic acid show wander-cells containing globules and granules which stain black, and which resemble the globules and granules of food material in the gut-wall. Such amœboid cells are especially to be seen in the dorsal organ and in the radial hæmal strands. This appearance suggests that they travel along the hæmal strands to minister to the nutrition of the tissues; in development it has been abundantly shown, in other animals, that leucocytes are used in the nutrition, not only for purposes of construction but also of destruction (vide 39).

Over and above the function of producing new leucocytes (or pigment) that the hæmal system may have, the following considerations render it probable that it assists in carrying on the nutrition of the animal:—(1) It contains a highly albuminous fluid; (2) it is in connection with the gut on the one hand, and with various organs of the body on the other, of which we may mention the generative organs, perhaps, as

those requiring much nutriment ; (3) in other Echinoderms (Echinids and Holothurians) there are more definite vessels which have a " parallel " distribution.

Since in Asterids the tubes containing fluid and the leucocytes infiltrating the walls of these tubes are associated together everywhere, I cannot agree with Hamann in considering that "blood lacunæ" RUN ON the " chromatogen organ :" the whole of the hæmal system is " chromatogen organ," and contains "blood lacunæ;" the two are inseparably bound together in Asterids, though in other Echinoderms there is more differentiation between the corpuscle-producing, and the hæmal fluid conveying portions.

Cuénot denies the existence of the radial hæmal tracts, though he allows that there is a " glandular " tract in the radial septum ; yet he figures (pl. iv, fig. 10) a tube dilated with its transparent colourless blood-fluid taken from that situation.   What Cuénot describes as oblique septa in the radial perihæmal tract simply appear to be the hæmal tracts passing off to supply the sucker feet, &c. (vide Ludwig, 7).   Of series of sections of the arm of Ophioglypha lacertosa, some sections show a well-marked hæmal radial strand with blood-fluid dilatation ; others might lead one to deny the existence of the strand, so slight is it in size.   Thus because at any one point no easily recognisable strand can be seen, we must look further before we conclude that it does not exist.

The following is a summary of the functions carried on by the hæmal system :

1. Nutrient substances (proteids, &c.) absorbed from the gut are distributed in a state of solution to the various organs.

2. Nutrient substances are distributed by means of amœboid cells (in the young certainly, in the adult possibly) which use the strands of the system, as it were, like railway lines.[1]

[1] Cuénot (No. 3, p. 50, and No. 22) advances a theory that certain granules contained in " amibocytes " consist of an " albuminogenous " ferment, which has the power of converting dialysable into non-dialysable proteids, and thus prevent loss by osmosis ; he maintains that this ferment is to be found in all animals from Echinoderms to man.   It is now well known that there is a considerable difference between osmosis through a dead, and

3. It is the site (together with "Tiedemann's bodies," "Polian vesicles," &c.) for the production of amœboid corpuscles (vide Prouho, Cuénot, et alt.). In Asterids this function is carried on all over the system, in Echinids it is limited certain situations (dorsal organ, &c.).

4. It has some concern with the working up of effete material. Some evidence has already been given (vide Hamann, Perrier, Koehler, &c.). Sarasin (19), from his study of Asthenosoma, considers the dorsal organ to be a typical nephridium with cœlomic funnels, and communicating with the exterior through a canal (ureter) which opens in common with the water-tube at the madreporite. The morphological aspect of this idea will be considered below. The dorsal organ has been compared (supra) to the pericardial gland of molluscs (p. 33), with which organ it agrees in taking up carmine (Kowalewsky, No. 40).

I would strongly insist, with Prouho (No. 11, p. 336), that there is no communication between the cavities or lumina of the hæmal system itself with other spaces, which some authors have described, and which Shipley (No. 57) quotes. The communicating cavities which they describe are hollowed out in the substance (inter-canalicular) of, e. g., the dorsal organ, but they are not in continuity with its hæmal (intra-canalicular) channels. As Prouho remarks, the only methods of communication between these hæmal channels and other spaces are (i) by osmosis, (ii) by diapedesis of corpuscles.

The following method of regarding the relations of the water-tube, dorsal organ, axial (perihæmal) sinus, and the madre-

transudation through a living membrane; otherwise it would appear paradoxical, if we accepted his theory, that non-dialysable egg-albumen when injected into the circulation should pass through the renal epithelium and appear in the urine as egg-albumen. The presence of a living membrane appears to be sufficient to prevent any such loss of dialysable bodies, at any rate until our methods are adequately refined for testing the validity of his hypothesis. It is also interesting to note that (No. 3, p. 49) he can prove that certain granules do not consist of hæmoglobin, although spectroscopically they appear identical with that substance, by means of a magnifying power of 1500 diameters !

poric or water pores, has, I believe, never been formulated; it has the advantage of bringing the different arrangements which have been described into harmony, and will put an end to the battles which have been fought over the point.

First of all we must refer to Bury's (No. 16) discovery, that the central water vascular apparatus is developed in three pieces—(i) the water-tube, (ii) an ampulla of anterior enterocele, (iii) the water-pore. He further promises to prove (p. 443) that the left anterior enterocele becomes the so-called "schlauchförmiger Kanal" (what is here termed the axial perihæmal sinus).

In specimens of Cribrella, 2 mm. in diameter, I find that there is as yet but a single water-pore, which communicates with the cavity of the axial sinus ; into the latter the free end of the water-tube opens; thus these three spaces are in communication with one another at a comparatively early period.

Now this free communication may remain throughout life in many forms, as Cuénot (No. 4, p. 92) proves:—" J'ai constaté par les coupes que des canaux madréporiques débouchent directement dans le sinus axial chez l'Echinaster sepositus, divers Astropecten, la Liudia iliaris, l'Asterias glacialis, et l'Asterina gibbosa; chez les grandes espèces, Asterias glacialis et Astropecten aurantiacus, on peut, par l'injection et la dissection, trouver les orifices sans faire de coupes. On voit que le fait est parfaitement constant dans toutes les familles," and as I showed in Cribrella oculata ('Proc. Roy. Soc.,' vol. xliii, p. 330).

Now the cavity of the axial sinus extends amongst the strands which form the dorsal organ ; these spaces we will term intercanalicular, as distinguished from the intracanalicular, which are the actual cavities of the strands themselves; and between these there is no free communication, as has already been stated.

In the dorsal organ of Echinids there exist epithelium-lined cavities which communicate together, and with a cavity extending longitudinally along the organ; this is termed the " canal aquifère annexe" by Prouho (No. 11), and the spaces

"Kanäle zum Wassergefäss gehörend" by Hamann[1] (1, 'Die Echiniden,' v. taf. xii, fig. 8, &c.) in Spatangus purpureus.

Into this space or system of spaces there is free communication, on the one hand, with the water-tube, and on the other with the madreporic pores; but this only occurs in certain forms—Spatangus (Hamann), Dorocidaris (Prouho). Hamann denies that there is any such communication in the regular Echinids he investigated: this space, therefore, bears exactly the same position in these Echinids that the axial (perihæmal) sinus holds in the Asterid; in fact, the one is the homologue of the other. The presence or absence of free communication with the water and madreporic tubes depends upon whether the embryonic developmental condition has been retained or lost. There is some difference in the arrangement of the axial sinus in the Asterid and the Echinid, for whereas in the former the sinus contains the dorsal organ, in the latter it is nearly surrounded by the tissue of that organ; that is, in the former the wall of the sinus has only given origin to hæmal strand tissue along one line, whilst in the latter this tissue has been developed from all parts of the wall except a narrow strip on either side of the water-tube. If we imagined the wall of the axial sinus of an Asterid to contract upon the contained organ, and ultimately come in contact and fuse with its surface, except along the stone canal, we should obtain a condition closely resembling that described by Prouho in Dorocidaris; some alteration would have to be made in the structure of the dorsal organ at the same time, for it does not consist so definitely of a number of anastomosing tubular structures as it does in the Asterid. Furthermore, we may predict that if as Bury shows that in Asterids the axial sinus is derived from the left anterior enterocœle, careful investigation will show that the

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[1] In Spatangus purpureus Hamann (loc. cit., p. 133) describes a space surrounding the upper end of the dorsal organ (vide also Prouho and Sarasin), which he considers homologous to the axial sinus of Asterids; probably this has been shut off from the rest of the axial sinus by collapse of intercanalicular spaces during development.

"canal aquifère annexe," or axial sinus of Echinids, is similar in its development.

In Ophiurids an axial (perihæmal) sinus exists; but according to Hamann (1, ' Die Ophiuren') it does not communicate with the water vascular apparatus in the adult.

This view seems to me to reconcile the discrepancies in the descriptions which have been published of the anatomy of the region ; the differences having apparently arisen from the retention or loss of the embryonic condition of the individual examined.

Lastly, we have to deal with Sarasin's (19) conception that the organ is to be regarded as a nephridium, with nephrostomes connecting its cavity with the general body-cavity. Taking into consideration the arrangement found in Asterids and Ophiurids, and believing that the organ is homologous through these groups, it is more probable that the communications are secondarily acquired, and have nothing to do with nephrostomes; moreover, if study of development shows that the cavity of the organ and Sarasin's "ureter" are in reality derived from enterocele cavities, the dorsal organ will no more fit in with what is understood as a true "nephridium" than will Hartog's (17) view that the whole water vascular system is in reality a left nephridium, a view which Cuénot has very properly criticised (5).

I have searched for "nephrostomes" in one large specimen of Spatangus purpureus, and have been unable to find a trace of any such structures. Three specimens[1] of Echinus sphæra have been examined with a similar object. There are numerous pits on the surface ; these are lined by more or less cubical epithelium. I have failed to find any communication between the cavity in the organ and the lumina of these pits by careful examination of both longitudinal and transverse serial sections. The pits are cæcal, and their function is, perhaps, to allow wander-cells to pass from the reticulum of the organ to the cœlom, and vice versâ, without traversing

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[1] One preserved with sublimate, one with Flemming's mixture, and the third with osmic acid.

the larger hæmal spaces which exist on the surface of the organ, whereby they might get carried to other regions—an accident which would not be likely to occur in the close mesh-work of the deeper parts of the organ, spaces of which, though permeated by, are not distended with, hæmal fluid. In the specimens examined cilia are not apparent in the pits.

We may conclude, therefore, that there are no free communications between the cœlom and the axial sinus in Echinus sphæra and Spatangus purpureus, such as occur in Asthenosoma.

V. Note on Technique: a Combined Method for fixing and flattening Paraffin Sections.

This method is a modification of one mentioned by Haidenhain (No. 35), originally practised by Canini ('Arch. f. Anat. und Physiol.,' 1883, p. 155), in which alcohol is used as a means of causing the sections to adhere to the slide. It was in performing the method that I contrived a modification, which I venture to think is an improvemement.

The following is the routine :—The section or sections are placed upon either a dry slide or one moistened with ordinary methylated spirit diluted to 70 per cent. alcohol. The slide is then placed upon a horizontal metal plate which is kept warm; its temperature should be only sufficient to soften and not completely melt the paraffin used; another slide with a ribbon of useless sections of the same paraffin forms a good "thermoscope." As soon as the slide has been placed on the warm plate more alcohol (70 per cent.) is run on by means of a pipette, the amount required being learnt by practice; if the sections are small a small quantity will suffice, if they are large a greater quantity will be requisite. As the slide becomes warm the paraffin softens, and any little wrinkles disappear, the sections floating flat on the top of the alcohol. When they all appear flat the excess of alcohol may be removed with a pipette. If too much alcohol has been added the ribbons tend to get seriously displaced; they can, however,

be easily replaced by means of a mounted needle. If too little alcohol has been used they will not have been enabled to extend and become flat, and they will be wrinkled more or less, according to their previous condition.

When all the alcohol has evaporated, the paraffin may be just melted and then dissolved with benzol or xylol, or seven or eight parts of benzol to one part of turpentine, the latter preventing absolute drying should the benzol evaporate. Canada balsam may then be dropped on and the cover-glass applied, or the slide may be put through absolute alcohol and stained and mounted in any desired manner.

Care should be taken that the warm plate is not too hot. If it is, the paraffin melts completely, the sections are unsupported, and are liable to be torn by the connection currents of the heated alcohol. Care should also be taken that all the alcohol is evaporated before steps are taken to mount in balsam, or a cloudy result will ensue.

By this method sections can be mounted quite flat without any wrinkles; indeed, apparently hopelessly crumpled sections can be mounted with success. It is much less trouble than Gaskell's warm water method, and is as satisfactory.

If staining on the slide is intended, I recommend the use of alcohol as fixative rather than egg-albumen-glycerin, or clove-oil collodion, because no stained material (albumen or collodion) results, as sometimes occurs in those methods. As regards its fixing power, I may mention that I have put slides straight from absolute alcohol to a watery stain without displacement— the use of intermediate grades of alcohol is recommended; and also have stained, restained, and remounted a slide three times successfully. I have used the method for nearly two years.

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EXPLANATION OF PLATE I,

Illustrating Mr. Herbert E. Durham's paper "On Wandering Cells in Echinoderms, &c.; more especially with Regard to Excretory Functions."

FIG. 1.—From a section of tissues taken from a specimen of Dytiscus ten days after injection of Indian-ink particles into the abdominal cavity. Two nodules, in close approximation, are seen; the central mass of the left-hand nodule consists of distinct leucocytes, containing carbon particles; in the centre of the right-hand nodule are a few scattered nuclei, but the abundance of the carbon prevents many being well seen. Surrounding the central masses is the zone of crescentic cells, some with carbon particles; on the free surface of this capsule are a few unaltered leucocytes, most of which contain carbon. *Fat* = cell of fatty body. *Trach.* = small tracheal tubes cut in the neighbourhood. Cam. luc., $\frac{1}{6}$ in., oc. 2; some details filled with $\frac{1}{15}$ in. oil imm.

FIG. 2.—From a section of the madreporite of an old specimen of Spatangus purpureus. *m. ep.* Epithelium lining the madreporic tubule. *b.* Non-pigmented spheruliferous corpuscle. Pigment-holding corpuscles are seen passing through the epithelium. *m. n.* are nuclei of the madreporic tubule epithelium, which have been carried before the intruding pigment-corpuscles. The nuclei of the epithelium of the madreporic tubules are pencilled; other nuclei are tinted purple. The colour of the granules is fairly correct. Cam. luc., $\frac{1}{6}$ in. obj., oc. 2.

FIG. 3.—From section of skin of Amphidotus cordatus, showing migration of pigment-cells through external epithelium. *n.* indicate nuclei of pigment-cells. *c. t.* Connective tissue. *ep.* Epithelium. Cam. luc., $\frac{1}{6}$ in., oc. 2.

FIG. 4.—Isolated strands of dorsal organ of Asterias rubens, fixed with gold chloride and dissected out under a lens. *dil.* Dilatations. *ana.* Anastomoses. Cam. luc., 1 in., oc. 2.

FIG. 5.—Part of an isolated strand of dorsal organ of A. rubens. Note the reticulum of cells on the surface of the dilatation (*dil.*). Cam. luc., $\frac{1}{6}$ in., oc. 2. Weak chromic acid, picro-carmine.

FIGS. 6 and 7.—From sections of the siphonal portion of the mantle of Anodonta. The cilia are not preserved. Z_1, Z_2 point to aggregations of pigment-granules which have reached the free surface (? pigment-corpuscles). *ext.—x.* Points to the free external surface. *conn. tiss.* Connective tissue. *p. g. c.* Pigment-cells, situate in the connective tissue. *p. g. c′* (in Fig. 7). Two corpuscles containing orange-brown granules. Notice the outward-pointing pseudopod in one of them. *ep. pig.* Pigmented epidermal cells. *n.* Nuclei of epidermal cells. Cam. luc., $\frac{1}{15}$ oil immersion, oc. 2.

On the Nature of the Excretory Processes in Marine Polyzoa.

By

Sidney F. Harmer, M.A., B.Sc.,

Fellow and Assistant Tutor of King's College, Cambridge.

With Plates II and III.

THE observations described below were made during an occupation of the Cambridge University table at the Zoological Station at Naples, in the course of the Easter vacation, 1891. The results obtained by Kowalevsky[1] on the excretion of carminate of ammonia and of indigo-carmine in various Invertebrates had suggested an inquiry, by means of the same methods, into the physiological meaning of the periodic formation of "brown bodies" in Ectoproctous Polyzoa, since there seemed reason to suppose that this process might serve as a means of excretion, a view which has been most definitely formulated by Ostroumoff.[2] My experiments made with artificial pigments confirm the view that the marine Ectoprocta are not provided with definite nephridia; and appear to show that the excretory processes are carried on principally by the "brown bodies," the funicular (connective) tissue, and the free mesoderm-cells contained in the meshes of the latter.

The species which, by reason of their transparency and of their abundance at Naples, were principally employed for these

[1] A. Kowalevsky, " Ein Beitrag zur Kennt. d. Excretionsorgane," ' Biolog. Centralblatt,' ix, 1889–90, pp. 33, 65, 127.

[2] A. A. Ostroumoff, " Cont. à l'Ét. Zool. et Morphol. des Bryozoaires du Golfe de Sébastopol," ' Arch. Slaves de Biol.,' t. ii, 1886, p. 339.

observations were **Flustra papyrea,** Pall., **Bugula neritina,** Linn., and **Bugula avicularia,** Linn.[1] Each of these species was exposed to the action of the following pigments dissolved in sea-water—(1) Indigo-carmine; (2) carminate of ammonia; (3) Bismarck-brown; and was further placed in sea-water containing carmine-powder in suspension. After leaving the animals for various periods, as explained below, in the solution of the pigment, the colonies were transferred to pure sea-water in a tank, and the changes undergone by the pigment were examined in the living animal from day to day. It may be at once remarked that the colonies remained living and apparently healthy, even after prolonged immersion in solutions of the pigment.

I have to acknowledge my indebtedness to Dr. Paul Mayer for the suggestion that Bismarck-brown might be expected to yield interesting results, a suggestion which I followed up with some success. Kowalevsky[2] has, however, pointed out that Bismarck-brown may be used with advantage in investigations of this kind, and implies that it is taken up by those parts of the excretory organs which absorb carminate of ammonia, a result which is largely confirmed by my own observations.

One of my most interesting results has been the observation of the fact that the tissues of different forms, even of two species of the same genus, do not necessarily react in the same

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[1] For the synonymy of these species see Miss Jelly's ' Synonymic Catalogue of the Recent Marine Bryozoa' (London, 1889).

My specimens of **Flustra papyrea** correspond very closely with F. papyrea, var. Mazeli, Marion ("Draguages au large de Marseille," ' Ann. des Sci. Nat.,' 6ᵉ sér.; 'Zool.,' viii, 1879, article No. 7, p. 33). It appears to me that Marion's statement that the forms described by Busk (' Cat. of Marine Polyzoa, ... Brit. Museum,' part i, 1852) were composed of narrow linear segments was due to a misapprehension of the fact that the numbers of Busk's plates xlix and l have been transposed, as pointed out by Waters ('Ann. Mag. Nat. Hist.,' ser. 5, iii, 1879, p. 119); and that the "var. Mazeli" does not really differ from the normal form of F. papyrea.

For further remarks on the species see Hincks, ' Brit. Mar. Polyzoa,' vol. i, p. 124.

[2] Loc. cit., p. 76.

way to the action of the same pigment; and that the taking up of the pigment by particular tissues may have a definite relation to the normal pigmentation of those tissues.

The bearing of this fact on the results arrived at by Eisig,[1] with regard to the excretory value of certain normal pigments, will be subsequently pointed out.

I. THE NORMAL CHARACTERS OF THE LIVING ZOŒCIUM IN THE SPECIES INVESTIGATED.

a. Bugula neritina, L.

This species, which is well figured in its natural colour by Costa,[2] is very common at Naples; the specimens examined by me being, as described by most authors, invariably destitute of avicularia.[3] The colony, as is obvious from Costa's figures, possesses a dark colour which varies from yellowish brown to purple.[4] The characteristic colour is almost entirely due to the presence of a pigmented funicular tissue, already noticed by Costa.[5] This tissue is shown in Pl. III, fig. 17.

It will be noticed that the funicular tissue agrees with that

[1] H. Eisig, "Monographie d. Capitelliden d. Golfes v. Neapel," 'Fauna and Flora . . . . G. v. Neapel,' xvi Monogr., 1887 (see especially the "Physiologischer Theil").

[2] O. G. Costa, "Fauna del Regno di Napoli," 'Zoofiti,' Napoli, 1838, Tav. v, fig. 1 [belonging to the chapter headed "Ordine III. Cellariee"].

[3] A. W. Waters ('Ann. and Mag. Nat. Hist.,' ser. 5, vol. xx, 1887) describes an Australian form of this species in which numerous avicularia are present.

[4] For an account of the pigments of B. neritina see MacMunn, 'Quart. Journ. Micr. Sci.,' xxx, 1890, p. 78, and the reference there given to Krukenberg.

[5] Costa, loc. cit., p. 15. The zoœcia are described as being "ripiene di sostanza granellosa, tinta di rosso di tutte le gradazioni."

[6] See W. J. Vigelius, 'Biolog. Centralblatt,' iii, 1883–4, p. 708; and his larger work, "Die Bryozoen . . . . 'Willem Barents,'" 'Bijd. tot de Dierkunde,' 11e Aflev., 1884 ("Parenchymgewebe"), and other writers.

of other marine Ectoprocta[6] in forming a continuous network stretching through the cavity of the zoœcium. It usually forms a thin layer on the inner side of the ectocyst, and another thin layer on the outer surface of the alimentary canal and tentacle-sheath, the two layers being connected by the network which traverses the body-cavity. A special region of this network is often distinguishable as a definite tract which passes from the apex of the cæcum of the stomach, in company with the colourless retractor muscles, to the proximal end of the zoœcium. In young zoœcia the network is much denser, and the young polypide-buds are constantly enveloped by a specially dense concentration of the network. The pigment, which is readily soluble in fresh water, is contained for the most part in numerous minute granules scattered through the funicular tissue, and occurs in two principal colours—purple and yellowish brown, both of which may occur in the same zoœcium.

A similar pigment occurs in the growing-points (which are very densely pigmented), in the tentacles, round the apertures,[1] and in the ovicells (the young ones being, like the growing-points, densely pigmented). It is probable that the pigment in all these cases is contained in the funicular tissue, although some of it may possibly be in the ectoderm.

The meshes of the funicular tissue contain numerous transparent cells, which are colourless or faintly yellow. These cells consist of an aggregation of vacuoles filled with a transparent fluid, and they are often produced into long, fine processes (fig. 19[2]), by means of which they are suspended in the funicular network. They play an important part in the absorption of indigo-carmine, and although not exactly similar to ordinary white blood-corpuscles, they will be alluded to in the remainder of this paper as the leucocytes.

These so-called leucocytes have been well figured by

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[1] S. delle Chiaje ('Mem. sulla Storia e Notomia degli Animali senza Vertebre del Regno di Napoli,' vol. iv, Napoli, 1829, p. 147) : "Osculis margine subfusco cinctis."

[2] Representing these cells after exposure to the action of indigo-carmine.

Claparède[1] in Bugula avicularia, who describes them as "gelbe Tropfen" (p. 153), and states that they only occur in zoœcia in which the polypide has degenerated. My own observations on the same species show that they are of normal occurrence in all the zoœcia, and Claparède's failure to find them universally present was probably due to the fact that they are often colourless in the young zoœcia. The yellow colour of these cells, so obvious in Claparède's figures, probably implies that they had taken up excretory pigments from other tissues during the degeneration of the polypide; and Claparède himself regards them as being excretory in nature.

These cells are mentioned by various other authors, as by Joliet,[2] who states that they are of very general occurrence in the Polyzoa. They are no doubt identical with some of the structures alluded to by Smitt[3] as "Fettkroppar" or as "floating cells," and are described by Cuénot[4] as "amibocytes." They are described by Prouho,[5] in the larva of Flustrella hispida, as very refringent aggregations of spherules, of a mulberry-like appearance, a description which I can confirm from my own observations.

Most of the alimentary canal has a pale, diffuse, yellowish-brown colour; but the walls of the stomach and cæcum contain numerous pigmented (brown) granules. A clear space, devoid of granules, always occurs at the base of the cæcum, in the position shown in fig. 16, which, however, refers to the next species.

[1] "Beitr. z. Anat. u. Entw. d. Seebryozoen," 'Zeits. f. wiss. Zool.,' xxi, 1870, Taf. viii, figs. 1 B, 1 C, t.

[2] L. Joliet, "Cont. à l'Hist. des Bryozoaires des Côtes de France," 'Arch. de Zool. Exp. et Gén.,' t. vi, 1877, p. 233.

[3] F. A. Smitt, "Om Hafs-Bryozoernas Utveckl. och Fettkroppar," 'Öfvers. af k. Vet.-Akad. Förh.,' xxii, 1865 ; and in other places.

[4] L. Cuénot, "Ét. sur le Sang," &c., 'Arch. de Zool. Exp. et Gén.,' 2e sér., t. vii, 1889; 'Notes et Revue,' p. III.

[5] H. Prouho, "Rech. sur la Larve de la Flustrella hispida," ibid., 2e sér., t. viii, 1890, p. 427.

b. Bugula avicularia, L.[1]

This form grows in short, bushy tufts, of a grey colour. The avicularia are usually large, although they vary a good deal in size; they are, in many cases, absent on the older parts of the colony, and are hence probably deciduous. The three spines on the upper margin of the aperture are very small in most of the specimens which I obtained at Naples. The Neapolitan form of this species has been well figured by Claparède.[2]

The funicular tissue contains no pigment. "Leucocytes" similar to those of B. neritina occur in the meshes of the connective tissue, and are either colourless or distinctly brownish grey. The colour, if any, is contained in a diffuse form in the vacuoles which compose most of the cell, and which sometimes contain, in addition, one or two minute granules. The connective tissue of this species will be described more fully in the later parts of this paper.

The pharynx and intestine are colourless. Yellowish-brown granules occur in the walls of the proventriculus, cæcum, and part of the stomach, the granules being most numerous in the cæcum. A saddle-shaped area at the base of the cæcum is devoid of granules (Pl. III, fig. 16[3]), just as was the case in B. neritina.

The walls of the rectum contain granules, which are either almost colourless or are faintly yellow in tinge.

c. Flustra papyrea, Pall.

The ordinary funicular tissue may be non-granular, or may

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[1] This is probably referred to by Costa (loc. cit.) as one of the forms assumed by B. neritina. This "second generation" arises from the "piedi della prima," and is "bianchissima, translucida come il cristallo, fragillissima." The individuals figured by Costa on Tav. vi possessed avicularia, and presumably belonged to this species.

[2] 'Zeits. f. wiss. Zool.,' Bd. xxi, Taf. viii, figs. 1, 1 B.

[3] The granules are, indeed, not represented in this figure; but the blue or green colour, due to the indigo-carmine which had been taken up by this individual, gives a perfectly correct idea of their distribution.

resemble that of B. neritina in containing numerous granules, which are, however, quite colourless, instead of being deeply pigmented, as in that species. They are spherical in form, and are highly refractive. When treated with iodine they assume a colour precisely similar to that taken on by starch under the same conditions.[1] This observation was made just before my departure from Naples, and I was unable to investigate the subject with more care. Dr. A. Hansen has, however, been kind enough to undertake to examine the reactions of these granules, which differ from starch in being readily soluble in dilute alkalies or acids. I am inclined to regard the granules, which were noticed in the tentacles as well as in the general body-cavity, as a reserve supply of nutritive (perhaps carbo-hydrate) material. This is supported by the facts that they are most numerous at the growing edges of the colony, and that relatively few are present in any zoœcium which contains a brown body and an old embryo; indicating that the nutritive substance of the granules has been employed for the nutrition of the embryo. This granular funicular tissue is best developed on the sides and on the front wall of the zoœcium.

Depositions of orange pigment may be seen attached to various parts of the ectocyst, and enclosed in structures which are very similar to the "Excretbläschen" described by Eisig[2] in the Capitellidæ. They may assume the form of a number of minute granules or vesicles scattered through the substance of a cell, or of coloured granules contained in the interior of a more faintly coloured vacuole (fig. 13). They appear to be

[1] According to H. J. Carter ("On the Identity in Structure and Composition of the so-called Seed-like Body of Spongilla with the Winter-egg of the Bryozoa, and the Presence of Starch-granules in each," 'Ann. Mag. Nat. Hist.,' ser. 3, iii, 1859, p. 331), starch-grains are present in the statoblasts of a species of Lophopus; while K. B. Reichert ("Verg. anatom. Unt. üb. Zoobotryon pellucidus," 'Abhandl. k. Akad. d. Wiss. zu Berlin,' 1869, p. 281) describes, in the endocyst of Zoobotryon, certain bodies which he calls "Amyloidkugeln," which take on a "Granatfarbe," or even a violet colour, under the action of iodine.

[2] 'Fauna u. Flora G. v. Neapel,' xvi Monographie.

most numerous near the growing edges. On the formation of a brown body they are collected into much larger masses, which occur here and there in the zoœcium. Similar orange granules were also observed in the leucocytes.

Most of the tissues of the polypide, such as the tentacles and the several parts of the alimentary canal, have a diffuse yellow colour, the pharynx, the circumoral region, and the intestine being of a brighter orange-yellow colour. The first part of the stomach and the whole of the cæcum contain, in addition, pigmented granules, the number of which gradually shades off towards the region connected with the intestine, so that a zone between the two granular parts is devoid of granules.[1]

II. The Absorption of Various Pigments.

A. Indigo-carmine.

A saturated solution of indigo-carmine in sea-water was added to the sea-water containing the living colonies until the solution was of such a strength that the animals were not easily seen through a thickness of more than one and a half inches of the solution. After being left for two or three days in this solution the colonies were transferred to ordinary sea-water in a tank, and were examined from day to day. It may be pointed out that even after this treatment, and in the other experiments described below, the colonies remained perfectly capable of producing " brown bodies," fresh polypide buds, and new zoœcia at the growing edges of the colony.

a. Bugula neritina.

On examining a colony which has been immersed for some hours in indigo-carmine, it is at once obvious that while most

[1] The arrangement of these granules is well shown by Haddon in the same species ('Quart. Journ. Micr. Sci.,' xxiii, Pl. XXXVIII, fig. 12). Haddon has identified this species as F. carbasea, and the part of the alimentary canal which he has described as "intestine" (*int.*) is the part which I have alluded to throughout as "rectum."

of the tissues have remained uncoloured, the leucocytes have taken up a large quantity of the pigment. The appearance of a zoœcium after exposure to indigo-carmine is shown in fig. 17, where numerous coloured leucocytes are seen in the meshes of the pigmented funicular tissue. The whole of the blue pigment is contained in the vacuoles of the leucocytes (fig. 19) ; and, as in the analogous cases noticed by Kowalevsky,[1] the nucleus is unstained.

The bright blue colour is acquired only in the younger zoœcia, most of which possess functional polypides. It cannot, however, be supposed that the indigo-carmine has been first absorbed by the alimentary canal, and then passed on to the free mesoderm-cells, as the pigment is taken up quite readily by the leucocytes of zoœcia which possess no polypides, at the growing-points. Nor, for the same reason, can it be supposed that the blue colour is derived from pigment introduced into the body-cavity directly from the tentacle sheath of the poly-pide, in the manner described by Pergens.[2] I have, indeed, no observations which confirm Pergens' results with regard to the mechanism of the extrusion of the polypide, although I am not prepared to deny the accuracy of those results. The indigo-carmine observed in the leucocytes has probably been derived from traces of the pigment which have diffused through the walls of the zoœcia ; the leucocytes absorbing the whole of the pigment which enters the zoœcium in this way, and so protecting the other tissues from the action of the pigment.

That the leucocytes of older zoœcia, with " brown bodies " developed or developing, are in a condition physiologically differing from that of the zoœcia which are nearer the growing-points is shown by the fact that the cells, in most of these cases, are of a distinct, though not very bright, green colour, instead of being blue.[3] The bright blue colour always appears

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[1] 'Biolog. Centralblatt,' Bd. ix, p. 47, &c.

[2] 'Zoolog. Anzeiger,' xii Jahrg., 1889, p. 508.

[3] According to the results of Kitasato and Weil ('Zeits. f. wiss. Mikrosk.,' vii, 1890, p. 241), this would apparently point to the existence of the pigment in a reduced condition.

in the leucocytes situated in the growing-points or in the younger zoœcia, and sometimes in older zoœcia.

No part of the alimentary canal takes up any recognisable trace of indigo-carmine.

b. Bugula avicularia.

The blue colour is taken up by the leucocytes as in the last species. These cells may consist of a comparatively small number of large vacuoles (figs. 20, 21), or of a much larger number of small vacuoles, or both kinds of vacuole may occur in the same cell. In any case the pigment is found diffusely colouring the vacuoles.

The alimentary canal, unlike that of B. neritina, takes up large quantities of indigo-carmine (fig. 16). The pigment observed in the walls of the alimentary tract is probably derived from pigment which has been actually swallowed, as those polypides which are still so young that they do not communicate with the exterior have no deposition of pigment in their gut-walls.

The absorption of indigo-carmine takes place in the walls of the stomach, cæcum, and rectum (fig. 16). No pigment appears in the clear area on the inner side of the base of the cæcum, nor in the wall of the pharynx or of the intestine. The indigo-carmine is deposited in granules, which, as may easily be seen, are identical with the normal yellowish-brown granules; and the admixture of these two pigments naturally produces, in most cases, a greenish colour. In young polypides in which the granules are less strongly pigmented, and in the rectum of all the polypides, the bright blue colour of the indigo-carmine is not materially altered by the presence of any other pigment.

c. Flustra papyrea.

Indigo-carmine is taken up by leucocytes which are similar to those of Bugula. These cells become intensely blue, the pigment being diffused through the cell; darker blue granules, associated in some cases with the orange granules already

noticed in the normal zoœcium, occur here and there in the diffusely coloured portions of the cell (figs. 23 and 24). In many cases large complexes of these cells occur in various parts of the zoœcium (fig. 23). The absorption of the indigo-carmine is independent of the functional activity of the alimentary canal. The general funicular tissue remains uncoloured.

In many of the zoœcia occur structures which are figured by Haddon[1] in Pl. XXXVIII, fig. 12 (*l. c.*). These structures occur near the posterior wall of the zoœcium, either on one or on both sides. They are not found in all the zoœcia, and are not recognisably present in those which are near the growing edges of the colony. They do not appear to have any special connection with the exterior or with the rest of the funicular tissue. They consist externally of a membrana propria, outside which are a few orange granules, the interior of the cord being finely fibrillated.

Freese[2] mentions the finely granular character of these cords in Membranipora. Nitsche[3] had previously termed them "funiculi laterales" or "lateral cords," and had stated that they passed from one rosette-plate to another. Their contents are sometimes granular according to Nitsche.[4]

When still incompletely developed the lateral cords are not affected by indigo-carmine; but in their older stages they invariably take up a large quantity of this pigment, which appears to be contained in the membrana propria, outside which is a layer of cells containing the orange-yellow granules (Pl. II, fig. 7).

Indigo-carmine is taken up by the granules of the functional alimentary canals only. The first part of the stomach and

[1] 'Quart. Journ. Micr. Sci.,' vol. xxiii, 1883.

[2] W. Freese, "Anatom.-histol. Unt. von Membranipora pilosa," &c., 'Arch. f. Naturg.,' Jahrg. 54, Bd. i, 1888, p. 16.

[3] H. Nitsche, "Ueb. d. Anat. u. Entw. von Flustra [Membranipora] membranacea," 'Zeits. f. wiss. Zool.,' Bd. xxi, 1870, pl. xxxv, fig. 1; pl. xxxvi, fig. 9.

[4] Loc. cit., p. 425, pl. xxxvii, figs. 19, 20.

the whole of the cæcum of old polypides consequently appear deep blue-green. In younger (functional) polypides, which are normally but slightly pigmented, the blue colour of the indigo-carmine is more obvious.

B. Carminate of Ammonia.

The colonies were placed for about two days in sea-water containing this substance in solution, and were then transferred to a tank containing ordinary sea-water.

a. Bugula neritina.

The pigment is taken up by the granules contained in the walls of the alimentary canal of those polypides only which have actually swallowed solid (precipitated) carmine particles.[1] The pigment is most copiously deposited in the blind end of the cæcum, gradually shading off thence on to the rest of the stomach. The wall of the rectum and the inner borders of the cells of the pharynx also contain carmine deposits. The clear patch on the cæcum never contains the pigment.

In some of the zoœcia carmine is taken up in granules by cells belonging to the funicular tissue. These cells are distinct both from the network of pigmented cells and from the leucocytes, and belong to the type shown for the next species in fig. 22. The growing-points are usually brightly coloured with red pigment.

b. Bugula avicularia.

The pigment is taken up by the same parts of the alimentary canal and of the funicular tissue as in B. neritina, being most copiously present in the latter position in the growing-points. The leucocytes contain no trace of the pigment. About twelve days after the commencement of the experiment it was noticed that the tips of many of the branches were

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[1] C. Vogt (" Sur le Loxosome des Phascolosomes," 'Arch. de Zool. Exp. et Gén.,' v, 1876, p. 320) has shown that carmine given as food is deposited in the yellow " hepatic cells " of the stomach of Loxosoma phascolosomatum, but in no other part of the alimentary canal.

growing out into abnormally long and slender growing-points, in the funicular tissue of which carmine-particles derived from those originally taken up by the growing-points were present in the form of sharply circumscribed spherules, which contained (normal) yellowish granules in addition to the red pigment (fig. 22).

c. Flustra papyrea.

None of the tissues of this animal were shown to take up carminate of ammonia, the experiments with which, in the case of this species, were not altogether successful.

C. Bismarck-Brown.

A weak solution, sufficient to give the sea-water a yellow tinge, was found to give the best results.

a. Bugula neritina.

No part of the alimentary canal could be shown to take up Bismarck-brown ; but as the granules of the alimentary canal are normally very dark in this species it was not possible to be quite sure that none of the pigment had been absorbed by them. All the other structures which are naturally pigmented, i. e. the funicular tissue, the pigmented part of the tentacles, the strongly pigmented region round each aperture, and the pigmented parts of the growing-points, take up the Bismarck-brown freely. The fact that the alimentary canal in this species takes up neither indigo-carmine nor Bismarck-brown points to a physiological difference between it and that of allied species. It may be suggested at once that this difference is associated with the pigmentation of the funicular tissue. If it is assumed that the pigment-granules of the alimentary canal are in part of excretory nature—a question which will be further considered below—the difference between Bugula neritina and other species may be expressed by saying that some of the excretory functions normally possessed by the alimentary canal are here performed by the funicular tissue, which is hence pigmented.

The Bismarck-brown is deposited only in the granules of the funicular tissue. Thus the finer processes of that tissue, which are devoid of granules, remain quite colourless after the action of the pigment. The nucleus thus becomes visible, in most cases, as a clear area, unstained by the Bismarck-brown (fig. 18, *A*).

b. Bugula avicularia.

The Bismarck-brown is taken up in intensely brown-red granules by the tentacles and by the proventriculus, cæcum, stomach, and rectum of the functional polypides, the cæcum being the part which becomes most deeply pigmented. The inner borders of the cells of the pharynx become distinctly brown; but the pigment is here diffuse, and not in the form of a granular deposit. No pigment is deposited in the œso-phagus (region between pharynx and proventriculus), nor in the clear area on the base of the cæcum, nor in the intestine. These parts may, however, acquire a faint yellow tinge. In "brown bodies" which are in process of formation the remains of the stomach, &c., take up the pigment, none of which is absorbed by the fully-formed "brown bodies," nor by the polypide-buds which do not yet communicate with the exterior.

Most of the funicular tissue remains uncoloured, but in some cases groups of intensely brown spherules are observed here and there in the body-cavity. This is especially the case in the region surrounding a "brown body." The leucocytes become hardly tinged with yellow.

The Bismarck-brown is taken up freely by young, growing cells. The growing-points and the young avicularia hence become strongly pigmented. Most of the pigment is deposited in these regions in the funicular tissue; but some of it appears to be in the ectoderm, which can also be traced in some of the older zoœcia as a series of small cells, separated from one another by considerable intervals, and stained brown by the action of the pigment. The tactile bodies of the old avicu-laria and the surrounding regions also become pigmented.

A remarkable form of cell, shown in Pl. III, fig. 20, was constantly noticed in this species, but in none of the others examined by me. Each cell consisted of a group of large vacuoles, some of which contained small granules or concretions, while others were completely filled with a number of minute granules, which always exhibited an active Brownian movement.

The larger concretions in the former kind of vacuole are normally colourless, but in colonies treated with a mixture of Bismarck-brown and indigo-carmine most of them became a pale brown colour, while some of them had taken up a small quantity of indigo-carmine (fig. 20).

These cells always occur in a special zone, in the growing-points, at the junction of the comparatively solid distal funicular tissue, and of the space in which the young tentacle sheath lies. They are perhaps in some way concerned in the excavation of this space out of the more solid funicular tissue of the younger part of the zoœcium. They are also found, here and there, in the cavities of the older zoœcia.

They have been noticed in the same species by Claparède,[1] who describes them as finely granular, brown structures, occurring only in the cavities of the buds, and only found in this particular species.

c. Flustra papyrea.

The granular parts of the stomach and cæcum become intensely coloured by the Bismarck brown, which also appears in smaller quantities in the rectum. The intestine, the inner borders of the pharyngeal cells, and the tentacles may become diffusely coloured. Small quantities of the pigment may appear, in spherules, in cells belonging to the funicular tissue (never in cells of the type shown in fig. 14).

D. Carmine Particles in Suspension in Sea-Water.

The carmine particles are readily swallowed by the polypides, whose alimentary canals become filled with dense masses of

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[1] 'Zeits. f. wiss. Zool.,' xxi, pp. 141–2, pl. viii, fig. 1.

solid carmine.   In some of these cases a small quantity of
carmine becomes deposited in the granules of the cæcum and
stomach in B. neritina.   In B. avicularia a small amount
of carmine was deposited in the rectum and proventriculus as
well ; while the funicular tissue of the growing-points took up
a little of the pigment.   This was no doubt derived from the
small quantity of carmine which dissolved in the sea-water,
the colour of which was distinctly red.

III. The Formation of the "Brown Body" and the Further History of the Absorbed Pigments.

Flustra papyrea.

The formation of the " brown body " in the normal zoœcium
takes place as follows.[1]

The tentacles lose their distinctness and shorten themselves,
then forming a mass situated at the proximal end of the
tentacle-sheath, which is also degenerating.   The alimentary
canal at the same time begins to degenerate, its granules
collecting into two masses, one placed at the apex of the
cæcum, and the other at the opposite end of the stomach.[2]
The tentacle-sheath loses its connection with the aperture,
and the three masses formed respectively by the tentacles
(with some part of the remains of the alimentary canal) and
the two sets of granules fuse into a small rounded mass, which
ultimately acquires a bright red colour.   The granules of the
alimentary canal can be distinguished for some time as a
darker mass in the " brown body."

The new polypide-buds have a distinctly bilateral origin,
which, so far as I know, has not been noticed by any previous
observer.   The inner layer of the bud contains orange granules,
which give it a distinct colour ; this layer is derived from the
two angles of the operculum.   At each angle a rounded

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[1] The account which follows, as well as that given of the origin of the new
polypide-bud, is almost identical with that given by Haddon ('Quart. Journ.
Micr. Sci.,' xxiii, 1883) for the same species (Haddon's F. carbasea).

[2] Cf. Haddon's fig. 13 (pl. xxxviii).

swelling, doubtless formed from ectoderm, grows out towards the middle line, immediately beneath the ectocyst (fig. 10). The two swellings unite (figs. 11 and 12) and form a single rounded mass, which remains connected with the angles of the operculum by a tract of modified ectoderm, which soon disappears, on each side. The outer layer of the bud is colourless, and is formed from ordinary funicular tissue.

In some cases the end of each bud-rudiment is considerably swollen before fusion takes place (fig. 11); in other cases no swelling appears until after fusion. In the former case the zoœcium appears to possess two buds placed side by side.

This mode of origin of the buds is no doubt the cause of an abnormality, frequently noticed, in which two polypide buds occurred side by side in the same zoœcium.[1] There was no doubt that in these cases each half of the polypide-bud had developed into a complete polypide, the normal fusion of the two halves having been, for some reason, prevented. Double polypides were observed in various stages of development, from the small ovoid stage, when the two halves should normally fuse, to the condition in which the tentacles of the two polypides are well developed. Each polypide then usually possessed a distinct tentacle-sheath, although in one case observed the two tentacle-sheaths fused distally, although separate at an early period of their development, when the double bud was first noticed.

In a particular experiment, all the colonies which had been treated with indigo-carmine were observed 313 hours later to be remarkable for possessing a considerable number of zoœcia with twin polypides; as many as six of these zoœcia having been noticed in one small area of a colony. This probably indicated that the indigo-carmine abnormally present had acted

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[1] This abnormality is recorded by Haddon (loc. cit., p. 520), as well as by Ostroumoff ('Arch. Slaves de Biol.,' t. ii, 1886, p. 341). Ostroumoff found that the two alimentary canals were always united by their stomachs; which he explains by assuming that there were at first two buds, which later became united through the intermediation of a common " brown body." I am unable to say whether a union of this kind would have been effected later in the cases of twin polypides which I observed in their immature condition.

on all the polypide buds which were in their critical state immediately preceding fusion in such a way as to prevent fusion, and so to cause each half to develop into a complete bud.

In a few cases which appeared to be abnormal the young bud made its first appearance in close contact with the " brown body." Buds developed in this position differed from the normal buds in being more spherical at a stage when the tentacles were first seen, and in some other respects. The differences subsequently became less marked, and the bud appeared to develop into a normal polypide, although my time was not sufficient to allow me to watch the last stages in this process.

At the growing edge of the colony the polypide-buds are formed at the extreme proximal edge of the zoœcia.

The development of the young bud into a complete polypide may be considered in connexion with the indigo-carmine experiments, the behaviour of the developing buds being, in those experiments, exactly similar to that of the bud developing under normal circumstances.

The zoœcia are found, after the action of indigo-carmine, to have a brilliant blue colour owing to the absorption of the pigment by the leucocytes, as already described. Some of the zoœcia may be dead, and are then readily recognised by the fact that they are stained intensely, but diffusely, blue, the operculum being usually open. In these zoœcia, Infusoria and Nematodes soon make their appearance.

In zoœcia which are still alive the pigment is confined to the cells already described. The leucocytes after several days tend to group themselves in masses, and some of them may be seen closely surrounding the " brown bodies " (fig. 1).

About 114 hours after the commencement of the experiment which turned out most successfully, all the functional polypides of the colony were noticed to be degenerating, the evidence of which ´was given by the fact that the fæces had ceased to rotate in the intestine. At the 165th hour the indigo-carmine taken up by the two groups of granules in the wall of the alimentary canal was, in many cases, becoming aggregated

into two round masses, one situated at the apex of the cæcum, and the other at the opposite end of the stomach. This is exactly what happens in the degeneration of the normal alimentary canal.[1]

At 238 hours from the first immersion in indigo-carmine new polypide-buds were commencing to appear in a good many of the zoœcia, many of which, at the 264th hour, had the appearance shown in Pl. II, fig. 1. The degenerating tentacles can be distinctly made out, as well as the two masses of indigo-carmine (mixed with the normal pigment of the stomach, and so of a green colour) derived from the wall of the alimentary canal. The leucocytes containing the absorbed pigment are grouped in a dense mass round the " brown body," and are enveloped in funicular tissue, strands of which radiate out to various parts of the body-wall. Some of these strands contain blue leucocytes, intermingled with which were seen, in some cases, granules of the brown or orange pigment found in the normal zoœcia (Pl. III, figs. 23, 24). The funicular tissue and the muscles shown in the sketch are perfectly colourless. The whole of the indigo-carmine taken up by the animal is contained in the " brown body" and leucocytes.

One of the strands of funicular tissue passing from the " brown body" is thicker than the rest. It is directed towards the operculum, and the polypide-bud is attached to it, having shifted its original position, and slipped down the strand of funicular tissue in the direction of the " brown body." The polypide-bud is pointed at the end turned towards the operculum, and its walls are here thinner than elsewhere. This portion will become the tentacle-sheath of the new polypide.

Somewhat later (286th hour) many of the polypide-buds had developed tentacles. The tentacle-sheath had become very thin, and was sharply differentiated from the proximal end of the bud. The tentacles were present as a bilateral incomplete ring of small tubercles developed on the side of the bud turned towards the opercular surface of the zoœcium, the ring being open on the side nearest the operculum. The intestine and

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[1] Haddon, loc. cit., fig. 13 (pl. xxxviii).

stomach were growing out from the other (posterior) side of the bud in the manner described by Haddon.[1]

The tentacles soon become arranged in the form shown in fig. 2, the bilateral series bending away from the opercular side of the zoœcium on each side as far as the emargination which marks the point from which the stomach and intestine have been evaginated. The retractor-muscles of the polypide are by this time developed as a group of fibres converging from the polypide to the " brown body."

The tentacles next rotate completely, so as to point towards the aperture, and their bases now form a simple circle, the plane of which is transverse to the long axis of the zoœcium, and at right angles to its opercular surface (fig. 3). The tentacle-sheath has become prolonged along the strand of tissue connecting it with the operculum, and has met a semi-circular thickening lying beneath the operculum, and destined to give rise to the new aperture.

The apex of the evagination which forms the rectum, intestine, and stomach corresponds to the apex of the cæcum. It is attached to the strong funiculus already described. Fig. 3 (305 hours from the beginning of the experiment) shows the commencement of the pharynx and œsophagus as a new outgrowth from the front surface of the bud. The " brown body" is more spherical than before, and has retreated to the proximal end of the zoœcium, a position which it normally occupies at this stage. The tentacle-sheath has met the developing aperture.

At the 353rd hour the two green parts of the " brown body" of the individual shown in fig. 3 were commencing to fuse. The end of the cæcum was of a lighter colour than the rest of the alimentary canal, and had passed, guided by the funiculus, very nearly as far as the " brown body."

At the 376th hour (fig. 4) this part of the cæcum had met the " brown body," in which the two green masses (seen through the " brown body") were in the act of fusing; the " brown body" was commencing to be retracted away from the

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[1] Loc. cit.

proximal end of the zoœcium. The first part of the stomach was being developed as an outgrowth of the cæcum, passing towards the pharynx. The tentacles were obviously increasing greatly in length, although their number had been for some time complete.

At a later period (401st hour) the rectum was distinctly differentiated from the rudiment which gives rise to the stomach, intestine, &c., and contained the so-called "meconium." The tentacles were individually contractile, and the stomach had met the pharynx, but without yet fusing with it.

At the 424th hour (same individual) the "brown body" was further covered by the cæcum, and the intestine was becoming bent over to one side of the zoœcium. The funiculus was present as before, passing along the back of the bud to the distal end of the zoœcium; it has been omitted from most of the figures for the sake of clearness.

At the 448th hour (fig. 5) the cæcum had grown over about half of the surface of the "brown body," which had by this time retreated still further from the proximal end of the zoœcium. The tentacles had increased greatly in length, and the intestine was still further bent over to one side. The intermediate tube, connecting the rectum with the tentacle-sheath, is clearly seen in fig. 5. This tube, which is a normal feature of the polypide, was first noticed in this individual at the 353rd hour.

At the 496th hour the curvature of the intestine to one side was becoming still more pronounced (fig. 6), and the "brown body" was by this time not far from the middle of the zoœcium. A projection of its substance into the lumen of the cæcum indicated its approaching dissociation into fragments destined to pass into the alimentary canal. It had become so dark in colour that the mass of indigo-carmine contained in its interior could no longer be seen, the "brown bodies" being, in individuals of this colony, much darker than the brown-red colour so characteristic of the normal "brown body."

At the 520th hour the "brown body" projected still further

into the cavity of the alimentary canal, but there were no other changes of importance.

It may be here expressly pointed out that the history of this experiment, as traced from the 286th hour, refers specially to a single polypide, which was carefully examined and drawn at intervals of about twenty-four hours, so that the amount of time taken by the development was actually determined. Numerous other zoœcia in the same colony completely confirmed the observations made on this particular individual.

My own observations unfortunately came to an end at the 520th hour, owing to my departure from Naples at that time.

In the particular individual whose history has been traced to the 520th hour after the commencement of the experiment no special change had taken place in the leucocytes, which still contained the bright blue pigment. In some other individuals of the colony, however, a much larger quantity of indigo-carmine had been taken up; so much so, indeed, that it did not at first appear possible that the zoœcia could recover. After a time, as shown in fig. 9 (476th hour), the pigment was deposited in dense masses, no longer contained, as it seemed, in the leucocytes, but probably deposited by these cells in a more or less insoluble form. These masses were found in various positions in the zoœcium, usually in the angles formed by the intersection of neighbouring sides, but sometimes running across the body-cavity. It appeared probable that in fig. 9 the " lateral cords " had formed a kind of focus, into which the pigment had been deposited from the neighbouring leucocytes.

In the same individual (which was certainly alive, and in which the young polypide-bud may be seen in the figure) a mass of indigo-carmine had been deposited by leucocytes at the proximal end of the " brown body," while a similar process seemed to be taking place all round the " brown body," though to a less marked extent than at the proximal end. It was further noticed that, in many individuals, the aggregation of the blue leucocytes round the " brown body " was much

more dense than in the specimens figured on Plate II; and other instances were noticed in which indigo-carmine had almost certainly passed from these leucocytes into the periphery of the "brown body." I at first expected to find that the leucocytes which had taken up indigo-carmine would, in some manner or other, discharge their pigment into the "brown body," with which it would be removed from the zoœcium through the agency of the young polypide. My experiments were, unfortunately, incomplete when I had to leave Naples, so that I cannot speak with any certainty on this point. I am inclined to believe that the greater part of the indigo-carmine taken up by the leucocytes is deposited, in an insoluble form, in masses situated in various parts of the zoœcium (as shown by fig. 9 and, to a less extent, by fig. 5), but that some may pass into the "brown body," as indicated in fig. 9, and so leave the zoœcium. The unusually dark colour of the "brown bodies" in the individuals of the colony treated with indigo-carmine was possibly due to an admixture of indigo-carmine derived from the leucocytes with the normal red pigment of the "brown body."

It has already been pointed out that indigo-carmine is freely taken up by the "lateral cords." During the formation of the "brown body" these cords shrivel, still containing the pigment, and sometimes becoming divided into two or more pieces (fig. 8). The fragments which are left at the distal end of the zoœcium appear to deposit their pigment as a densely blue mass on some part of the inner surface of the wall of the zoœcium. Other parts of the lateral cords may become closely connected with the "brown body," with which they may, perhaps, ultimately pass to the exterior.

The colony on which these observations were principally made did not belong to the experiment previously described. Very little indigo-carmine had been taken up, and nearly all of it was in the lateral cords and in the granules of the alimentary canal; very few of the leucocytes having taken up any blue colour.

I am very much indebted to my friend Mr. A. H. L. New-

stead, of Christ's College, Cambridge, for having made and communicated to me, after my departure from Naples, some further observations on the history of the experiment which I had commenced.

The following statements, referring to F. papyrea, are given on the authority of Mr. Newstead, to whom I express here my very best thanks.

At the 622nd hour the disintegration of the "brown body" had already progressed some distance, and dark masses derived from it were observed in the rectum.

In the following days the disintegration of the "brown body" continued, fragments being broken off from it and passing into the lumen of the alimentary canal. The fragmentation of the "brown bodies" was taking place actively at about the 743rd hour; in some of the individuals the greater part of the "brown body" was by this time accumulated in the rectum.

At the 959th hour the rectum had, in some cases, emptied itself, although a small portion of the "brown body" was still left at the apex of the cæcum (as in Haddon's pl. xxxvii, fig. 11).

During the process of fragmentation of the "brown body," Mr. Newstead was able to observe most conclusively that the indigo-carmine which was contained in the "brown body" passed into the cavity of the new alimentary canal, where it was seen as distinct blue masses in the intestine, rectum, &c. This is especially alluded to in Mr. Newstead's notes referring to the 790th—808th hours.

At the 880th hour these blue fragments had entirely disappeared in some of the individuals, and there can be no doubt that they had left the alimentary canal, with other parts of the "brown body," by a process of defæcation.

In other cases blue fragments were still observed in the intestine and rectum as late as the 1744th hour, the last observation recorded.

During the fragmentation of the "brown bodies" which contained indigo-carmine, Mr. Newstead was unable to ob-

serve that the walls of the alimentary canals took up any of the blue pigment contained in their cavities, with the doubtful exception of the part of the cæcum immediately overlapping the " brown body." This shows that the pigment taken up by the first generation of alimentary canals is really excreted, and that there is no (or at least very little) secondary absorption of the pigment by the new polypides.

I may remark incidentally that my observations, and Mr. Newstead's continuation of them, give some information with regard to the amount of time necessary for the development of a " brown body " and the complete formation of a new polypide in this species of Flustra.

One of the effects of the indigo-carmine on the colony was to induce the degeneration of all the polypides. This process was observed to be commencing rather more than four days after the immersion of the colony in the solution of the pigment. The further history of the experiment is explained by the following table.

Colony treated with a Solution of Indigo-carmine.

5th day (114 hours) . Polypides commencing to degenerate.

7th „ (165 „) . Aggregation of granules of stomach and cæcum into two masses.

10th „ (238 „) . Appearance of young polypide buds.

12th „ (286 „) . Tentacles developed, the lophophore having become circular in the oldest individuals.

13th „ (305 „) . Stage shown in fig. 3.

15th „ (353 „) . Fusion of two masses of granules of stomach, &c.

16th „ (376 „) . Tip of the cæcum becoming connected with " brown body " (fig. 4).

19th „ (448 „) . Fig. 5.

21st „ (496 „) . " Brown body " preparing to break up (fig. 6).

26th „ (622 „) . " Brown body " broken up into numerous fragments, contained in the alimentary canal of the new polypide.

40th „ (959 „) . " Brown body " almost completely absorbed.

The process of absorption of the " brown bodies " was not, however, completed in all the individuals at the seventy-third day (1744 hours), the last observation recorded. It is, how-

ever, obvious that the formation of new polypide-buds commences (and therefore ends) at different periods in the several zoœcia.

The account which has just been given of the growth of the end of the cæcum round the "brown body," and of the manner in which the latter passes into the lumen of the alimentary canal, is supported by the fact that precisely similar processes were noticed in fresh zoœcia which had not been exposed to the abnormal condition of being placed in indigo-carmine.[1]

Mr. Newstead did not find that any of the indigo-carmine contained in the leucocytes passed with the "brown body" into the new alimentary canal; and at the end of the series of observations (seventy-third day) all the living zoœcia still contained blue-coloured leucocytes. Further observations will be necessary to show what is the ultimate fate of these leucocytes. It is not impossible that the pigment contained in them may be excreted in small quantities with successive generations of "brown bodies;" but it appears to be more probable that the pigment is simply left behind in the zoœcia.

Bugula neritina and B. avicularia.

The "brown body" in these species has a very different history from that which has just been recorded in Flustra, inasmuch as it is not taken up by the alimentary canal of the newly formed polypide.

In B. avicularia the formation of a new polypide in an old zoœcium was, in my specimens, comparatively rare. The older parts of the colony merely contained "brown bodies," smaller masses of pigment, leucocytes, and connective-tissue cells of various kinds; and polypides were usually only found in the neighbourhood of the growing-points.[2]

In B. neritina new polypides are freely regenerated in the old zoœcia. They are developed in the middle of a dense network of funicular tissue situated between the front wall of the zoœcium (i. e. the surface which bears the aperture) and the

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[1] See also Haddon's account ('Quart. Journ. Micr. Sci.,' xxiii, 1883).

[2] See Hincks, 'Brit. Marine Polyzoa,' vol. i, 1880, p. 52.

remains of the old polypide. The cæcum of the young poly-
pide soon projects beyond the proximal side of the " brown
body," which is ultimately left near the distal end of the
zoœcium, and at its posterior side (fig. 17).

Nearer the base of the colony the zoœcia usually contain at
least two " brown bodies,"[1] sometimes three, which always lie
at the back of the zoœcium, and which have probably been
formed by the disintegration of as many successive polypides.
The older zoœcia also contain numerous brown spherules,
which have resulted from the disintegration within the zoœcium
either of the older " brown bodies" or of the pigmented
funicular tissue.[2] The leucocytes, in normal old zoœcia, retain
their ordinary, slightly yellowish, clear colour, and the zoœcium
further contains a certain quantity of ordinary funicular
tissue.

No change of importance was noticed in the blue-coloured
leucocytes at any period of the experiments in either of these
species of Bugula. To the end of the observations they
retained their bright blue colour, and were scattered in the
meshes of the funicular tissue. In some cases they became
more or less aggregated into masses situated near the " brown
body" (see fig. 17). In other cases some of the pigment
appeared to pass from the leucocytes into the " brown body,"
apparently by immigration of the entire leucocyte into the
latter; but it was not quite certain that this was a normal
process.

The effect of immersion in indigo-carmine on B. neritina
was, in some cases, to induce the degeneration of most of the
polypides. This was noticed, for instance, in one set of
colonies at the beginning of the fourth day (seventy-three
hours) after the first treatment with indigo-carmine solution.
New polypide buds were being formed in most of the zoœcia
at the fifth day (114 hours), and tentacles had appeared at the
seventh day (148 hours).

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[1] ' Brit. Marine Polyzoa,' vol. i, 1880, p. 53.
[2] Claparède, 'Zeits. f. wiss. Zool.,' xxi, p. 154; pl. ix, fig. 1 C; pl. x,
fig. 2.

The amount of pigment in the funicular tissue had considerably diminished by the eighth day (170 hours), probably indicating that it had been used for the nourishment of the young polypide, a view which is confirmed by the facts that the bud is invariably surrounded by a dense network of funicular tissue, and that the tissue in question is very largely developed at the growing-points. By the end of the same day the cæcum of the young polypide projected considerably beyond the proximal side of the " brown body," and one or two of the blue leucocytes had passed into the ring-canals of the young polypides.

At the eleventh day (243 hours) the polypides had reached the stage shown for Flustra in fig. 4 (sixteenth day). The " brown body " was fully formed and the aperture was in process of development. The funicular tissue appeared to be appreciably diminished in amount, and had lost most of its granules. Although some of these granules are probably used for the nutrition of the young polypide, others appear to give rise to the spherules of brown matter seen in various parts of the body-cavity. These spherules may be regarded as effete portions of the funicular tissue which are deposited, with the " brown body " itself, in the body-cavity.

At the end of the fourteenth day (288 hours) the funicular tissue was regaining its purple colour, which had been lost during the occurrence of the processes already described. I am inclined to regard this purple pigment as being of nutritive rather than of excretory value.

Fig. 17 shows the condition of the polypides regenerated since the beginning of the experiment at the fourteenth day (316 hours). The polypide had by this time become very irritable, but was apparently not yet capable of protrusion. No essential change had taken place in the leucocytes, which were still bright blue. In the individual shown in the figure a large mass of these leucocytes occurred at the distal end of the zoœcium. Twenty-four hours later food was circulating in the intestine, and the aperture appeared to be completely formed.

In Bugula avicularia the "brown bodies" are usually situated in a similar position, at the distal end of the zoœcium, and the leucocytes are in most cases aggregated around them. There was not much reason to suppose that the pigment taken up by the leucocytes passed into the "brown bodies."

In this species, parts of the wall of the alimentary canal took up indigo-carmine. In one case observed, the fæces circulating in the intestine of one of these polypides contained bright blue particles, indicating that some of the pigment taken up by the walls of the cavity may be excreted with the fæces. Most of the indigo-carmine, however, does not escape in this way; and the "brown body," when formed, is very obviously blue-green in colour, owing to the admixture of the indigo-carmine with the natural pigments of the stomach.

At 261 hours after the commencement of the experiment it was noticed that many of the old zoœcia had developed new growing-points at their tips; and some of these already possessed polypides. Blue-coloured leucocytes had in these cases passed in an apparently unaltered state from the old zoœcia into the young growing-points. No obvious alteration of the indigo-carmine contained in these leucocytes took place during the whole of this experiment, which lasted about 350 hours.

Carminate of Ammonia.

B. neritina.—The pigment taken up by the walls of the alimentary canals is left in the "brown bodies" formed by their degeneration. The part of the "brown body" formed from the tip of the cæcum is at first very brilliantly coloured by the pigment. By the 286th hour the colonies contained numerous compact "brown bodies," which were most distinctly carmine-coloured. Many of the polypides whose development had commenced since the beginning of the experiment were nearly mature. At the time of the last observation made (478th hour) no change of importance had taken place. The zoœcia were left with "brown bodies" containing carmine, and probably destined to remain permanently

in the zooecia, while the body-cavity contained small quantities of vesicles or spherules containing carmine, probably derived from the small quantities of that substance taken up by the funicular tissue ; or, in some cases, formed by the fragmentation of the "brown body" within the zooecium.

Precisely similar results were obtained with B. avicularia. The "brown bodies" formed by the degeneration of polypides whose alimentary canals had absorbed the carmine into their walls were of a bright red colour.

At the 480th hour—the end of this series of observations—these red "brown bodies," together with spherules containing carmine (as shown in fig. 22) were left behind in the body-cavities of the zooecia.

BISMARCK-BROWN.

Bugula neritina.—It has already been pointed out that the pigmented funicular tissue of this species takes up Bismarck-brown very freely. The leucocytes are at first unaffected by this pigment; but 120 hours after the beginning of one of the experiments they had become bright orange-yellow in colour, presumably by the absorption of Bismarck-brown from other tissues, since they had remained quite uncoloured by the Bismarck-brown for some hours at least after being transferred to pure sea water. The vacuoles of the leucocytes contained, in some cases, a few deposited granules of the pigment.

Thus, although apparently not adapted to take up Bismarck-brown directly, the leucocytes do their best, at a later period, to remove portions of this pigment which have been absorbed by other tissues; and this is an additional argument in favour of the view that these cells possess excretory functions.

In one of the experiments the action of the Bismarck-brown had been so strong that the processes of the cells of the funicular tissue had been to a large extent retracted; and it at first appeared that the zooecia had been killed, the movements of nearly all the polypides having completely ceased.

Subsequent observation showed that the animals were not really dead. At 48 hours from the beginning of the experiment, the pigment taken up by the funicular tissue was commencing to be deposited in intensely brown (almost black) spheroidal masses of granules in various parts of the funicular tissue. This process continued as time went on, and fig. 18 represents the appearance of the tissue at the 143rd hour. It will be observed that the leucocytes have by this time taken up the pigment, but that in three of those which are drawn the pigment is not contained in all the vacuoles of the cell; so that the cell consists partly of coloured and partly of uncoloured vacuoles. The Bismarck-brown taken up by the funicular tissue is obviously, for the most part, segregated into dense deposits of granules, while the remainder of the tissue has become absolutely hyaline and colourless, containing, however, occasional granules of Bismarck-brown. The parts of the tissue which are now hyaline are, in their normal condition, filled with coloured granules, the absence of which clearly shows that the granules, all of which originally took up the Bismarck-brown, have accumulated in the dense brown masses seen at the nodal points, and have thus succeeded in ridding most of the tissue of the obnoxious foreign substance.

At the same period some of the zoœcia contained minute polypide-buds.

At a later period (238th hour) the leucocytes were still bright yellow, most of them having deposited a few granules of Bismarck-brown in their vacuoles. The funicular tissue retained its previous appearance, the deposited pigment usually tending to accumulate round the " brown bodies," in the form of a dense brown mass at the back of the zoœcium. The tissues which were in course of regeneration, i.e. the polypide-buds, the new muscles, and the greater part of the funicular tissue, were quite transparent and colourless.

At the 312th hour the leucocytes were still more brightly coloured, and contained deposited granules of Bismarck-brown, indicating a continued effort on their part to remove the deleterious pigment from other tissues. The funicular tissue had

deposited nearly all the pigment taken up by it in deep brown masses of granules.

Finally, at the 378th hour, the funicular tissue, having rid itself of the granules which had taken up Bismarck-brown, was commencing to redevelop fresh granules, which had an appearance perfectly similar to those of the normal zoœcium.

During the actual excretion of the Bismarck-brown by the funicular tissue, the deposited granules are contained in vacuoles like those which have already been noticed as concerned in taking up carminate of ammonia. At a later period the vacuoles disappear, and the pigment is in the form of the dense masses already described.

The zoœcia to which the above statements refer were obviously living. Some of the zoœcia were, however, dead, and in these cases the ectocyst and the tissues generally were stained deeply, but diffusely, by the Bismarck-brown. A similar fact was noted with regard to the action of indigo-carmine on dead zoœcia of Flustra papyrea.

In B. avicularia the leucocytes are at first uncoloured by the Bismarck-brown. At a later period, as was the case in B. neritina, they may become coloured by that pigment. An analogous phenomenon was observed with regard to indigo-carmine, the blue colour of the leucocytes increasing in intensity after the first absorption of the pigment. This probably implies that a certain amount of the pigment occurred at first dissolved in other parts of the zoœcium, and that the leucocytes had charged themselves with the office of removing all the indigo-carmine with which the other tissues were infiltrated.

The alimentary canal and tentacles in this species take up large quantities of Bismarck-brown. In some cases such polypides simply degenerate into " brown bodies " which have an intensely orange-brown colour, in consequence of the artificial pigment contained in them.

In other cases, however, the alimentary canal undoubtedly excretes the pigment into its lumen. This process takes place by the separation of small round vesicles from some part of

the wall of the alimentary canal, and probably from the cæcum.[1] These vesicles contain granules of Bismarck-brown, and may be seen in the stomach, intestine, or rectum, where they are no doubt on their way to the exterior. The effect of the continuation of this process was that, whereas the alimentary canals were brightly coloured with Bismarck-brown shortly after their immersion in that fluid, many of the polypides, at the 143rd hour, possessed brightly coloured tentacles, whilst the alimentary canals had become almost colourless, or at least were much less brightly coloured than at an earlier period.

At the 192nd hour the alimentary canals of all those polypides which were functional at the beginning of the experiment, and which had not since degenerated to " brown bodies," had got rid of almost every trace of Bismarck-brown, while the tentacles still remained brilliantly coloured by that pigment. At the 212th hour the alimentary canals of some of these polypides with coloured tentacles were quite colourless, and it was obvious that the polypides had been obliged to discharge all the granules normally contained in the wall of the stomach, &c., in order to excrete the Bismarck-brown. The pigment taken up by other parts of the colony (growing-points, &c.) showed no appreciable diminution in quantity. In those zoœcia in which the " brown bodies " were stained, the body-cavity contained a considerable quantity of irregular masses of granules coloured by Bismarck-brown. This fact further supports the view which has been already suggested, that the " brown bodies " may undergo a certain amount of fragmentation in the body-cavity.

At the 378th hour, polypides with tentacles stained by Bismarck-brown were still left, although the colonies had been kept, since their original immersion in Bismarck-brown, in pure sea-water. The leucocytes had got rid of nearly all their pigment, having probably excreted it into the masses of brown granules seen in various parts of the body-cavity.

[1] The vesicles were frequently noticed in the normal animal, and in one case in B. neritina.

Flustra papyrea.

Some days after the beginning of the experiment, the leuco-
cytes, as in other species, are found to be coloured by Bismarck-
brown. In a colony which had been treated first with indigo-
carmine and afterwards with Bismarck-brown, the leucocytes,
at the 188th hour, were either yellow or green (in the latter
case obviously containing the two pigments), and contained,
within their vacuoles, granules coloured by Bismarck-brown
or by indigo-carmine, or by both substances.

In one experiment it was noticed that the "lateral cords"
of nearly all the individuals had the appearance shown in
fig. 15, 213 hours after their first immersion in Bismarck-
brown. The contents of the lateral cords appeared granular,
but enclosed a number of round vacuoles of a yellow colour.
This is probably to be interpreted as an excretory process.

IV. Summary and General Conclusions.

The experiments made with various artificial pigments
showed conclusively that the tissues did not all react alike to
these pigments, the absorption of which was, in each species,
limited to certain definite tissues. As Kowalevsky has pointed
out for other Invertebrates, the action was precisely the same
if the animals were immersed in a solution of two pigments
mixed. Thus, when indigo-carmine mixed with Bismarck-
brown was used, the leucocytes were at first blue (although
they subsequently absorbed Bismarck-brown from other tis-
sues), just as if the former pigment had been used by itself.

The results on the absorption of the pigments may be
summarised as follows :

Leucocytes.—These cells, in all the species examined, readily
absorb indigo-carmine. Although they do not appear to take
up Bismarck-brown directly, they abstract it from other tissues
at a later period. They are not in the least affected by
carmine in suspension, nor by carminate of ammonia.

Alimentary Canal.—The pigmented granules of the stomach

and cæcum of B. avicularia readily take up indigo-carmine, carminate of ammonia, or Bismarck-brown, and become less obviously red when the animals are fed with carmine in suspension.

In B. neritina the same parts of the alimentary canal take up carminate of ammonia or carmine, but appear to be quite unaffected by indigo-carmine or Bismarck-brown. In F. papyrea the granules take up indigo-carmine or Bismarck-brown. The experiments made with carminate of ammonia and with carmine were unsuccessful.

Funicular Tissue.—This tissue is deeply pigmented in B. neritina, and readily takes up Bismarck-brown. In other species investigated the funicular tissue was for the most part colourless, and did not take up Bismarck-brown in the manner characteristic of B. neritina. Small quantities of this pigment and of carminate of ammonia might, however, appear in spherules apparently contained in cells of a specialised type, which may be regarded as belonging to the funicular tissue; these spherules are often pigmented in their normal condition.[1]

Young, slightly Differentiated Tissues of the Growing-points.— These tissues readily took up considerable quantities of carminate of ammonia and of Bismarck-brown.

It now remains to consider how far the experiments already described throw any light on the normal excretory processes of the marine Polyzoa.

It can hardly be doubted that the pigments experimented with were actually excreted. This was clearly seen in the action of Bismarck-brown on the funicular tissue of B. neritina. The pigment was taken up so freely that it at first appeared extremely improbable that the animals could recover; but at a later period it was deposited in an apparently insoluble form in various parts of the funicular tissue, leaving the remaining parts of that tissue quite free from it. In order to bring about this result the granules normally present in the funicular tissue had to be deposited with the Bismarck-brown

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[1] For a more complete account of the absorption, by various tissues, of the pigments experimented with, see the earlier part of the paper.

they had taken up, and fresh granules had to be developed later.

Similarly the alimentary canal of B. avicularia was able to rid itself of Bismarck-brown, a process involving the loss of its normal granules, by excreting it into the lumen of the alimentary canal, enclosed in spherules noticed as of normal occurrence in this species; these spherules are probably concerned in the excretion of some of the normal pigments of the alimentary canal, although their function may be in part a digestive one.

Again, the pigments taken up by the alimentary canal, whether carmine, indigo-carmine, or Bismarck-brown, passed for the most part into the "brown bodies" at the time of the degeneration of the polypides. In F. papyrea it was definitely proved that indigo-carmine contained in the "brown bodies" left the zoœcia by way of the alimentary canals of the new polypides. In the two species of Bugula the "brown bodies," coloured by pigments taken up at an earlier period, were left behind in the zoœcia.

The leucocytes charged with indigo-carmine did not appear to take any further active part in the life of the zoœcium; but they certainly play the important part of retaining the greater quantity of the pigment which originally soaked into the tissues. The other tissues thus remain without the slightest trace of a blue coloration; and it can hardly fail to be admitted that the leucocytes are performing an excretory function, so far as they are exercising a selective absorption of an abnormal substance like indigo-carmine from the other tissues. In dead zoœcia all the tissues become at once diffusely stained with indigo-carmine. So long as the zoœcium remains alive the leucocytes retain this pigment, and shield other tissues from its action.

On the regeneration of a polypide the growing tissues are invariably quite free from the artificially introduced pigments. The exposure to the action of these bodies may in some cases result in the degeneration of the polypide or of the funicular tissue; but the young polypide-bud and its muscles, as well as

the freshly formed funicular tissue, do not develop with their cells impregnated with the pigment in question. It will probably be admitted that the pigment is deleterious, and that certain tissues capable of taking it up retain it in order to allow the newly formed tissues to escape its injurious action.

It may thus be taken for granted that certain tissues of the animals investigated have an excretory function, so far as certain pigments which are abnormally introduced into the animal are concerned. Can it be further shown that these tissues, or any of them, are to be regarded as normal excretory tissues?

In the first place it may be noticed that the marine Polyzoa are not known to possess specific excretory organs. The "intertentacular organ" described by Farre,[1] Hincks,[2] Prouho,[3] and others, has not been shown to be of sufficiently general occurrence in marine Polyzoa to allow it to be regarded as the principal excretory organ. Hincks has, indeed, stated that he has "repeatedly seen a mass of excrementitious matter pass into the organ from below," and that it is "at last ejected, as a pellet, through the terminal orifice."[4] This observation seems to show that, in those Polyzoa which possess this organ, it may really serve for the excretion of the effete products of the metabolism of various tissues, and this, together with the discharge of the spermatozoa, is the function ascribed to it by Hincks. But in spite of the known occurrence of this organ in a few species, and of structures like the semilunate pore of Microporella Malusii, stated by Pergens[5] to open directly into the body-cavity, it is obviously necessary to look for other excretory arrangements in the vast majority of marine Polyzoa in which no special structures of this kind have been detected.

It may next be pointed out that one or two of the pigments used in these observations are, in other animals, specially

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[1] 'Phil. Trans.,' 1837, pp. 408 and 412.
[2] 'Brit. Marine Polyzoa,' vol. i, p. lxxxix.
[3] 'Comptes rendus,' t. cix, 1889, p. 197.
[4] Loc. cit., p. xc.
[5] 'Zoolog. Anzeiger,' Jahrg. xii, 1889, p. 507.

selected by organs which are known on other grounds to have an excretory function.

Kowalevsky,[1] starting from the well-known facts with regard to the excretion of indigo-carmine and carminate of ammonia in Vertebrates,[2] has shown that both these substances are removed from the body, in many Invertebrates, by organs which are undoubtedly excretory in nature. Indigo-carmine injected into the body is excreted, for instance, by the Malpighian vessels of Insects, by the tubules of the green gland of the Crayfish (but not by the end-sac), by the organs of Bojanus in Pecten and in other Lamellibranchs, by the kidney in Gasteropods, by the brown tubes of Phacolosoma, &c., all of them organs which are admitted to have an excretory function. Carminate of ammonia was excreted by the end-sac of the green gland of the crayfish, by the nephridia of Nereis, by the excretory tubes of Tænia, &c. That the removal of indigo-carmine is really a process of excretion is shown, for instance, by the experiment recorded with regard to Paludina. On injecting this animal with a mixture of indigo-carmine and carminate of ammonia, the tissues were at first violet; after one or two days the blue colour was entirely taken up by the kidney, and the animal became red, and the red colour itself disappeared after a further interval.

Additional proof that the removal of these pigments is a process analogous to the normal excretory processes is afforded by Kowalevsky's observations that the crystals of (excreted) indigo-carmine appear, in the organ of Bojanus of Pecten, in the very vacuoles which actually contain normal excretory concretions, and that in Phallusia and in Molgula crystals of this substance make their appearance in a similar relation to the concretions which are normally present.

Kowalevsky's observations further show that these two pigments may be excreted by different parts of the same excretory

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[1] 'Biolog. Centralblatt,' Bd. ix, p. 33, &c.

[2] See L. Hermann's 'Handbuch der Physiologie,' Bd. v, Theil i; 'Absonderungsvorgänge,' by R. Heidenhain, Leipzig, 1883, p. 345, and the references there given.

organ, as in the case of the green gland of the crayfish, where the end-sac excretes carminate of ammonia and the tubules excrete indigo-carmine, or as in the well-known case of the Vertebrate kidney. Similarly, the facts recorded above with regard to the Polyzoa show that the function of taking up these and other pigments is by no means restricted to one set of cells,—the leucocytes, for instance, taking up indigo-carmine, but being quite unaffected by carminate of ammonia.

Kowalevsky's results on Nereis are of special interest in connexion with my own results on the Polyzoa. In that animal Kowalevsky showed[1] that indigo-carmine is taken up principally by the blood-corpuscles, but also by certain segmentally arranged organs consisting of glandular cells lying on the dorsal side, and containing, in the normal animal, accumulations of brown or yellow bodies. This latter statement agrees closely with Eisig's results on the excretion of carmine in Capitella,[2] in which carmine taken up and digested by the alimentary canal is ultimately excreted into pigmented granules which normally occur in the skin. Eisig shows that there is considerable reason for regarding the cutaneous pigment of Capitella and of many other animals as an excretory product.[3]

The taking up of indigo-carmine by the cells which have been above described as "leucocytes" is analogous to its absorption by the blood-corpuscles of Nereis. This pigment as well as the others employed may, however, be taken up by certain normally pigmented cells occurring principally in the walls of the alimentary canal.

This fact recalls the observation of Chrzonszczewsky[4] that

[1] Loc. cit., p. 71.

[2] 'Fauna u. Flora G. v. Neapel,' Monographie xvi (" Capitelliden "), 1887.

[3] The interesting results of H. E. Durham (" The Emigration of Amœboid Corpuscles in the Starfish," 'Proc. Roy. Soc.,' vol. xliii, 1888) have not much bearing on my own observations, inasmuch as Mr. Durham investigated the ingestion of granules of precipitated pigments by leucocytes, whereas my observations concerned the excretion of solutions of pigments permeating the tissues.

[4] N. Chrzonszczewsky, "Zur Anat. u. Physiol. d. Leber," 'Virchow's Archiv,' xxxv, 1866, p. 157.

indigo-carmine is excreted in large quantities by the liver of Vertebrates, as well as by the kidney; the bile, like the urine, becoming blue soon after the injection of indigo-carmine,— suggesting a new analogy between the so-called "liver-cells" of Polyzoa and the liver of Vertebrates. Without going into the question of the excretory value of the processes which take place in the Vertebrate liver—a question I am not competent to discuss—I may express my conviction that the appearance of pigments like indigo-carmine, carminate of ammonia, and Bismarck-brown in the granules of the walls of the alimentary canal in Polyzoa, taken in conjunction with the normal appearance, in the same place, of a natural pigment, and the ultimate passage of much of that pigment into the "brown body," is to be regarded as—in part at least—a process of excretion. As has been already pointed out, Ostroumoff[1] has definitely formulated the view that the occurrence of "brown bodies" is correlated with the absence of nephridia, and indications of a similar manner of regarding these bodies are not wanting in the writings of other observers.

It is certainly a significant fact that, while the young polypide-bud is, in most cases, at first quite colourless, brown pigment soon appears in the wall of the stomach, &c.; and that when the polypide degenerates, the most conspicuous feature of the "brown body" is the pigment whose presence has suggested that name for the degenerated polypide. In some Ectoprocta the "brown body" leaves the zoœcium by way of the alimentary canal of the new polypide; in others it is simply left behind in the zoœcium, just as, in many Tunicates, the excretory concretions are stored up in various parts of the body without ever finding a way to the exterior.

It has already been pointed out that in B. avicularia Bismarck-brown was excreted into the lumen of the alimentary canal, enclosed in spherules which were also noticed in the normal condition. This fact tends to show that the spherules in question are, in part at least, excretory, although their

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[1] A. A. Ostroumoff, "Cont. à l'Ét. Zool. et Morphol. des Bryozoaires du Golfe de Sébastopol," 'Arch. Slaves de Biol.,' t. ii, 1886, p. 339.

formation may not be altogether unconnected with the process of digestion. Instances of excretory processes carried on by the walls of the alimentary canal are, however, by no means unknown in other animals.

In this connexion, too, Eisig's results on the Capitellidæ[1] must be further cited. Eisig gives a most elaborate discussion of the excretory value of many natural pigments, not only in the Capitellidæ, but also in various other animals.[2] The facts observed by myself with regard to the appearance of indigo-carmine and other pigments in cells which are normally pigmented is in complete accord with Eisig's results; tending to establish the conclusion that these normal pigments are to be interpreted as, to a considerable extent, excretory in nature. As instances of this may be mentioned the deposition of these pigments in the granules of the wall of the alimentary canal, and in the granules of the funicular tissue of B. neritina; the appearance of carminate of ammonia in cells of the type shown in Pl. III, fig. 22, these cells also containing natural brown pigments; and the association of indigo-carmine with natural pigments in the leucocytes of F. papyrea (figs. 23 and 24).

The general conclusion of the experiments described above is that excretion is performed, in the marine Ectoprocta, partly by the cells which have been described as " leucocytes," partly by the walls of the alimentary canal, and partly by the funicular tissue. The so-called " lateral cords " probably play some part in excretion, although I am by no means confident that this is their principal function.

A few remarks may be made with reference to Cuénot's recent paper[3]—the general conclusions of which had been

[1] Loc. cit.

[2] I have had the opportunity of looking through the manuscript of a paper which is to be shortly published by Mr. H. E. Durham, who has brought together a large series of observations tending to establish the same conclusion. (*See paper preceding this; p. 81–121.*)

[3] L. Cuénot, " Études sur le sang et les glandes lymphatiques dans la série animale. 2e Partie: Invertébrés," 'Arch. Zool. Exp. et Gén.,' 2e sér., t. ix, 1891, pp. 12 and 365.

given in the preliminary note already referred to,—which has
come into my hands since the above was written.   Cuénot
throws doubt on the value of the evidence given by the reaction
of the seve ral tissues to pigments such as indigo-carmine in
elucidating the nature of the excretory processes ; and supposes
that the absorption of carmine by the pericardial tissue of
Insects, for instance, is due to the affinity of that tissue for
colouring matters (see p. 398).   The enumeration given by
Cuénot of the details of the absorption of various pigments by
certain tissues to which an excretory function is usually not
ascribed does not seem to me so convincing, in Cuénot's sense,
as the demonstration by Kowalevsky and others that these
pigments are selected by organs known to be excretory in
nature in the opposite sense.   Cuénot shows that the leuco-
cytes of Polyzoa are quite similar to the "amibocytes" of
other animals (see p. 407), the most important function of
which would appear to be the transformation of the peptones
thrown into the blood by the alimentary canal into non-
dialysable albuminoids.   The same author alludes to the
formation of "pseudo-plasmodia" by the "amibocytes" of
various animals (cf. the description given on p. 131 of a
similar process in Flustra) ; and mentions more than once
the occurrence, in various Invertebrates, of cells whose
granules exhibit a Brownian movement, and which he usually
regards as of respiratory nature ("hématies").   I have not,
however, found in Cuénot's figures or text an account of any
cells which can be exactly compared with the remarkable cells
above described in Bugula avicularia (see p. 135).

EXPLANATION OF PLATES II and III,

Illustrating Mr. Sidney F. Harmer's paper " On the Nature of the Excretory Processes in Marine Polyzoa."

(All the figures were drawn from living animals.)

PLATE II.

Flustra papyrea (indigo-carmine experiments : all the figures were drawn with a camera lucida, under a Zeiss' C objective).

Fig. 1.—264th hour. Front view. The blue leucocytes are grouped round the "brown body," in which can be clearly distinguished the remains of the tentacles and two green masses derived from the granules of the alimentary canal, which had taken up indigo-carmine in addition to their normal pigment. A young polypide bud, slung in a cord of funicular tissue attaching it to the " brown body," is already present.

Fig. 2.—286th hour. Front view, showing the condition of the lophophore when half rotated (leucocytes not represented).

Figs. 3—6.—Successive stages of a single polypide.

Fig. 3. 305th hour. Front view. The " brown body " is more compact than in Fig. 1, and is situated at the extreme proximal end of the zoœcium. Its two green masses are approaching one another. The tentacle-sheath has met the new aperture, which is in process of formation.

Fig. 4. 376th hour. Front view. The "brown body" has become much darker, and its two green masses (which are not very distinctly visible, lying, as they do, at the other side of the " brown body ") are beginning to fuse. The cæcum of the stomach has met the " brown body," and the proventriculus is growing out to meet the œsophagus.

Fig. 5. 448th hour. Back view. The cæcum has nearly half covered the " brown body." The intestine is bent over to one side, and the rectum contains the " meconium."

Fig. 6. 496th hour. Back view. The "brown body" is now much nearer the middle of the zoœcium than in earlier stages, and has a process projecting into the lumen of the alimentary canal, indicating its approaching absorption (leucocytes not represented).

For later stages in the absorption of the "brown body" in this species, see Haddon's figs. 9, 10, and 11 (this Journal, vol. xxiii, Pl. XXXVII).

Figs. 7 and 8.—From an experiment in which very little indigo-carmine had been taken up by leucocytes. 283rd hour. Back views. Polypide buds and other structures not represented. Notice the indigo-carmine contained in the two green masses in the "brown body."

> Fig. 7. The zoœcium has a somewhat abnormal shape. The "lateral cords" contain a quantity of indigo-carmine, and are less uniform in calibre than in zoœcia with functional polypides.
>
> Fig. 8. The lateral cords have broken up into several portions.

Fig. 9.—From an experiment in which the leucocytes, &c., had absorbed an unusually large quantity of indigo-carmine. 476th hour. Back view. A new polypide bud is developed. The "brown body" appears to be absorbing indigo-carmine from the leucocytes, which have, however, deposited the greater part of their pigment in continuous deep blue strands situated in the body-cavity.

PLATE III.

Figs. 10—12.—F. papyrea. Successive stages in the formation of the (ectodermic part of the) polypide bud (Zeiss, DD).

Fig. 13.—F. papyrea. Normal excretory (?) cell from endocyst, with natural pigment (Zeiss, $\frac{1}{12}$ oil immersion).

Fig. 14.—F. papyrea. Normal funicular tissue, with colourless refractive granules, giving a starch-like reaction with iodine (Zeiss, F).

Fig. 15.—F. papyrea. Bismarck-brown. 213th hour. Portion of one of the lateral cords (Zeiss, F).

Fig. 16.—Bugula avicularia. Indigo-carmine. 72nd hour. Side view. Rough sketch of the alimentary canal, to show the distribution of the granules which take up indigo-carmine. Parts of the stomach, &c., are green, as the natural blue of the indigo-carmine contained in the granules is masked by the normal pigment of these structures. In the rectum, in which the granules are practically colourless, the blue colour is not obscured (Zeiss, DD).

Fig. 17.—B. neritina. Indigo-carmine. 316th hour. Back view. The polypide, which has been completely developed since the beginning of the experiment, is not quite mature. The arrangement of the leucocytes in the meshes of the funicular tissue is well seen (Zeiss, C).

Fig. 18.—B. neritina. Bismarck-brown. 143rd hour. Portion of the funicular tissue of a colony in which a very large quantity of the pigment had been absorbed. At A is seen a cell whose granules have an almost normal appearance. The greater part of the pigment taken up by the funicular tissue has been deposited in the form of dense brown masses, leaving the rest of the tissue hyaline. Most of the vacuoles of the leucocytes contain Bismarck-brown in solution, but one or two of them still remain colourless (Zeiss, F).

Fig. 19.—B. neritina. Indigo-carmine. 64th hour. Three leucocytes (Zeiss, F).

Figs. 20—22.—B. avicularia.

Fig. 20. Bismarck-brown and indigo-carmine (mixed). 40th hour. Three leucocytes containing indigo-carmine, and two of the cells which contain minute granules exhibiting a Brownian movement, or larger concretions. These cells appear to have taken up minute quantities of Bismarck-brown and of indigo-carmine (Zeiss, F).

Fig. 21. (Same treatment as Fig. 20.) Two leucocytes and one of the cells with colourless granules found in the body-cavity of this species (Zeiss, F).

Fig. 22. Carminate of ammonia. 330th hour. Two leucocytes, whose natural colour is unaltered, and several of the well-defined spherules, found especially near the growing-points, and containing carmine as well as natural brown pigments (Zeiss, F).

Figs. 23 and 24.—F. carbasea.

Fig. 23. A complex of leucocytes which have taken up indigo-carmine, and which contain granules of deposited indigo-carmine as well as natural pigments (Zeiss, F).

Fig. 24. An individual leucocyte (Zeiss, F).

Spermatogenesis in Myxine glutinosa.

By

J. T. Cunningham, M.A.,

Naturalist on the Staff of the Laboratory of the Marine Biological
Association, Plymouth.

With Plate IV.

In a paper published in this Journal in 1886 (2) I described
the generative organs and the peculiar protandrous herma-
phroditism of Myxine. That description included a brief and
incomplete account of the spermatogenesis, of which I had not
been able, for want of leisure and material, to make a more
deliberate investigation. In 1888, Dr. Fridtjof Nansen, then
Zoological Curator at the Bergen Museum, published in the
'Aarsberetning,' or Annual Report of that Institution, the
results of a study of the reproductive organs of the animal in
question (3). Dr. Nansen's researches were, as he himself
states, suggested to him by my paper, and in a great many
important matters he fully confirms my results. But with
regard to the development of the spermatozoa he contradicts
and rejects with perhaps unnecessary emphasis all my state-
ments, and comes to the conclusion that I never saw the
normal spermatozoa at all.

I was therefore naturally desirous of going into the subject
again and investigating it more fully. I was led to believe
also that it was possible to obtain living Myxine with less
trouble and less expense in the neighbourhood of Bergen than
on our own coasts. I therefore decided to go to Bergen, and
not only to study the spermatogenesis anew, but to make
further attempts to obtain the fertilised ova. I was enabled to

them is seen to be made by an intermediate mass of clear
protoplasm, as in fig. 2, *d*.   Besides the elements already
mentioned there are seen in each preparation several others.
There are spindle-shaped bodies produced into an attenuated
process at each end (fig. 2, *a*) ; in these a nucleus is not usually
visible.   There are also a large number of less regularly shaped
masses of clear protoplasm running out into similar attenuated
processes, some being bipolar, some multipolar.   In the
majority, though not in all of these are seen one or two, or a
number of bodies in all respects similar to the heads of sperma-
tozoa.   In some cases these bodies, which may conveniently be
called sperm-nuclei, are seen to be connected, as in fig. 3, *a*,
fig. 1, *a*, with a mass of protoplasm by a thin protoplasmic
filament similar to the tail of a spermatozoon.   In other cases
the sperm-nuclei are situated in the substance of processes of
the protoplasmic masses, and in other cases again there are
several of them scattered through the main body of these
masses.   The various conditions seen irresistibly suggest the
idea that the sperm-nuclei are capable of motion, that they are
pushing themselves out of the protoplasmic masses, drawing a
shread of the viscous protoplasm behind them, and that when
the connection between the mass and the thread breaks, the
nucleus with the portion of the thread attached to it forms an
independent spermatozoon.   I believe that all this really takes
place, but that it is the clear protoplasm which effects the
changes, the sperm-nuclei remaining passive.   For several
times I have seen the protoplasm actually in motion like the
tail of a spermatozoon.   For instance, the filament connecting
b and *c* in fig. 2 was slowly vibrating from side to side and
gradually lengthening while under observation, although there
is no sperm-nucleus in either *b* or *c*.   At *b* in fig. 1 is seen a
small unipolar mass containing two sperm-nuclei ; the apex of
this mass was twitching when the drawing was made.   But it
must be remembered that as a rule no motion is seen except in
the tails of perfect spermatozoa ; so that when I say that the
protoplasm is the active agent in separating the spermatozoa
from the larger masses, I mean that it does so in the same

gradual way in which it divides a cell into two, or a spermato-
cyte in another animal into a bundle of spermatozoa.   Active
vibration of the tails of the spermatozoa in Myxine usually
begins only when they have reached their perfect form.

On the explanation I have adopted the attenuated processes
of the protoplasmic masses were originally continuous with the
tails of spermatozoa which have separated.   In many cases
sperm-nuclei are seen in the figures near the bases of these pro-
cesses, and it might perhaps be argued that the processes are
the tails belonging to these heads, and that the spermatozoa
are produced from the spermatocytes tail first as in other ani-
mals, not head first as I maintain in this case.   But it will be
noticed that in all cases the pointed apex of the sperm-nucleus
is turned outwards towards the process, while its broad base is
towards the centre of the mass of protoplasm.   Since in the
perfect spermatozoon the tail is attached to the base of the
sperm-nucleus, and not to the apex, it follows that my account
of their formation is correct.

I have to complete the history I read from the appearances
by saying that the spherical nucleated spermatocytes give rise
to the protoplasmic masses containing sperm-nuclei.   The
single nucleus of a spermatocyte doubtless divides into several
sperm-nuclei, producing structures like that shown in fig. 1, c.
It must be mentioned that the bipolar spindles, of which an
example is represented at a, fig. 2, are in most specimens of
Myxine, next to the spermatozoa, the most abundant elements,
multipolar cells being comparatively rare.   These spindles vary
very much in size, and in many of them neither sperm-nuclei
nor nuclei of any kind can be detected in them in the fresh
condition.   The explanation of these facts is, I think, that as a
rule the sperm-nuclei pass out successively in the same
direction, and so leave only two processes behind them.   This
view is clearly supported by the condition of many of the
spermatocytes represented in figs. 1, 2, and 3, where a sperm-
nucleus is seen at the base of a process which was formed by
the production of a spermatozoon already separated.

I will now give the results I have obtained from the study

them is seen to be made by an intermediate mass of clear protoplasm, as in fig. 2, *d*.   Besides the elements already mentioned there are seen in each preparation several others. There are spindle-shaped bodies produced into an attenuated process at each end (fig. 2, *a*) ; in these a nucleus is not usually visible.   There are also a large number of less regularly shaped masses of clear protoplasm running out into similar attenuated processes, some being bipolar, some multipolar.   In the majority, though not in all of these are seen one or two, or a number of bodies in all respects similar to the heads of spermatozoa.   In some cases these bodies, which may conveniently be called sperm-nuclei, are seen to be connected, as in fig. 3, *a*, fig. 1, *a*, with a mass of protoplasm by a thin protoplasmic filament similar to the tail of a spermatozoon.   In other cases the sperm-nuclei are situated in the substance of processes of the protoplasmic masses, and in other cases again there are several of them scattered through the main body of these masses.   The various conditions seen irresistibly suggest the idea that the sperm-nuclei are capable of motion, that they are pushing themselves out of the protoplasmic masses, drawing a shread of the viscous protoplasm behind them, and that when the connection between the mass and the thread breaks, the nucleus with the portion of the thread attached to it forms an independent spermatozoon.   I believe that all this really takes place, but that it is the clear protoplasm which effects the changes, the sperm-nuclei remaining passive.   For several times I have seen the protoplasm actually in motion like the tail of a spermatozoon.   For instance, the filament connecting *b* and *c* in fig. 2 was slowly vibrating from side to side and gradually lengthening while under observation, although there is no sperm-nucleus in either *b* or *c*.   At *b* in fig. 1 is seen a small unipolar mass containing two sperm-nuclei ; the apex of this mass was twitching when the drawing was made.   But it must be remembered that as a rule no motion is seen except in the tails of perfect spermatozoa ; so that when I say that the protoplasm is the active agent in separating the spermatozoa from the larger masses, I mean that it does so in the same

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spermatocytes represented in figs. 1, 2, and 3, where a sperm-
nucleus is seen at the base of a process which was formed by
the production of a spermatozoon already separated.

I will now give the results I have obtained from the study

of sections prepared, since my return, from material preserved in Norway. The testicular tissue was preserved with Flemming's mixture of chromic, osmic, and acetic acids. I have found the best staining liquid to be an aqueous solution of saffranin. In all the pieces of testis which I have cut into sections I have found that only a small number of the capsules contain ripe spermatozoa. A great many capsules are always full of cells containing large nuclei in the resting condition: examples of these cells are represented in Plate IV, fig. 4. Other capsules are full of similar cells preparing to divide, the chromatin of the nuclei being in the skein form, as shown in fig. 5 of the same plate. In other capsules, again, cells of the same large size exhibit the stage of the equatorial plate, as in fig. 6, the chromatin being in the form of a number of densely stained rods at the equator of a protoplasmic spindle. In my preparations the capsules in the last condition are not full, the cells being separate from one another, not in contact as in other cases.

The spermatozoa are seen with other elements in a few capsules of a section. Figs. 7, 8, and 9 exhibit the cellular elements seen in a single ripe capsule in one section. In fig. 9 are shown one perfect spermatozoon, several spindle-shaped cells, and one other structure. The spindle a is destitute of chromatin: it has an axial core more deeply stained than the rest of the cell, and continuous with the attenuated processes. This core consists of very faint longitudinal striæ. One of the processes in the spindle b is connected with a sperm-nucleus, but there is no chromatin in the body of the spindle; the axial core is distinct. At c is seen another spindle in which the core is not distinguishable, and in which there is a dense small nucleus similar in appearance to a sperm-nucleus, but more rounded in shape. At d is a smaller spindle very faintly stained, containing a small nucleus, whose structure is that of an ordinary resting nucleus and not of a sperm-nucleus. g is a spindle in which a sperm-nucleus is situated at the base of one of the processes; h is similar to c, except that the core is visible as well as the nuclear body. Comparison of

b and *g* shows that the explanation I have given of the forma-
tion of the spermatozoa is correct : the sperm-nucleus in *g* is
evidently about to separate from the spindle, so as to pass first
into the condition of *b*, and finally to become the head of a
spermatozoon. The nucleus in *c* may be one which would
afterwards have formed a sperm-nucleus, but it is possible that
the whole of the chromatin of a spermatocyte is not always
used up in the formation of sperm-nuclei, and that the nucleus
in *c* is a portion left in the spindle which would remain there
unused. In *d* a portion has remained behind and resumed the
structure of an ordinary nucleus. In any case chromatin is
sometimes present in the spindles, sometimes not. The struc-
ture shown at *f* is a rounded mass of protoplasm connected by
a filamentous process with a spermatozoon-head of unusually
large size, the sperm-nucleus consisting of discontinuous
chromatin. In some capsules numbers of spermatozoa have
heads of this structure, from which it may be concluded that
the degree of concentration of the chromatin in the sperm-
nuclei at their first formation is somewhat variable. In all
probability when the chromatin of the sperm-nucleus is at first
reticulated, it concentrates afterwards into the solid mass of
smaller dimensions which is characteristic of the head of the
ripe spermatozoon.

Pl. IV, figs. 7 and 8, represent cells with nuclei in the
resting state, from the same capsule as the elements shown in
fig. 9. Some of these cells are as large as the spermatocytes
of an unripe capsule, others are somewhat smaller, but probably
all are spermatocytes which have not yet begun to produce
spermatozoa.

Fig. 11 shows elements from a ripe capsule from another
specimen. The three spindles here containing several sperm-
nuclei agree in all respects with similar elements which were
described above in fresh preparations. They prove conclusively
that my interpretation of the elements as seen in the fresh
condition was correct ; the correspondence between the sperm-
nuclei in these spindles and in the heads of spermatozoa is
exact. Moreover it is clear from the condition of *b* and *c* that

three or more sperm-nuclei may escape successively from a spermatoblast without forming more than two processes.

In the series of sections from which fig. 12 was taken, although there were plenty of perfect spermatozoa, and spindles like those in other sections, there were several abnormal structures like those represented at *a* and *b*. I believe these were not produced by imperfect preparation of the tissue, but were really present in this particular testis. They are obviously due to a coalescence or excessive enlargement of spermatocytes, the nuclei having segmented in the peculiar manner shown.

Thus I think it is clear that the process of spermatogenesis to be deduced from the structures, seen both in fresh teased preparations and in stained sections of fixed and hardened material, is as follows :—The cells found in unripe capsules (Pl. IV, fig. 4) are spermatocytes which multiply by karyokinetic division. At a certain period of development these cells cease to divide in this way, and commence to form spermatozoa; the nucleus of the spermatocyte loses its ordinary structure, and the whole of its chromatin is formed into a number, probably six or more, of pear-shaped bodies which may be appropriately called sperm-nuclei. By the activity of the protoplasm of the spermatoblast these sperm-nuclei separate one by one from the latter, passing out point foremost, and trailing a slender thread of protoplasm behind them. The thread breaks near the spermatocyte, and the free portion then forms the tail of a perfect spermatozoon. The spermnucleus is doubtless surrounded in the head of the spermatozoon with a thin envelope of cell-plasma, which expands behind the nucleus into a small protoplasmic mass often called the body of the spermatozoon. After one spermatozoon has separated, another sperm-nucleus may pass out of the spermatocyte along the process left behind by the former. In this way the remnant of the spermatocyte usually forms a spindle-shaped body with a process at each end, but sperm-nuclei may separate at three or more points instead of two, and then multipolar bodies are formed.

I regret that I have not been able to trace satisfactorily the

successive steps in the transformation of the nucleus of the spermatocyte into a number of sperm-nuclei. I believe, however, that *d*, *e*, and *c*, Pl. IV, fig. 11, exhibit stages in this process; *d* and *e* are spermatocytes with two and three nuclei respectively, and it seems that the original nucleus of the spermatocyte simply divides without any process of karyo-kinesis into first two, and then more, smaller resting nuclei. The chromatin in these small nuclei then undergoes concentration to form the solid sperm-nuclei, all other parts of the nucleus disappearing. If the membrane of the resting nucleus is chromatin, it must, of course, cease to exist as a membrane after the concentration, while the achromatic substance (Kern-saft) doubtless fuses with the cell-plasma. In this way the spermatocyte reaches the condition seen in *c*, fig. 11, or fig. 1.

Another point on which I am uncertain is the history of the capsules after the spermatozoa have been discharged from them. I think there is little reason to suppose that a given capsule can produce more than one crop of spermatozoa. The capsules closely resemble the follicle of the ovary, and we know that the follicles are gradually atrophied after the discharge of their ova. But some of the follicles of the ripe testis contain a large number of small separate cells whose nuclei are in the resting condition ; cells from such a follicle are represented in Pl. IV, fig. 10. Similar small cells are also often seen in addition to the other elements in a follicle which contains ripe spermatozoa. Since these cells are considerably smaller than the spindle-shaped cells from which the spermatozoa are derived, I cannot believe, as Dr. Nansen does, that they have anything to do with the production of the spermatozoa. It seems to me much more likely that they are derived from the remnants of the spindle-shaped cells left after the spermatozoa have been formed, and that capsules containing them are in process of degeneration.

In my former paper (2) on the reproductive elements of Myxine I gave the following description of the spermatozoa and their genesis :—" The spermatozoa possess a pear-shaped head which is very highly refringent, and has a distinct out-

line ; round the posterior thicker end of the head is a translu-
cent protoplasmic body which is produced into a long tail."
" In some cases two spermatozoa were connected by their tails,
and on the connecting thread thus produced were slight dila-
tations composed of clear protoplasm. In other cases a cell
somewhat spherical in shape gave off two processes, one of
which was the tail of a spermatozoon, while the other termi-
nated in a point, the head of the spermatozoon belonging to
the process having probably become detached in the operation
of teasing.   There were also seen cells in which were present
one or more structures resembling the heads of spermatozoa ;
these heads had, however, no tails."   " It is evident that the
cells and spermatozoa described were derived from the sphe-
rical cells of the testicular capsules.   These cells apparently
develop the heads of the spermatozoa, each of which then grows
out from its cell, trailing a thread of protoplasm which forms
the tail.   The curious thing about the spermatogenesis observed
in Myxine is that the spermatozoa are attached to the spermato-
blast by their tails, and not by their heads as usually occurs."

It will be seen that the results described in the present paper,
though more detailed and complete, are in no respect in con-
tradiction to the statements I have just quoted from my former
paper.   But Dr. Nansen, after quoting the above passages,
writes thus :—" Those strange statements are completely erro-
neous as regards the structure of the normal spermatozoa, as
also the process of spermatogenesis ; my investigations have
led me to no such surprising conclusion, as will subsequently
be seen, though the testis and spermatogenesis of Myxine are in
several respects very remarkable."

The account of the spermatogenesis which Dr. Nansen
offers in substitution for mine is as follows :—The spherical
cells which completely fill the unripe capsules he calls sper-
matocytes, a term therefore synonymous with spermatoblasts
in my former description.   When the spermatocytes have
become reduced by subdivision to a certain size the testicular
capsule is rapidly enlarged, and the spermatocytes are isolated
from one another.   The spermatocytes, however, continue to

subdivide, becoming still smaller; when subdivision ceases they become spermatides. By an elongation of the nucleus, as well as the whole body of the cell, these spermatides are now gradually transformed into ripe spermatozoa. Concerning the manner of this transformation Dr. Nansen writes:—"As to the details of the development of the spermatides into spermatozoa, I will give no circumstantial description here; my investigations of that branch of the subject are not yet finished. From the little I have seen I think, however, that the spermatozoon is formed from the nucleus as well as from the protoplasm of the spermatide, i. e. the whole spermatide is transformed into a spermatozoon. As to the tail, that is perhaps formed partly by an elongation of the nucleus, partly by the protoplasm of the spermatide."

Thus the spindle-shaped cells which I have described are, according to Nansen, stages in the transformation of a single spermatide into a spermatozoon. There is no difference between us as to the elements seen in the testis of Myxine, but only as to their interpretation. But although the elements I have described are recognisable in Nansen's figures, they are not accurately represented. He figures the slightly more deeply stained core of the spindle-shaped cell as the nucleus, and supposes that this becomes the head of the spermatozoon. The true sperm-nuclei figured by me in the interior of the spindles are nowhere figured by Nansen. This error vitiates entirely his figures and description. Two obvious difficulties in his interpretation he does not notice. One is that the spindle-shaped spermatides even in his own figures have a filamentous process at each end; if one of these is the tail of the spermatozoon what becomes of the other? The second difficulty is the enormously greater size of the spindle-shaped cells as compared with the spermatozoon. How could one of the former dwindle away to the dimensions of one of the latter? This difference in size is of course a necessary consequence of the mode of formation of spermatozoa which I have described.

There are two other points, both discussed in Dr. Nansen's paper, to which I have given some attention. One is the

nature of the follicular epithelium lining the wall of the spermatic capsules, the other is the origin of the capsules. The two points are so closely connected as to form a single question. I have figured the appearance of the follicular epithelium as seen on opposite sides of a partition between two capsules in Pl. IV, fig. 13. The outlines of the cells in my preparations are not distinct, the nuclei are considerably smaller than those of the spermatocytes, and vacuolar in appearance; they contain little chromatin. These cells, in my preparations at least, and in Nansen's figures also, are irregularly arranged. Some of the nuclei are constricted, and some divided into two, as though they multiplied by direct division. I agree with Nansen in thinking that they have nothing to do with the formation of spermatozoa. It seems to me very probable that their function is to supply nutriment to the growing and multiplying spermatoblasts, and perhaps they perform this function by proliferation and actual dissolution.

Figs. 14 and 15 are from sections of very young undeveloped testes; they show the proliferating genital tissue, which in other Vertebrates has been described as germinal epithelium. The structure of this tissue corresponds almost exactly to the germinal epithelium in Teleostean fishes as described by Hector Jungersen. It is proliferating tissue, the cells being naked at the surface, and without regular arrangement; that is to say, they do not form a definite epithelium. In this tissue it is easy to distinguish two kinds of cells—a large number of small, and a few much larger scattered at intervals among the others. The smaller cells are stroma-cells (s. c.), and resemble ordinary embryonic mesoblast-cells, as in fact they are. The larger cells (g. c.) are primitive germ-cells, and do not differ in any way from the primitive germ-cells, or, as they are often called, primitive ova, of Teleostean embryos. As the tissue increases in bulk the inner portion of it forms testicular capsules, of which several in different stages of development are seen in the figures. In many cases a single germ-cell is seen surrounded by stroma-cells. The stroma-cells afterwards form the follicular epithelium of a spermatic capsule, while the multi-

plication of the germ-cell gives rise to the spermatoblasts. It may be that each capsule originally contains but a single germ-cell, but I am not sure of this. Possibly two or more may sometimes be enclosed in a single developing capsule. The remaining stroma-cells are differentiated into the fibrous connective tissue which forms the walls of the capsules and the intermediate tissue of the testis.

Thus Nansen's conjecture, that originally each capsule contains a single large cell or spermatogonium, is to some extent verified by the condition seen in the germinal proliferating tissue, where the separate germ-cells may be seen surrounded by stroma-cells. I have never seen a capsule with fibrous walls containing only a single spermatogonium; by the time the walls of the capsule are definitely formed the original germ-cell has increased to several.

The existence of the germinal proliferating tissue is by no means confined to the earliest stage of development of the testis. I have found it in portions of the surface of the testis at all stages, even in ripe testes, although in the older organ it is in small quantity, and occurs only at scattered points. The rest of the testis is not bounded by a definite flat epithelium, but simply by irregularly arranged small cells.

Theoretical Considerations.

So far as I have been able to discover, the spermatogenesis of Myxine is in many respects unique, not resembling closely that observed in any other form, either vertebrate or invertebrate. I am not, however, in a position on the present occasion to discuss the facts I have described above in the light of all the various theories of spermatogenesis that have been recently brought forward, or to attempt to compare the spermatogenesis of Myxine with the complicated history of the spermatozoa in other Vertebrates, such as Elasmobranchs, Birds, and Mammals. Such a discussion demands a more extensive and more detailed acquaintance with both the literature and the phenomena than I yet possess. I will therefore confine myself to the fundamental features of the process.

It seems to me that spermatogenesis in **Myxine** resembles that which occurs in certain Invertebrates more closely than that which occurs in the higher Vertebrates. In Chætopods and Molluscs, e.g. in the earthworm and Helix, according to Blomfield's description (**5** and **6**), a large cell (spermatospore or spermatogonium) undergoes a process of division which leads to the formation of a number of small round cells (spermatoblasts) surrounding a larger central mass of protoplasm, the blastophore. Each of the spermatoblasts in these cases becomes a single spermatozoon, while the blastophore has only a subsidiary supporting function, and, after the spermatozoa have separated from it, degenerates. Moreover, in the transformation of spermatoblast into spermatozoon, the tail appears first, projecting away from the blastophore, and the nucleus of the spermatoblast does not acquire the characters of the head of the spermatozoon till after the tail has begun to appear. In the testis of Myxine I can only compare the spindle-shaped body, from which the spermatozoa separate, with the blastophore of Chætopods and Molluscs; and the striking peculiarity is, as I pointed out in my first description, that the spermatozoa in Myxine are, when first differentiated, attached to the blastophore by their tails, and not by their heads, as in Chætopods and Molluscs. This peculiarity is connected with another, namely, that the heads of the spermatozoa, or sperm-nuclei, as I have called them, are in Myxine fully differentiated within the undivided spermatocyte (spermatospore, Blomfield) before any differentiation of the tails has taken place; whereas, in all other cases described, a partial or complete separation of spermatoblasts or spermatides takes place first, and the nuclei of these only subsequently acquire the characters of sperm-nuclei. The only process at all similar to that which I have described in Myxine, so far as I can discover, is one described by Jensen (**4**) in the Mollusc Triopa clavigera. According to this description, young spermatogemmæ consist of a small number of cells all similar, and forming morulæ adjacent to the wall of the tubule of the testis. The cells multiply by indirect division until their number is considerable. Then a

few of the cells of each spermatogemma, namely, those near the wall of the tubule, undergo a " chemical dissolution "—that is, they lose their original structure, fuse together, and form the cytophore (blastophore of Blomfield). The other cells are transformed into spermatozoa, and are called by Jensen spermatocytes. These spermatocytes are for the most part uninuclear, but not invariably; often they are multinuclear. The uninuclear spermatocyte forms a single spermatozoon, and the transformation is thus effected. The outer end of the spermatocyte grows out into a thin filament, the tail of the spermatozoon; the protoplasm of the spermatocyte collects on the outer side of the nucleus, the latter being next to the cytophore. The filament after a time can be traced through the protoplasm to the nucleus, and, after this stage is reached, the protoplasm of the spermatocyte descends along the filament, forming drops of various sizes, which are afterwards used up to increase the thickness of the tail.

Now the feature which resembles what I have described in Myxine is this :—When the spermatocyte has several nuclei each of these may develop into a sperm-nucleus. In this case, instead of one filament projecting away from the spermatocyte there are several in a bundle, one for each nucleus. The nuclei usually remain at their original level, and the multinuclear spermatocyte forms a number of spermatozoa. But in other cases one nucleus remains in its original position, while the rest are carried down the bundle of filaments in the drops of protoplasm; some of these nuclei dissolve and disappear, but others often develop into sperm-nuclei within the drops, and in this way a condition is produced resembling in some degree some of the stages I have figured on Plate IV. For instance, in Jensen's pl. ii, fig. 38, there is an elongated bundle of filaments connecting three drops of plasma, each of which contains a sperm-nucleus. But in Jensen's cases the points or apices of the sperm-nuclei are invariably turned in one direction, towards the cytophore, and the base of each is connected with a filament which can be traced distinctly through the drop of plasma and down the bundle. These conditions do not obtain in

Myxine. Moreover, according to Jensen, in Triopa the filament is formed first before the sperm-nucleus is differentiated, and has a definite existence apart from the drops of plasma. According to my observations in Myxine there is no indication of a filament as a distinct structure at all ; the sperm-nuclei are differentiated within the spermatocyte, and then the cell-plasma is merely drawn out into viscous threads which form the tails of the spermatozoa.

But although I have compared the multinuclear spermatocyte of Triopa with that of Myxine, I cannot regard them as morphologically homologous. As I have stated above, I believe that a considerable portion of the spindle-shaped cells in Myxine remains unused after the separation of the spermatozoa, and represents the blastophore or cytophore in other cases. In Triopa, on the contrary, the cytophore is separated before the spermatocytes of Jensen's nomenclature are constituted, and the whole of the protoplasm of these spermatocytes is used up in the formation of the spermatozoa. In comparing the testicular cells of Myxine with those of Molluscs either the whole contents of a follicle must be regarded as the equivalent of a single spermatogemma or sperm-polyplast, or each of my spermatocytes must be regarded as that equivalent. The former hypothesis seems to me untenable, because in the multiplication of the spermatic cells in the follicle there is no indication of anything corresponding to a cytophore or "blastophoral corpuscle." On the other hand, after the separation of the spermatozoa from each spermatocyte there is a blastophoral remnant left behind.

It must, therefore, be understood that I have used the term spermatocyte in its etymological sense, and that the element thus designated in Myxine corresponds, not, as Nansen supposed, to a cell which is bodily transformed by change of structure into a single spermatozoon, but to what in other cases has been called a spermatogonium or spermatospore, which gives rise to a bundle of spermatozoa.

It is interesting to note the extreme simplicity of the structure and development of the testicular follicles in Myxine, a

simplicity much greater than is found in the more highly organised Invertebrates.   As a consequence of this simplicity the history of the testis approximates very closely to that of the ovary.   In fact, we cannot help regarding the male follicle and the female follicle as exactly, or almost exactly, homologous. My sections of developing testes lead me to conclude that only a single germ-cell is usually included in a follicle.   In the female follicle the germ-cell undergoes no multiplication after its isolation from the germinal tissue, while in the male follicle the included germ-cell multiplies by division, and each of the numerous spermatocytes produced gives rise to several spermatozoa.   It seems to me probable that future researches upon the development of the testes in the higher Vertebrates, in the light of our knowledge of the testis of Myxine, will afford us a better comprehension than we yet have of the complicated structure of the former.

W. Müller (1) has shown that the testis of Petromyzon is composed of follicles and cells similar to those of the testis of Myxine.   Probably the development of the testis and the process of spermatogenesis are also similar, but these have not yet been investigated.

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DESCRIPTION OF PLATE IV,

Illustrating Mr. J. T. Cunningham's paper on "Spermato-
genesis in Myxine glutinosa."

FIGS. 1, 2, and 3.—Elements seen when a piece of the fresh testis is teased
on the slide; each figure from a different specimen.   Drawn with Abbé's
camera, Zeiss E, oc. 3.

All the remaining figures drawn with the aid of Abbé's camera, under the
combination Zeiss F, oc. 3.

FIG. 4.—Three spermatocytes with resting nuclei, from an unripe follicle.

FIG. 5.—Spermatocytes with nuclei in the skein stage, from an unripe
follicle.

FIG. 6.—Spermatocytes with dividing nuclei, from a follicle in which the
spermatocytes were separate from one another, but which contained no
spermatozoa.

FIGS. 7, 8, and 9 are various elements seen in a single ripe follicle in one
section.   Figs. 4 and 5, spermatocytes with resting nuclei.   Fig. 6, stages of
spermatogenesis.

FIG. 10.—Elements seen in certain follicles from which the spermatozoa
have probably escaped.

FIG. 11.—Elements seen in a ripe follicle in another section from another
specimen.

FIG. 12.—Elements from another ripe follicle.

FIG. 13.—Portion of the connective-tissue partition between two unripe
follicles, with the follicular epithelium on either side of it.

FIG. 14.—Portion of a section of a young testis.   *s. c.* Stroma-cells.   *g. c.*
Germ-cells.

FIG. 15.—Portion of a section of a somewhat older testis.   *s. c., g. c.,* as
before.

Notes on some Aquatic Oligochæta.

By

W. Blaxland Benham, D.Sc.Lond.,
Aldrichian Demonstrator of Comparative Anatomy, Oxford.

With Plates V, VI, and VII.

Contents.

DURING the present spring (1891) I had under observation at various times a number of aquatic Oligochæta, for the purpose of illustrating my lectures to the students here who were working at the group; and I came across several interesting facts, some of which are worth recording in these pages.

Wishing to obtain examples of Oligochæta living on the sea-shore, I wrote to Mr. W. H. Shrubsole, of Sheerness, and he was good enough to obtain for me several gatherings from the neighbourhood. I wish to convey to him my best thanks for the energy and promptness which he displayed in acceding to my requests.

Amongst these gatherings, living in dark, smelling, decaying organic detritus, I found Hemitubifex ater, Paranais littoralis, Clitellio arenarius, and Pachydrilus sp., together with a worm originally diagnosed very briefly by Claparède about thirty years ago, which appears to have

escaped the notice of naturalists since that time.   To it he gave the name Heterochæta costata; the generic name referring to the interesting arrangement of chætæ, and the specific to longitudinal ridges, or to the distinctly annulate character of the segments.

Of these worms I found abundant specimens in some of the earlier gatherings (in May) ; but more recently, in the latter end of June, I have been able to find only a few specimens, though I still find a few fully mature forms now in July.

Heterochæta costata, Claparède, 1863.

In his studies on Invertebrates from the coast of Normandy ('Beobach. u. Anat. und Entwickel. wirbellöser Thiere') Claparède gives (p. 25) a brief diagnosis of a new genus, of which the following is a translation :

"Heterochæta, n. gen.

"Chætæ bundles in two rows (on each side) ; those of the upper row, on Segments v to viii, are hollowed out at the free end in the form of a cup. The remaining chætæ are all crotchet-shaped [hakenförmig].

" H. costata, n. sp., Taf. xiii, figs. 16 to 19.

" Body 16 mm. in length, $\frac{1}{2}$ mm. in breadth ; skin ribbed by longitudinal grooves ; each segment divided into rings by about four constrictions."

He further states, during a brief extension of the diagnosis, occupying only eight lines, that all his specimens were immature and without a clitellum ; that the peculiar chætæ (which he figures) have a constriction just below the cup (or " becher ") ; that the ordinary chæta has a swelling (or " node," as I will call it) about midway along its length.   The vascular system consists of a dorsal vessel, a ventral vessel, and a loop in each of the hinder segments.

This is essentially all that is known of this interesting worm ;

and Vejdovsky makes no remarks upon it, merely giving Claparède's diagnosis almost textually (' System und Morph. d. Oligochæta,' p. 84). Vaillant, in the ' Hist. nat. d. Annélides,' has no more to say of it.

In the above diagnosis there is one error, which I will point out at once. The chætæ referred to as " becherförmig " are not cupped; they are what is known as fan-shaped (or palmate, as I shall call them), such as are characteristically found in Psammoryctes.

I have been able to extend our knowledge of this worm, and will now give my own observations on Heterochæta.

During life the worm is pink in colour, the sperm-sacs and ova in mature animals showing up white.

The length is about five eighths of an inch when fully mature, and exceptionally slightly longer; but I have seen none that attain a length of one inch. The white region which marks the position of the genital cells occupies about one eighth of an inch, commencing about the same distance from the anterior end (fig. 1). The number of segments is about forty.

In habit it resembles Hemitubifex, &c., amongst which it lives; and I found it sometimes by itself in the mud, with posterior end protruded therefrom, as is the habit of other members of the family, or twisted and coiled amongst the Hemitubifex in the " balls " which the latter form.

When disturbed, an individual will roll itself into a spiral, or twist itself into a knot (fig. 2).

It is not by any means easy to distinguish Heterochæta by the naked eye from such forms as Paranais littoralis, Pachydrilus, or even some paler forms of Hemitubifex. I have found it impossible to identify them without picking them out and using a low-power microscope for their examination; and even then, unless they happen to present their dorsal surface to view, it requires great practice to appreciate the exact shade of pink which serves to distinguish them from the other worms mentioned above.

If, however, the back is uppermost, then one can readily

recognise the fan-shaped bundles of dark palmate chætæ on Segments v to xiii [1] (fig. 3).

The ventral chætæ (figs. 12, 13) are practically all alike, to wit, crotchets or "furcate" chætæ; although the size of the prongs and the angle between may vary in different parts of the body. The dorsal chætæ, with the exception of those on Somites v to xiii (both inclusive), are similar to the ventral chætæ. These are shown in figs. 10, 11.

The dorsal chætæ of Segments ii, iii, iv, have the two prongs very nearly equal, the lower (or proximal) prong being slightly smaller than the upper or distal prong; the two prongs are not greatly divergent (fig. 10). The dorsal chætæ of the hinder segments are slightly stronger than the anterior ones; the proximal prong is shorter than the distal prong, and has a decided curve away from the latter, so that the angle between them is slightly more obtuse than that of the anterior dorsal chætæ (fig. 11).

The ventral chætæ present a similar difference according to their position—namely, those of the more anterior segments (fig. 12) have less divergent prongs than have those of the more posterior segments (fig. 13); and there is the same difference in size of the prongs as occurs in the dorsal bundles. These differences agree with those figured by Professor Lankester for Psammoryctes umbellifer ('Ann. Mag. Nat. Hist.,' 4th ser., vii, 1871, p. 92). Similar differences have been noticed in other members of the family Tubificidæ.

The dorsal chætæ of Segments v to xiii closely agree in appearance with those described and figured by Lankester as characteristic of Psammoryctes umbellifer; where they occur, however, in Segments ii to x.

Each of these palmate chætæ has the appearance presented by fig. 4. The stalk or axis of the chæta is straight, swollen at the point where it passes through the skin in a protruded

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[1] The numeration that I here adopt is that usually followed by recent writers on Oligochæte anatomy—namely, to regard the first chætigerous segment as the second [ii] body-segment; the buccal or peristomial segment being the first [i] segment.

state; then narrows, as Claparède stated, before dilating to form the "head."

This "head" is flattened from in front backwards in the natural position of the chæta as seen in a living worm, and expanded laterally; it is not cup-shaped : this appearance is an optical illusion. When seen from in front this head is seen to be formed by seven or eight blunt teeth ; and each is slightly curved forwards, so that the free edge of each "tooth" is marked by a thicker line. The "teeth" appear at first sight to be separated from one another by very fine spaces, but I believe these spaces are in reality occupied by an extremely thin transparent membrane, as in Psammoryctes and Tubifex.

I was at first inclined to regard the "teeth" as separate from one another; but, as Professor Lankester remarked to me, if this were so, the lines between the "teeth" would appear much more pronounced, owing to refraction along the edges, than is the case.

And further observation served to assure me that there is such a membrane ; it can be distinguished by the use of Zeiss's homogeneous immersion, with compensating eye-piece 4. Moreover if the chætæ, during the movements of the bundles to and fro, be looked at from above (with a Zeiss' E, and No. 2 eye-piece), so that the free edge only of the structure be seen in focus, we get a curved beaded appearance (fig. 7) ; the thicker parts are the "teeth," the thinner are the intervening membranes. This interpretation is further confirmed by a very lucky find of an abnormal variety of the chæta, in which the "teeth" are scarcely differentiated, and we have a continuous membrane with extremely faint lines across it (fig. 9). This is not a young chæta, but occurred in middle of a bundle of palmate chætæ, and was of same size as these.

The whole of the "head" is curved at its sides, as shown in fig. 7, and at the same time is bent forwards, as in fig. 6. Hence the whole "head" can never be in focus at one and the same time under a high power. This leads to the appearance represented in fig. 5, where the two most laterally placed or outer teeth or ridges are in focus, whilst the remainder are

only imperfectly focussed; hence the outermost ridges appear more strongly defined than the rest (see Lankester's and Vejdovsky's figures of Psammoryctes), but by careful focussing it appears to me that all the ridges are equally developed.

Now, if one of these chætæ be observed from above during life, so that we look down it, we shall of course see the head foreshortened, and we have the appearance portrayed in fig. 8. This has somewhat the appearance of a cup, one side of the cup being formed by the free edge of the teeth, whilst the two outer ridges and the thick stalk immediately below the head form the curve which may be mistaken for the other side of the cup; and I believe this appearance deceived Claparède. These palmate chætæ vary in number on different segments, as the following table and fig. 3 show; there is also individual variability for each bundle.[1] The chætæ of a bundle, when in movement, diverge from one another in a fan-shaped manner, the edges of the various chætæ almost touch, and are so regularly arranged as to form part of a curve. The whole bundle is moved backwards and forwards—striking the water, that is to say, with the flat faces of the chætæ, which no doubt serve as oars. It is interesting to note that the furcate chætæ are freqnently rotated on their own axes, in addition to their to and fro movement; and that not unfrequently the angle between the prongs, which is normally directed downwards, is directed upwards. There appears to be a much greater freedom of movement in all directions in the furcate bundles than in the palmate bundles.

Although these facts can be to a very great extent observed in a living worm, slightly compressed by a cover-glass to prevent too active a movement, yet it is necessary, in order to properly ascertain the character of the chætæ, to treat them with caustic potash. This I did, and have mounted them in glycerine jelly, which, as Professor Lankester has observed, is a most useful mounting medium for chætæ.

When treated with KHO, however, the embedded ends of

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[1] That is, the bundle of the corresponding somite in two or more worms does not always have the same number of chætæ.

the chætæ swell up to nearly twice their real diameter, so that this method alone would give a wrong impression as to their real shape; the terminal parts appear to be harder, and are not so affected.

Table of Arrangement of CHÆTÆ.

Segment.	Dorsal Bundles.		Ventral Bundles.	
	Right.	Left.	Right.	Left.
II.	2 or 3	2 or 3	3 or 4	3 or 4
III.	4 (+ 1)	4 (+ 1)	3 (+ 2)	3 (+ 1)
IV.	4 (+ 1)	4 (+ 1)	3 (+ 2)	4 (+ 1)
V.	6 or 7 (+ 2 or 3)	6 (+ 2 or 3)	3 (+ 1)	4 (+ 1)
VI.	7 (+ 3)	8 (+ 3)	3 (+ 1)	3 (+ 1)
VII.	7 (+ 3)	10 (+ 1)	3 (+ 1)	3 (+ 1)
VIII.	9 (+ 3)	11 (+ 3)	3 (+ 1)	3 (+ 1)
IX.	11 (+ 2)	11 (+ 1)	3 (+ 1)	3 (+ 1)
X.	9 (+ 1)	9 (+ 3)	2	2
XI.	6 (+ 1)	5 (+ 2)	2 (or 1)	2
XII.	6 (+ 3)	6 (+ 2)	2 (+ 1)	1 (+ 1)
XIII.	4 (+ 2)	6 (+ 1)	1 (+ 1)	1 (+ 1)
XIV.	3	3	1 (+ 1)	1 (+ 1)
XV.	2 or 3	2 or 3	2	2
XVI.	2	2	2	2
XVII.	2	2	2	1 or 2
XVIII.	2	2	2	2
XIX to XXIV.	2	2	2	2
XXV to end.	2	2	1	1

NOTE.—The strong figures represent the palmate chætæ; the (+ x) represents young chætæ, which do not protrude.

But I have found, not unfrequently, examples which show a slight difference in the position of the palmate bundles.

In two or three specimens the chætæ of Segment xiv were palmate, like those of xiii.

In another specimen the dorsal bundle of one side of Segment xiv consisted of four palmate and one furcate chætæ, that of the other side only of furcate chætæ.

In another a similar asymmetry of Segment xv occurred; i. e. on one side the bundle consisted of one furcate and two palmate chætæ, those of Segment xiv being all palmate.

Still further, in one specimen, whilst the chætæ of the dorsal bundles of Segment xiii were as usual palmate, those of Segment xii were furcate.

Seeing that in Psammoryctes and in Tubifex we get certain chætæ which are neither simple crotchets nor completely palmate (see Vejdovsky's figs. 11 and 4, pl. viii), I looked carefully for similar intermediate forms in Heterochæta, and for a long time I looked in vain, but ultimately I was successful in finding somewhat similar "intermediate" or "multidentate" chætæ. The one figured (fig. 15) was from one of the dorsal bundles of Segment xiv of one specimen; it occurred with three typical palmate forms, and there were no crotchets. Curiously enough it was not at the side of a bundle, but between the 2nd and 3rd palmate chætæ. These "intermediate" chætæ have one or two teeth between the prongs.

One specimen I noted, and here represent (fig. 16), in which a great amount of divergence from the typical arrangement was accompanied by intermediate conditions of the chætæ. This may be tabulated thus:

<div align="center">Abnormal Specimen.</div>

Segment.	Right Dorsal Bundle.	Left Dorsal Bundle.
II.	3 furcates	3 furcates.
III.	{ 1 furcate (most dorsal of bundle) { 2 intermediates	} 3 furcates.
IV.	{ 1 furcate (dorsal) { 3 intermediates	{ 2 furcates. { 1 large palmate. { 2 small palmates.
V.	5 (+ 1) palmates . . .	5 (+ 1) palmates.
VI to XIII (inclusive) were normal.		

<div align="center">Dorsal Chætæ.</div>

Segment.	Right.
XIV.	3 palmates.
XV.	1 furcate and 1 intermediate.
XVI.	1 palmate and 1 furcate.
XVII, &c.	2 furcates.

The palmate chætæ are usually considered as special modi-

fications of the furcate or crotchet-shaped chætæ, with the multidentate forms as an intermediate condition.

I am inclined to regard them in a different light, for—

(a) If a palmate bristle be viewed from its edge, I have shown that the tips of the teeth are all curved, and this curve resembles the curve of a furcate chæta if the lower tooth were removed.

(b) The divergence of the two outer teeth or ridges of the comb is different from that of the prongs of the fork, in which both prongs are directed towards the same side of the stalk, and the tip of each is frequently curved downwards in the same direction.   In the palmate bristles it will be seen that neither of these things occurs (see also Pl. VII, figs. 33, a, b, 36, c, d).

(c) The plane of the head, i. e. the two prongs of the furcate chæta, is in a state of rest parallel with the long axis of the body, whereas that of the palmate bristle is at right angles to the axis of the body.

(d) The so-called "intermediate" forms, or "multidentate" forks, do not represent a stage in the formation of the ctenate bristles.   The two prongs are similar to those of the simple furcate forms in relative size, curvature, divergence, &c.

(e) I would rather regard the ctenate chæta as having been derived from a simple "sigmoid" chæta (such as is common amongst earthworms, some Lumbriculidæ, some Enchytræidæ, and in Phreoryctes) by a flattening and expansion of the dorsal extremity, so as to form the "membrane," which then becomes thickened or ribbed to give rise to the palm-leaf-shaped arrangement; whilst the furcate chætæ, which are so common amongst the Oligochæta generally, may have been derived similarly from the "sigmoid" form by the appearance of a notch at the extremity, which became deepened to form the angle between the two prongs, these in their turn becoming more and more developed, the plane of two prongs being at right angles—with respect to the axis of the original chæta— to the plane of the "membrane" of the palmate forms.

The " multidentate " chætæ will then be a further develop-

ment of the fork, but not in the direction of the palmate type.

The sigmoid form itself is not necessarily the primitive form of chæta ; it is possible that a straight spine, such as we find in the Enchytræids, is an earlier form, which may have given rise to the capilliform shape by elongation, and to the sigmoid form by curvature, and hence to these other forms.

Amongst other external features which are of interest may be mentioned the distinct annulation of the anterior segments of the body (fig. 3). This is distinctly visible during life, and can be detected in longitudinal sections. Each segment is marked out by three grooves into four rings, of which the third is the largest, and on this the chætæ are embedded. The most anterior segments show only two of these grooves, and on the clitellum they are present only ventrally. This feature is mentioned by Claparède, and is of more frequent occurence in aquatic Oligochæta, as in earthworms, than is usually supposed.[1] It is indistinctly marked in some Naids. Claparède mentions the fact in Tubifex and Limnodrilus. It is present, in fact, in the majority of this family, and is carried to an extreme in Branchiobdella. It is present in Hemitubifex, though rendered less noticeable and even almost obscured by the dark cuticular papillæ. In Heterochæta, however, it is so distinct that it is almost the readiest means of distinguishing, under a low power, this worm from other forms with which it occurs, for the characteristic chætæ are not visible if the animal presents its ventral surface upwards.

I am unable to say what may be the "Langsfürchen" in the skin, mentioned by Claparède. I see no such grooves. His figure may represent the bundles of longitudinal muscles which can be seen during movement of the worm, and will at times be thrown into wavy lines such as his drawing represents.

The shape of the prostomium is exhibited in my figs. 3

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[1] For example, Mr. W. Hatchett Jackson, in the 2nd edition of 'Forms of Animal Life,' states on p. 593 that "annulation of the somites is very rare."

and 16.   It is conical in its general outline, with a circular groove near the tip, giving it a pointed appearance.

The male pores (spermiducal pores) are, as is always the case in the Tubificidæ, in Somite xi slightly dorsal of the ventral chætæ.

The spermathecal pores are in a similar position in Somite x.   In both cases the cuticle dips inwards at the pore, and is folded around its lip.

The vascular system is of the usual Tubificid[1] type. There is a pair of lateral dilated hearts in Somite viii.   These contract not together, but alternately.   In the genital segments these vessels are contractile, and lie on the sperm- and ovi-sacs ; in other segments a pair of narrow convoluted vessels lie immediately below the body-wall.

The most important system of organs after the chætæ are, of course, the genitals.   These are shown in situ in fig. 17, which is taken from a careful sketch made from the living worm, sufficiently compressed to prevent movement, and to allow the organs to be seen without undue distortion or dis-placement.   The clitellum covers the dorsal surface of xi, xii, and part of x as far as the chætæ, sometimes even part of xiii.

The Male Organs.—A pair of testes in the 10th segment are attached to the anterior septum of this segment.

The funnels of the sperm-ducts lie against the posterior septum of the 10th segment; from the funnel on each side the narrow ciliated duct passes, with only slight undulations, back through the Segment xi and into Segment xii.   Here it enters the glandular " atrium ;" this narrows, loses its glandular coat, passes forwards into Segment xi, and here opens to the exterior.

The " cement gland," or prostate, lies in Somite xii, and here opens into the atrium.

[1] In a recent paper ("Monog. českých Tubificidů ") Stolc gives some excellent figures of the arrangement of vessels in several genera, and repre-sents several new points ; e. g. the lateral hearts in his two new genera Lophochæta and Bothrioneuron arise, not from the dorsal, but from a supra-intestinal trunk.

The male apparatus is represented in greater detail in fig. 18, as seen when "squeezed" out of the body, with exception of the funnel, which remained inside.

The funnel, as in all the aquatic Oligochætes, is simple, and not folded as in earthworms. In the Tubificidæ it is a very large, flattened, and shallow structure, as is also the case in the Lumbriculidæ and Phreoryctidæ. In the Enchytræidæ it has a characteristic form, being long, narrow, thick-walled, and projecting far into the cavity of its segment.

In Heterochæta the funnel, though usually lying flat against the face of the septum, undergoes, with the contraction and extension of the worm, a corresponding movement, in that the lips move to and from the septum, so that its other extreme position is represented in fig. 19.

The duct does not leave the funnel in the centre of the latter, but slightly to its outer side. This asymmetrical condition is shown by Beddard in Clitellio arenarius; but in all other figures by Claparède, Vejdovsky, &c., the duct is represented as leaving the funnel in its centre. Whether there is any differential character in this feature I do not know. I rather think that this is not the case, but that the drawings are to some extent diagrammatic, and make no pretence to represent the thing accurately.

The sperm-duct is thin-walled and ciliated internally, as is always the case.

The "atrium" (fig. 18) is divisible into two regions here, as in Psammoryctes—a region coated with granular cells, which give to it a dark appearance (*gl. atr.*); and a thin-walled, narrower region (*n. gl. atr.*), which passes to the penis.[1]

There are no cilia in the "atrium," using the word to include all that part of the apparatus after the entrance of the solid "prostate." The terminal part of the atrium passes into

[1] To this dark granular region Vejdovsky, in his description of Psammoryctes, gives the name "seminal vesicle," whilst the long narrower region between it and the penis is termed by him the "cement duct."

the protrusible penis, which is surrounded by a specially thickened chitinous coat. The figures 18, 21, 22, representing the arrangement of this region of the duct, aid the description. It will be seen that the male pore leads into a chamber (penial chamber, *a, b*) lined by cuticle invaginated at the male pore. Projecting from the bottom of this penial chamber (or " cloaca" of Claparède, or "ductus ejaculatorius" of Vejdovsky) is the " penis." This consists of a soft central part, the " glans," and a thick, refracting, chitinous coat (Vejdovsky's " penis tube"), which has a characteristic form. It is nearly cylindrical, the edge of the outer end of the cylinder being bent outwards so as to form a rim, and is much more noticeable than that of Psammoryctes, Spirosperma, &c. (cf. this with the penis of other members of the family).

The internal soft part is perforated nearly axially by the narrowed continuation of the atrium, the external aperture of this duct being excentrically placed on a protuberant " glans penis" (of Vejdovsky). I have not seen this penial apparatus in a protruded condition, but no doubt a similar process occurs here as in Tubifex.

When I first examined the genital duct, separated from the animal, I found that the " cement duct" (of Vejdovsky) and the " seminal vesicle" exhibited irregular dilatations, as seen in fig. 20, *a* and *b*. I therefore isolated some eight or nine ducts, in order to ascertain whether these dilatations were constant, and I soon found that such was not the case—that these swellings are only artificially produced. This was confirmed by the fact that in situ there are not such definite swellings.

In the fully mature worm Segments ix to xiv, or even xv, are occupied by developing ova and spermatozoa. In some cases, such as the one figured, we find both genital cells fairly equally developed; whereas in other specimens the spermatozoa are predominant, and no large ova are present, and vice versâ.

The developing spermatozoa are included in definite sacs, the sperm-sacs, which are provided with thin membranous

walls, upon which are greatly convoluted and contractile commissural blood-vessels.

These sperm-sacs are asymmetrically arranged. There is usually one in Segment IX, and another starting in Segment XI, and extending back as far as Segment XIV or XV, pushing the intervening septa in front of it. Very usually, though I believe not invariably, this sac crosses from one side of the body to the other dorsally of the gut.

Besides these two sacs (Claparède's "testicles") masses of developing spermatozoa more or less fill Segment X. The minute structure of various parts of the male apparatus deserves a brief description, not from any peculiarity special to the genus, but because we have but few details of the histology of these aquatic Oligochætes. The figures of Claparède, Vejdosky, Eisen, &c., are, in the main, somewhat diagrammatic, even where these authors intend to show detailed structure. Beddard has recently contributed some facts as to the minute structure of Hemitubifex and Phreoryctes, and Michaelsen has recorded something of the histology of Enchytræids.

The sperm-duct, when its surface is viewed, is seen to be striated transversely, as is the case in Tubifex, &c. The minute structure, as seen in sections, is exhibited in my fig. 23, which includes a transverse and a longitudinal section of the sperm-duct.

In transverse section there is a remarkable "striation" of the wall, which is apparently due to radial arrangement of the granules in the cells; this is seen even better in a series stained in hæmatoxylin than in the borax-carmine sections drawn. I can see no boundaries to the cells; and the nuclei are not elongated radially, but tangentially : they are, in fact, ovoid in shape, the long axis being at right angles to the axis of duct, so that in longitudinal section the nuclei have circular outlines. I have seen a similar arrangement in Tubifex, both as regards striation and nuclei.

In earthworms, e.g. Lumbricus, Perichæta, the striation is present, but the nuclei appear to be spherical, giving a

circular section in all directions. Beddard,[1] in his figure of the sperm-duct of Hemitubifex, shows a different arrangement.

The two parts of the atrium, already distinguished externally, are equally distinct in histological structure : the non-glandular part (fig. 23, *ngl. atr.*) is lined by flat cells, with horizontally elongated nuclei and granular protoplasm ; whilst the glandular region (*gl. atr.*) is lined by cells of a totally different shape. The epithelium here is more or less cubical ; the cells are glandular—at any rate vacuolated, as if a secretion had been discharged; the protoplasm, with darkly staining granules of comparatively large size, is chiefly found in the outer part of the cell, where, too, is the round nucleus (fig. 25); the spaces, or vacuoles, appear sometimes to be occupied by an extremely fine substance—so fine that it is impossible to detect whether it is homogeneous or finely granulated, and requires an apochromatic to be seen at all.

The prostate is a solid structure, built up of a mass of cells having essentially the same structure as the preceding, though differing in shape (fig. 24). These cells are pyriform ; the granules are larger and distinctly spherical ; probably the neck of each cell serves as a duct, and pours the secretion into the atrium independently of its neighbours.

Mr. Beddard's[2] drawing differs slightly from mine, chiefly as to details, which is perhaps due to the method of preservation. If the cells in Heterochæta were a little emptier, we should have an appearance something similar to that shown by him for Hemitubifex.

I may say that the isolated cells here figured are drawn from sections, and not from teased preparations.

The prostate, like the sperm-duct and atrium, is surrounded by a delicate membrane (*c. ep.*), with a few scattered nuclei ; this membrane is continuous, and is the cœlomic epithelium. The atrium has in addition a muscular coat, as shown in the figure (*mus.*).

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[1] "On Certain Points in the Structure of Clitellio," 'Proc. Zool. Soc.,' 1889, p. 485, pl. xxiii, fig. 5.

[2] Loc. cit., fig. 7.

In fig. 22 I have drawn a section along the penis, in order to show the character of the cells which give rise to the chitinous penial sheath, and their continuity with neighbouring epithelia. The figure is sufficiently explained, and confirms the drawing (fig. 21) which is made from a living specimen, but where of course only the cuticular structures (except the penis itself) are shown.

The Female Organs.—There is a pair of ovaries in Segment xi, usually concealed in a fully developed individual by other structures.

As is the case in other Oligochæta, eggs leave the ovary and undergo further development in an "ovisac;" this in Heterochæta occupies Segments xiii, xiv, and sometimes xv. Usually this sac contains two or three very large ova, one in each segment; there is a contractile vessel in the wall of the ovisac, as in the case of sperm-sacs.

The oviduct is a small funnel opening externally between xi/xii.

As in other members of the family, there is a pair of spermathecæ in Segment x. Each spermatheca consists of a short, narrow duct, and an elongated, wide, tubular sac. The duct is shorter and the sac longer than in most other genera, the duct being about the same size as in Limnodrilus Udekemianus, Claparède. The pore lies in a line with the spermiducal pore, and, like it, has the cuticle invaginated around it.

The sac of the spermatheca may be entirely confined to Somite x, or may extend into the neighbouring segment. In the specimen from which the figure is taken the spermatheca of the right side is wholly in Segment x; that of the left side had pushed its way into the succeeding somite. In other specimens I have seen the sac of one side extending into Somite ix, that of the other into Somite xi.

The spermatheca, though not externally divisible into very marked regions beyond duct and sac, shows internally certain peculiar cells near the pore (cf. Vejdovsky's picture of Tubifex, Pl. IX, fig. 17). The greater part of the sper-

matheca is lined by columnar cells (fig. 26), the nuclei of which are usually situated near the outer margin. These cells are very granular, and their internal margin sometimes indistinct. But near the neck the cells are pyriform (fig. 27), as in Vejdovsky's figures; and these cells are extremely finely granulated, the nucleus being in the dilated part of the cell, which projects into the cavity of the spermatheca, so that in this region the inner limit of the epithelium is quite irregular.

The spermathecæ are filled with the characteristic Tubificid sperm-ropes (I prefer this term to spermatophores), of which I counted in one specimen as many as thirty-five; some of which were completely formed, others incompletely. A completely formed "rope" is shown in fig. 28, its structure in figs. 29—31.

It is spindle-shaped; one end being drawn out into a point, the opposite end being truncated, slightly knobbed, and apparently perforated. The wall is highly refracting and forms a fairly thick coat, in which the heads of the spermatozoa are embedded (a); immediately below this is, in optical section, a layer of granules (b) (? sections of spermatozoa), and the interior is filled with loose spermatozoa in addition to those embedded in the wall, whose freely projecting tails give rise to the movement of the whole apparatus. When partially crushed, bundles of spermatozoa protrude from the interior through any breaks in the wall (fig. 30).

Though spindle-shaped like the sperm-rope of Psammoryctes or Limnodrilus, it lacks the peculiar "spines" on the neck which occur in the former, and is relatively much longer, narrower, and more pointed than in the latter.

The structure of spermatophore is further illustrated by fig. 31, which is drawn from a section through a spermatheca. The homogeneous layer (a) is in these preserved specimens very highly refractive and scarcely stained. By the use of an apochromatic, and regulation of the light, the heads of the spermatozoa could just be detected; the lines representing them in the figure are much too coarse.

I have added a figure of a specimen of the worm after sexual

maturity is over (Pl. VI, fig. 32), as it shows very distinctly
the shrunken sperm-sacs, the ovaries and testes.   The sperma-
thecæ are apparently undergoing degeneration ; they are filled
with highly refracting globules.   No trace of the sperm-duct
or atrium, nor even penis was present; but in other speci-
mens traces of the male apparatus could be detected.   This
disappearance of the penis appears to me particularly remark-
able, though I am unable to say whether it is due to its solu-
tion or absorption by the cells, or whether the hard coat is
thrown off.

Comparison of Heterochæta with other Tubificidæ.

The nearest genus to Heterochæta is undoubtedly Psam-
moryctes (Pl. VII, fig. 33), on which alone are found palmate
chætæ closely resembling those of the former genus.   In its
generative apparatus, too, there is a pretty close agreement,[1]
in the division of the atrium into two regions, and in the
general form of the chitinous penial coat; but this is quite
characteristic in Heterochæta.   The chief differences
between the two genera are (a) the restriction, in the latter
genus, of the palmate chætæ to Segments v to xiii, which in
Psammoryctes commence in Segment ii and extend to x;
and (b) the absence of capilliform chætæ in Heterochæta.
As the various genera of aquatic Oliogochæta occur pretty
abundantly in England, and as we have no brief summary of
generic characters, I have brought together the chief charac-
teristics in the form of a series of figures, and will point out
here the leading features of these genera.[2]

Commencing with the chætæ of the dorsal bundles—for
those of the ventral are essentially similar in all the family
except Telmatodrilus, we have a fairly ready means of dis-
tinguishing groups of genera.

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[1] I have not been able to see the dilatations in the atrial duct described by
Vejdovsky.

[2] Stolc, in a recent paper in the Czech language, adds considerably to our
knowledge, so far as I can judge from the plates.

Telmatodrilus, Eisen, possesses only one kind of chæta, simple, unforked, sigmoid forms, resmbling those of Enchytræus.

Limnodrilus and Clitellio likewise possess only one kind of chætæ (Pl. VII, fig. 35), namely, the furcate or " crotchet," which may vary in detail in different regions of the body.[1] In Hemitubifex, too, there is only one kind generally, though Eisen and Beddard mention capilliform chætæ as " sometimes " occurring. I have not seen them in H. ater. Tubifex (fig. 34) and Ilyodrilus (fig. 33) present us with capilliform chætæ, either only anteriorly in Segments III to X (or thereabouts) in the former, or throughout the body in the latter genus ; these chætæ are confined to the dorsal bundles, and with them occur furcate chætæ, which possess very usually two or more accessory prongs between the two main prongs in Tubifex,[2] or a membrane, without ridges or teeth, in Ilyodrilus. In both genera these modified furcate chætæ occur in anterior segments only.

Further, in Spirosperma (fig. 36) capilliform chætæ occur all along the body, and are accompanied throughout by a peculiar variety of palmate chætæ (see further on in this paper), which are small and inconspicuous.[3] They differ in shape and size from those of Heterochæta and Psammoryctes.

Another set of characteristic modifications affects the male apparatus.

The shape of the atrium, presence or absence of prostate, and penial coat are among these. The prostate is absent in Clitellio and Ilyodrilus. In the remainder it is a somewhat kidney-shaped, solid mass of cells communicating with the atrium at the point of entrance of sperm-duct.

[1] Bothrioneuron, Stolc, so far as chætæ are concerned, agrees with Limnodrilus.

[2] I have not seen the membrane between the forks, as figured by Professor Lankester.

[3] In Stolc's genus Lophochæta somewhat similar chætæ occur in the dorsal bundles, together with peculiar feathered capilliform chætæ ; but the exact distribution of these I am unable to give, as I have not yet had Stolc's paper translated.

In Telmatodrilus there are eight or more small isolated prostates.

The atrium is relatively short in Tubifex and Ilyo-drilus,[1] where it is glandular throughout, and there is no distinction of a non-glandular portion or atrial duct.

In Limnodrilus there is a short non-glandular region, which is larger in Spirosperma.

In Psammoryctes (see below) and Heterochæta[2] the glandular region is relatively small, and the non-glandular portions greatly extended.

I append figures of the chitinous coat or tube of the penis (Pl. VII, fig. 37), by which I understand a thick, refracting modification of the cuticle, such that it can be readily recognised.

Such a penial tube is absent in Tubifex and Ilyo-drilus.[3] It is short and nearly cylindrical in Spirosperma (37, c), Psammoryctes (37, c), and Heterochæta (Pl. VI, fig. 21). The last, however, has the outer edge turned out so as to form a rim.

In Telmatodrilus (37, b) and Hemitubifex[4] (fig. 37, a) it is a short truncated cone (I cannot find a description or figure of this part in Clitellio).

In Limnodrilus (including therein Camptodrilus, Eisen) the tube is usually very long and relatively narrow, cylindrical, or constricted near the middle, or trumpet-shaped (figs. d, e, f). In L. Hoffmeisteri it has a peculiar free end (37, g), and in L. silvani it is flask-shaped (fig. 37, h).

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[1] Stolc figures the male apparatus of Ilyodrilus: the atrium appears spherical, and is surrounded by a layer of large cells, which Beddard would call the "prostate," resembling that of Stylaria, &c.

[2] Lophochæta resembles these two genera. Bothrioneuron presents several peculiarities in the male apparatus.

[3] As also in Bothrioneuron.

[4] In Lophochæta also.

Spirosperma ferox, Eisen.[1]

This worm has hitherto been found only in Sweden, and has been observed by Eisen alone.[2]

I have found specimens in the Thames and in the Cherwell.

It is readily recognised by the naked eye, owing to its grey colour, with a bright white clitellum occupying Segment xi, and extending into x and xii. The grey is sometimes less marked, apparently in immature specimens, which are greyish red. The grey colour is due to numerous closely set papillæ, of rather irregular form, usually irregularly rectangular, with long axis, as Eisen states, at right angles to the worm's length; but they are not "dark," as he says. They look dark by transmitted light, but if the surface is viewed by direct reflected light they are white, the dark colour being due to the feebly yellow globules in the papillæ.

The worm is six eighths of an inch long, and relatively thick anteriorly. The chief anatomical point is that the chætæ of the dorsal bundles throughout the body are capilliform, accompanied by, in most cases, extremely delicate webbed chætæ. These are rather stouter in the first half-dozen segments than posteriorly, but throughout they are less than half the thickness of the capilliforms (fig. 36, c, d).

The shape of these "webbed" chætæ is quite distinct from the multidentate, or even the webbed chætæ of Tubifex, or the palmate chætæ of Psammoryctes and Heterochæta, though they approach the latter.

The ventral chætæ are not all alike (fig. 36, e, f); they are all crotchets, but in the first six bundles the proximal prong is shorter than the distal prong (f). Behind the reverse is the case, and the proximal prong is extremely stout and strongly recurved (e), somewhat as in Psammoryctes, but more so.

The capilliform chætæ are usually four per bundle up to

[1] "Oligochætological Researches," 'Annual Report of the Commissioner of Fish and Fisheries for 1883," Washington, 1885.

[2] Stolc has found it in Bohemia.

Segment x, behind which there are three per bundle. In Segments v and vi I noticed six per bundle.

The webbed chætæ are usually two, rarely three per bundle. The length of the latter is $\frac{8}{1000}$ mm., and of the capilliform chætæ $\frac{32}{1000}$ mm., though those of the Segments vi, vii, viii are $\frac{40}{1000}$ mm.

I have little to add to Eisen's description and figures. The body-wall is so opaque that it is difficult to see accurately the contained organs. By compressing the worm I released the genitals, which agreed almost exactly with Eisen's figures, except that the sperm-rope, of which I found only one in each spermatheca, is much less, and less regularly, coiled than he represents.

Note on Psammoryctes.

It is worth recording that a species which I, for the time, regard as Ps. barbatus, Vejd. (Tubifex umbellifer, Lankester), occurs in the Cherwell, in the mud amongst the roots of reeds. As far as I am aware it has not been recorded from a British locality since Professor Lankester[1] found it at Barking in brackish water.

The palmate chætæ (fig. 33, *a*), are rather different from those of Heterochæta, in that the " head " makes a slight angle with the " stalk," a fact which is not represented in Vejdovsky's nor in Lankester's drawings. It may, perhaps, be characteristic of a new species.

The ridges on the membrane are usually twelve or thirteen in number, and the head has the same curvature (fig. 33, *b*) as in Heterochæta. In existing drawings of these chætæ the outermost prongs are represented as stronger than the rest. This is not the case in my specimens. It is a matter of focussing, as in Heterochæta.

I figure also the ventral chætæ from different regions of the body. Those of the posterior segments (fig. 33, *e*) have a slightly different curve from that represented in existing figures. These, too, may turn out to be specific differences. But I will wait

[1] 'Annals and Mag. Nat. Hist.,' 4th ser., vol. vii, 1871.

till I obtain other specimens from Barking before giving a new name on these grounds.

I find no chætæ, either dorsally or ventrally, in Segment xi in mature worms.

In Segment x the ordinary ventral chætæ are replaced in mature forms by a single long rod-shaped chæta on each side, immediately in front of the pore of the spermatheca.[1]

I do not find the " dilatations " which Vejdovsky figures on the sperm-duct between atrium and penis so long as the duct is uninjured ; when, however, it is separated from the body of the worm by pressure there is a variable number of dilatations, as in Heterochæta.

Note on Chætæ of Tubifex rivulorum.

Professor Lankester[2] was the first to record the existence of secondary prongs or teeth in the fork of the dorsal chætæ of this worm, and mentions a web passing between the two main prongs. I have not been able to detect this web, but have not had a very large series of specimens under observation for this purpose. I treated the chætæ in the usual way, i. e. I dissolved the worm, on a slide, in KHO, mounted the chætæ in glycerine, and examined them with a Zeiss's homogeneous immersion lens, but I was quite unsuccessful. It is not impossible that the specimens which Lankester examined belonged to Eisen's genus Ilyodrilus, in which such a membrane exists. But I have noted some peculiar modifications of these dorsal chætæ, which I figure in Pl. VII, fig. 34, a, b, c, d ; one of the most curious of which is the occurrence in more than one case of a tooth outside the chief prong.

Stylodrilus Vejdovskyi, n. sp.

In a gathering made on July 17, just below Goring-on-Thames, I found a few small red worms about one third the

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[1] Since writing this paper I have seen Dr. Antonin Stolc's memoir on Bohemian Tubificidæ, in which he figures this chæta, which is grooved distally, and is inserted in a sac into which a pair of glands open, close to the pore of the spermatheca.

[2] Loc. cit.

size of an ordinary Tubifex—namely, about an inch in length.
1 found these worms amongst the roots of the bur-reed, which
I was examining for Criodrilus, and with them I found
a specimen of Spirosperma. Hitherto only two species of
Stylodrilus have been described : the first by Claparède,[1] St.
heringianus ; and the more recent species by Vejdovsky,[2] St.
gabretæ. The differences—and these appear to me slight—
between the two are such that had not Vejdovsky examined
both species, one would be inclined to regard these differences
as merely ones of observation. The specific characters of St.
Vejdovskyi are as follows:

Prostomium conical, two and a half times as long as the
buccal segment, and differing in shape from both that of the
previous species (Pl. VII, fig. 42).

The segments, after the first three, are biannulated, the
smaller annulus being anterior, as in St. heringianus. This
annulus is very small in anterior segments, but behind
the clitellum it is a third as large as the posterior annulus
(Pl. VII, fig. 43).

The chætæ are essentially all alike ; in the other two species
some of the ventral ones are simple sigmoid, unnotched chætæ.
In St. heringianus these are irregularly arranged, whilst in
St. gabretæ they occur only in the pre-genital bundles. But
in the present species, though with a low power some chætæ
appear sigmoid, a high power reveals an indication of the
notch ; and the young, non-protruded chætæ are notched as
markedly as in the dorsal and posterior ventral chætæ (fig. 44).
In fact, these anterior ventral chætæ appear to have had the
small upper tooth worn away.

In St. Vejdovskyi, then, all the chætæ are notched ; and,
like those of Lumbriculus, have the distal or upper tooth
much smaller than the lower.

The dorsal vessel is not dilated in any segment; while in
St. gabretæ there are dilatations in Segments VI and VII.

[1] Claparède, "Recherches sur les Oligochétes," 'Mém. Soc. Phys. et Hist.
Nat. Genève,' xvi.
[2] 'System und Morph. d. Oligochaeten.'

The sperm-sacs are paired, and have the normal arrangement extending as far back as Segment xvi; there are three large eggs in xv, xvi, and xvii respectively (in one specimen one egg occupied the 16th and 17th segments).

The spermatheca lies in Segment ix entirely; in St. gabretæ it extends also into the next segment. I found no crystals, such as Claparède found, in the spermatheca of St. heringianus.

The characteristic penis differs from that of both the previous species in shape and size. In St. Vejdovskyi it has a length just a little greater than half the width of the body (fig. 43). It is not so narrow relatively as that of St. gabretæ; it is not so pointed as in St. heringianus. In normal position its free end is on a level with the chætæ of Segment xi.

The nephridia have a very peculiar arrangement. The first nephridial funnel lies in Segment vi, just in front of the posterior septum; the tube passes backwards with only slight undulations and coils as far as the middle of Segment x, and then returns alongside itself into Segment vii, where it opens externally in front of the ventral chætæ.

The second nephridial funnel lies in normal position in Segment xii; its external pore is in xiii, and the looped tube passes backwards as far as the hinder part of Segment xv.

The third funnel is in Segment xv, the nephridiopore in xvi, and the loop lies wholly in this segment. This is the normal condition for the following nephridia; but I observed one case in which the tube passed through two segments.

This condition of the nephridium is very similar to that described by Vejdovsky for Phreatothrix, and is unknown elsewhere amongst the Oligochæta. The structure of the nephridium agrees with Claparède's figures and description, although in that species he states (p. 265) that the first nephridium lies in Segment vii; that there are none in viii, ix, x, xi, or xii, and that they reappear in xiii.

The length of St. Vejdovskyi is about an inch; none of my specimens exceeded this length. In colour they are bright red, with a tendency to orange; but the colour is much less

marked anteriorly and posteriorly, where it is dull pale yellow. They are very active little worms. I have found them not only in the Thames, but in the Cherwell, just above Oxford.

<div align="center">Nais elinguis, O. F. Müller.</div>

In a gathering from a ditch in the immediate neighbourhood of Oxford I found, together with a number of specimens of Chætogaster diastrophus, Gruithuisen, some Naids showing zones of budding. A glance at the dorsal chætæ of these latter determined the species as N. elinguis.

It may be useful to recall the characteristic chætæ of this species. In addition to the long capilliform chætæ of the dorsal bundles (fig. 38, a), which commence on the 6th segment, there are short needle-like forms, and a third kind, a straight spear-like chæta notched at the free end, with a swelling or node on the stalk at about one third or one fourth from the free end. There are usually two capilliform, two or three short needles, and two notched spears. The ventral chætæ present no important characters (fig. 38, b). The blood is red, as in Paranais littoralis and other species.

These specimens were gathered on May 10th of this year (1891), and were then actively reproducing asexually. They were, after examination, placed in a greenhouse; and on the 26th of May, when I examined them again, most of them were sexually mature, whilst some were still showing zones of budding.

In these asexual forms 1 find generally that $n = 13$, according to Professor Bourne's use of the letter;[1] but one case I noted in which $n = 15$.

The sexual worms agree, in respect of the position of the organs, with Stylaria: that is to say, there is a pair of testes in Somite v, in which also are situated the spermathecæ; a pair of ovaries in Somite vi, in which are also placed the atria and their apertures.

The sperm-sacs have the arrangement shown in fig. 39;

[1] " Notes on Naidiform Oligochæta," this Journal, vol. xxxii, p. 335.

namely, a pair in Somite VI, and a long unpaired, asymmetrically placed sac extending through Segments VII, VIII, IX, and into X.

Masses of developing ova were observed in Somites VII, IX, XI, and a large ovum in Segment X.

I have thought it worth while to figure the arrangement, as Dr. Bourne gives a different position for the "testes" and "ovaries" for Paranais littoralis, which I was unfortunately unable to find in a mature condition. He figures a large, asymmetrically placed mass occupying Segments VIII and IX, which he speaks of as "testes," and he indicates as "ovary" a single mass of developing ova in Segment X. Now, this would be a very exceptional position for the gonads in the family Naididæ; and the "testis" of Oligochæta is not a large structure, but a small organ, occupying only a part of one segment. I cannot help thinking that Bourne has made a slip in writing of these structures as the gonads; he meant probably to speak of them as the sperm-sacs and ovisacs respectively.

It is a well-known fact that in the sexually mature Naid genital chætæ replace the ventral bundles in Segment VI, and that the dorsal chætæ, like the ventral ones, drop out, but are not replaced (fig. 40).

I figure a portion of a nearly mature worm, i. e. with sexual organs, in which the dorsal chætæ are still in situ. The ventral chætæ of one side are also present, but on the other side have been replaced by the genital chætæ (fig. 41). These are stouter and longer than the ordinary ventral chætæ, and are not forked (fig. 38, c).

The Supposed Constancy of n in a given Species of Naid.

In a recent contribution ('Quart. Journ. Micr. Sci.,' xxxii, Part 2, June) on the Naididæ, Professor A. G. Bourne adds many facts to our knowledge of the family, and gives the position of the zone of budding for many of the species, but says nothing about it for N. elinguis. He regards the position of the zone of budding as a constant character for

the various species, and designates this position by n, which signifies the number of segments in front of the zone.

Now, from my recent observations on this phenomenon, some of which were made before the publication of Professor Bourne's paper,[1] I believe that he is rather too dogmatic on this point; for he only mentions one exception, and that for Pristina breviseta, A. G. B., whereas I have found considerable divergences in the value of n from the value given by him. For example, a number of Stylaria lacustris, collected in different places and at different times, were examined from this point of view.

Dr. Bourne gives for this species "$n = 27$." I find, however, as will be seen from the following table, a good deal of variation in the position of the zone. The list refers to two sets of specimens, each individual being indicated by a letter.

A	.	.	.	$n = 20$	F	.	.	.	$n = 30$
B	.	.	.	$n = 27$	G	.	.	.	$n = 24$
C	.	.	.	$n = 24$	H	.	.	.	$n = 24$
D	.	.	.	$n = 30$	J	.	.	.	$n = 25$
E	.	.	.	$n = 24$					

Six specimens were without zones.

In another lot—

K	.	.	.	$n = 27$	N	.	.	.	$n = 25$
L	.	.	.	$n = 27$	O	.	.	.	$n = 34$
M	.	.	.	$n = 30$					

Three specimens showed no zone.

In all these cases I am counting as Professor Bourne counts; namely, the 1st setigerous segment is Segment II, so that the first dorsal bundle occurs in Segment VI.

Again, in Nais barbata he states, on p. 344, "$n = 17$." I examined a limited number of these, and found the number by no means restricted to 17. I have unfortunately mislaid my notes on the point, but four specimens, taken at random before I had seen his paper, were stained and mounted. Of these two have $n = 14$, and in the two others $n = 15$.

[1] Professor Lankester kindly allowed me to see a proof of the paper, and my attention was thereby drawn more particularly to some of these points.

EXPLANATION OF PLATES V, VI, and VII,

Illustrating Dr. W. Blaxland Benham's "Notes on some Aquatic Oligochæta."

Heterochæta costata.

FIG. 1.—The worm of the natural size.

FIG. 2.—A specimen coiled, about twice natural size.

FIG. 3.—Side view of worm, enlarged, showing the characteristic arrangement of chætæ. The segments are numbered and the annulations shown. *Pr.* Prostomium. *Dors.* Dorsal bundles. *Vent.* Ventral bundles. *Cten.* Palmate chætæ.

FIG. 4.—One of the characteristic palmate chætæ from in front.

FIG. 5.—The "head" of a palmate chæta, more enlarged and in a different focus, showing now the apparently stronger outer prongs.

FIG. 6.—A palmate chæta from the side, to show curvature of head.

FIG. 7.—View of the free edge of a palmate chæta, showing curve, membrane, and ridges.

FIG. 8.—A palmate chæta seen obliquely from above, to suggest Claparède's possible mistake in describing these chætæ as "cup-shaped."

FIG. 9.—An abnormal palmate chæta, with ridges feebly marked.

FIG. 10.—A furcate chæta from the dorsal bundle of Segment III.

FIG. 11.—A chæta from a dorsal bundle behind Segment XV.

FIG. 12.—The free end of a furcate chæta from ventral bundle of Segment IV.

FIG. 13.—The free end of a chæta from a ventral bundle of a more posterior segment to show the recurved proximal prong.

FIG. 14.—The "head" of a furcate chæta from in front, the transverse ridge being the lower or proximal prong.

FIG. 15.—An abnormal chæta, with two teeth between the chief prongs, from dorsal bundle of Segment XIV of a certain specimen.

FIG. 15 *a.*—The dorsal bundle of Segment XIV, showing the abnormal multidentate chæta (which is drawn too large, relative to the normal ones).

FIG. 16.—Dorsal view of anterior end of a worm, showing abnormal arrangement of chætæ (see p. 194).

FIG. 17.—A few segments of the worm viewed ventrally by transparency,

showing arrangement of organs. Drawn from a sketch of a living specimen slightly compressed. Portions of body-wall are represented showing the pores of sperm-ducts and spermathecæ.

FIG. 18.—The male duct isolated by compression. *Sp. f.* Spermiducal funnel. *Sept.* Septum between Segments x and xi. *Sp. d.* Sperm-duct. *gl. atr.* Glandular region of atrium. *n. gl. atr.* Non-glandular region of atrium. The extent of cilia is shown. The chitinous coat of the penis is represented in black outline.

FIG. 19.—A spermiducal funnel, with lips partly closed during movement of worm.

FIG. 20.—Two atria, isolated by compression, in order to show the artificial character of the dilatations of atrium. *sp. d.* Sperm-duct. *prost.* Prostate. *gl. at.* Glandular region of atrium. *n. gl. at.* Non-glandular region of atrium.

FIG. 21.—Enlarged view of penis from living worm, slightly compressed. The drawing is sufficiently explained. *a, b,* point to the circumpenial and pre-penial regions of penial chamber lined by invaginated cuticle. *v. ch.* Ventral chætæ.

FIG. 22.—Longitudinal section through penis, to show character of the cells of this region. *a.* Circumpenial; and *b,* pre-penial portions of penial chamber. *ch. pe.* Chitinous coat of penis, which is continuous with cuticle lining penial chamber. *ep. b.* Epidermis. *atr.* Portions of atrium.

FIG. 23.—Longitudinal section through portions of atrium and prostate. *c. ep.* Cœlomic epithelium covering prostate, sperm-duct, &c. *ep.* Epithelium lining the non-glandular part of atrium (*n. gl. atr.*). *ep'.* Epithelium lining the glandular part of atrium (*gl. atr.*). *mus.* Muscular coat of atrium. *pro.* Prostate. *sept.* Septum between Segments xi and xii. *sp. d., sp. d'.* Transverse and longitudinal sections of sperm-duct.

FIG. 24.—A cell from prostate enlarged. *vac.* Vacuole.

FIG. 25.—A cell from epithelium of glandular part of atrium.

FIG. 26.—Section through spermathecal pore and neighbouring part of the organ. *ep.* Epithelium of spermatheca (the internal boundaries of the cells have been made too definite). *ep'.* Epithelium of neck. *ep. b.* Epidermis. *c. ep.* Cœlomic epithelium. *mus.* Muscular coat. *p.* Spermathecal pore.

FIG. 27.—A cell from epithelium of neck of spermatheca.

FIG. 28.—A sperm-rope.

FIG. 29.—A portion of sperm-rope, more highly magnified. *a.* Very highly refracting wall, with heads of spermatozoa. *b.* Layer of granules within. *c.* Mass of spermatozoa in cavity. *d.* Tails of those spermatozoa whose heads are embedded in *a.*

FIG. 30.—Portions of crushed sperm-rope, the wall of which has burst, and spermatozoa from within are escaping. *a, b, c,* as in Fig. 29.

Fig. 31.—A transverse section of a sperm-rope, from a series of longitudinal sections of the worm. *a, b, c, d,* as in Fig. 29.

Fig. 32.—View of genital segments of a spent worm, seen by transparency. Drawn from living specimen. *D. v.* Dorsal vessel (the dilatation is not a permanent feature, it merely represents a diastole of the vessel). *G.* Intestine. *ov.* Ovary. *sp. s.* Shrunken sperm-sac, with convoluted blood-vessel on its wall. *spth.* Degenerating spermatheca. *t.* Testis. *v. v.* Ventral blood-vessel.

Fig. 33.—Diagrammatic side view of Psammoryctes, to show arrangement of chætæ.

Fig. 33*a.*—A palmate chæta from dorsal bundle of anterior segments, seen from in front.

Fig. 33*b.*—Same in optical longitudinal section to show curvature of free edge.

Fig. 33*c.*—A multidentate chæta from dorsal bundle.

Fig. 33*d.*—A forked chæta from ventral bundle, anteriorly.

Fig. 33*e.*—A forked chæta from ventral bundle, posteriorly.

Fig. 34.—Diagrammatic side view of Tubifex.

Fig. 34 *a, b, c, d.*—Multidentate chætæ from anterior segments, dorsal bundle, after treatment of the worm with KHO and mounting in glycerine. *b* is a young chæta ; the others were in use.

Fig. 35.—A diagrammatic side view of Limnodrilus, Clitellio, and Hemitubifex.

Fig. 35*a.*—A dorsal chæta.

Fig. 36.—A diagrammatic side view of Spirosperma and Ilyodrilus Perrieri.

Fig. 36*a.*—Chæta from dorsal bundle of Ilyodrilus Perrieri.

Fig. 36*b.*—Ditto from other species.

Fig. 36*c.*—Palmate chæta from dorsal bundle of 3rd segment of Spirosperma.

Fig. 36*d.*—Palmate chæta from hinder segments. Same magnification (the lines are too coarse).

Fig. 36*e.*—A ventral chæta.

Fig. 36*f.*—A ventral chæta from one of the anterior six segments.

Fig. 37.—Outlines of chitinous coat of penis of various genera, after Claparède, Eisen, and Vejdovsky. *a.* Hemitubifex. *b.* Telmatodrilus. *c.* Psammoryctes and Spirosperma. *d.* Limnodrilus corallinus. *e.* L. igneus. *f.* L. alpestris. *g.* L. Hoffmeisteri. *h.* L. silvani.

Nais elinguis.

FIG. 38.—The chætæ. *a.* Of dorsal bundle. *b.* A ventral chæta. *c.* A genital chæta.

FIG. 39.—View of genital region from above by transparency (from a living worm compressed). *o, o, o.* Masses of ova at different stages of development which have dropped away from the ovary. *sp. sac.* Sperm-sac. *spth.* Spermatheca. *ne.* Nephridium.

FIG. 40.—Ventral view of anterior region of sexually mature worm, showing position of clitellum and the genital chætæ (*gen. ch.*). *d.* Dorsal chætæ. *v.* Ordinary ventral chætæ.

FIG. 41.—View of a nearly mature specimen, rather from the side, showing presence of genital chætæ (*gen. ch.*) on one side only. *d.* The still persistent dorsal chætæ of Segment VI. *v.* The still persistent ventral chætæ of the same segment on one side.

Stylodrilus Vejdovskyi.

FIG. 42.—Ventral view of head. *Pr.* Prostomium.

FIG. 43.—Ventral view of Segments X and XI, to show penis and annulation (*a, b*) of the segment. *v.* Ventral chætæ.

FIG. 44.—Chætæ. *a.* From post-genital region, ventral or dorsal. *b.* Pregenital ventral bundles.

On the Differentiation of Leprosy and Tubercle Bacilli.

By

Charles Slater, M.B.Cantab.

In the course of an investigation of a case of tubercular leprosy the question arose as to the nature of certain lesions of the lungs. It is well known that patients suffering from leprosy frequently succumb to an affection of the lungs closely resembling ordinary phthisis. Whether this phthisis is the result of an intercurrent tuberculosis, or is of the same nature as the undoubtedly leprotic lesions of viscera other than the lungs, is still a matter of dispute. While certain observers, such as Hansen, Neisser, and Leloir, regard the phthisis as an intercurrent disease attacking a weakened patient, others, as Bonomé and Arning, think that the lesions are due to the spread of the leprotic process to the lungs. As a subsidiary branch of the inquiry it was thought advisable to examine the statements of previous investigators as to the differential staining and morphological differences of the bacilli present in the two diseases, and to test their results on material supplied by this undoubted case of leprosy.

The material, for which I am indebted to Dr. Delépine, consisted of various internal organs, glands, nerves, and skin from different parts of the body. In addition there were specimens of sputa obtained some three days before death.

Distinctions between the bacilli of leprosy and tuberculosis have been sought by many investigators, with the general result that one after the other the differential criteria have been shown to be untrustworthy or less constant than was originally supposed.

These criteria have been sought in differences in—

1. The size of the bacilli.
2. The shape.
3. The staining properties, including the resistance to decolorisation.
4. The numbers and distribution of the bacilli.

The differences based on the number and distribution will be found fully discussed in a paper written in association with Dr. Delépine ('Trans. Path. Soc.,' 1891).

While all observers speak of the B. lepræ as resembling in size the B. tuberculosis, yet some, such as Flügge, give measurements for the former which are considerably greater than those given for the latter. Bonomé, on the other hand, speaks of the leprosy bacillus as shorter and thicker than B. tuberculosis.

There is also a general consensus of opinion that the B. lepræ is less variable in length and more rectilinear (Koch, Flügge, Babes); but Baumgarten confesses himself unable to establish any fundamental differences on these grounds, and, considering the extreme variability of the B. tuberculosis in various specimens of sputa, &c., it would be impossible to lay much stress on these differences.

According to Neisser the B. lepræ possesses pointed extremities, while Bonomé, on the other hand, regards a swollen pole as a distinctive mark of the bacillus. In the specimens obtained from the present case there is a decided enlargement of the poles of the bacilli, and in rapidly stained specimens the extremities are more strongly stained than the intermediate portions.

The possession of a capsule seems to be common to both organisms.

It is to the variations in the staining properties that most attention has been paid, and differences sought in—

1. The different staining properties of various dyes, and of the various solutions of these dyes, whether made with aniline oil, alcohol, water, &c.
2. The rapidity of the staining.

3. The resistance to decolorisation.

Originally Koch considered that while the B. tuberculosis required for its demonstration one of the complicated methods devised by himself or Ehrlich, the B. lepræ could be readily stained by Weigert's method of nuclear staining ; and also that while the B. tuberculosis was stained by alkaline methylene blue the leprosy bacillus remained unaffected.

Babes showed that various violets (gentian violet, &c.) in simple solution would stain the B. tuberculosis, but stated that this bacillus was not stained by simple solutions of red or violet fuchsine, methylene blue, or eosin, while all these colours would stain the B. lepræ.

Baumgarten pointed out that both the bacilli were stained by watery solutions of fuchsine, but in contradiction to Babes asserted that neither was stained by watery or alcoholic solutions of methylene blue or eosin.

Wesener found that it was possible to stain the B. tuberculosis with any dye which stained the B. lepræ, and agreed with Baumgarten that eosin and watery methylene blue stained neither.   Alkaline methylene blue in diluted alcoholic solution, however, stains both organisms, as does also (but very badly) acid solution of eosin.   Bismarck brown and vesuvin stain neither.

My own results agree in the main with those of Wesener. About such stains as fuchsine and the various violets all authors are agreed.   It is not, however, easy to decide in all cases whether the stain is really taken up by the B. lepræ or by the material present in the lepra cell.   If a section rich in bacilli or lepra cells is stained with simple methylene blue (2 per cent. diluted alcoholic), Löffler's solution, Friedlander's stain, logwood, or even Bismarck brown, it is perfectly easy to distinguish the lepra cells, and those parts of the section which can be shown by control staining to contain numerous bacilli. The cells are filled with a fairly strongly stained granular material, often of a different tint from the surrounding nuclei, and a similarly stained granular material interpenetrates those portions of the sections where bacilli are known to exist.

The cells contain numerous vacuoles apparently filled with colloidal material, and the web of granular material passing amongst the cells of the leprous granulomata seems to form the boundary of similar vacuoles.   The granules are arranged in lines which suggest the course of bacilli.   It is generally impossible to distinguish individual bacilli by the above stains, though Löffler's stain gives decided results; but the parts which are thus doubtfully stained are those which with fuchsine show definite bacilli.   If the section be rapidly stained by fuchsine, decolourised by acid alcohol, and counterstained by methylene blue, the cells are seen to be filled with a similar granular blue-stained material in which a few red-stained bacilli are embedded. It would, therefore, appear probable that the granular staining material is in large part made up of altered cell contents and possibly degenerated bacilli.

It is impossible to distinguish with certainty these two bacilli by the use of various stains or by modifying the material in which they are dissolved, since the simple watery, the aniline oil, the alcoholic, and the carbolised solutions all stain.

The rapidity with which the bacilli are stained by such a dye as fuchsine has been tried as a means of distinguishing between the two bacilli.   The statements of different observers are very contradictory.

Babes states that B. lepræ is alone coloured by staining for thirty minutes with simple Poirier fuchsine and decolourising by an acid.   Baumgarten gives two methods for staining the B. lepræ and leaving the B. tuberculosis unstained.   According to this author, dilute alcoholic fuchsine (5—6 drops of the alcoholic stain in a small watch-glass of water) stains the B. lepræ in twelve to fifteen minutes in sections, and on cover-glasses in five to six minutes, so as to resist decolorisation by acid alcohol (nitric acid 1 part, and alcohol 10 parts) for half a minute; or similar staining can also be effected in two to three minutes by Ehrlich's fuchsine solution.   The B. tuberculosis is said to be left unstained.

Bonomé, who was attempting to solve the question as to the nature of some lung lesions in leprosy, used Baumgarten's

methods and considered them satisfactory, though at the same time he pointed out various circumstances which might modify the result, more particularly the thickness of the sections.

Wesener repeated Baumgarten's experiments with tubercular material from various sources, and concluded that the methods afforded no absolute criterion by which to differentiate the two bacilli.   Both the methods would stain tubercle as well as leprosy bacilli.

My own experiments point in the same direction.   The material used was, for the leprosy, chiefly sections of skin hardened in alcohol; while the tubercular material consisted of cover-glass preparations of sputa, sections of bovine lung very rich in bacilli, and human tubercular lung.   It may be objected that results obtained with bovine tuberculosis are not satisfactory, but bovine and human tuberculosis are usually considered to be identical in origin, and it was important to use sections containing large numbers of tubercle bacilli in order to render them comparable with the leprosy sections.   It was impossible to find specimens of human tuberculosis which contained sufficient numbers of tubercle bacilli to make the negative results certain.

This question of the number of bacilli present is highly important in estimating the rapidity of staining.   As is well known, the staining properties of bacilli in the same specimen vary largely.   Some bacilli are stained in a period which is quite inadequate to stain others, and the process of decolorisation is also quite gradual.   The most striking thing in a leprosy section is the enormous number of bacilli present.   Now even if the two varieties of bacilli stain equally quickly, and are exposed to the stain for such a period as is sufficient to colour say 1 per cent. of the bacilli present, it is obvious that the leprosy bacilli would appear to stain more quickly and strongly than the B. tuberculosis, or even to stain in a time which is insufficient to stain tubercle bacilli. The difference is really due to the number, and not to the proper staining power of the bacilli.   Looking first at results obtained with cover-glass preparations of sputa, it was found that the B. tuberculosis

was quite certainly stained in five minutes in cold diluted alcoholic solutions of rubin, fuchsine, and magenta, prepared according to Baumgarten's directions. The preparations were decolourised by acid alcohol (v. supra) and counterstained with methylene blue. Cold concentrated aqueous solutions of these two dyes also stained tubercle bacilli perfectly well, and they resisted the same subsequent treatment as when stained by the dilute alcoholic solutions.

The cover-glasses spread with the sputum from the leper gave similar results. There was, however, in these specimens a decidedly greater tendency for many of the bacilli to have their red stain partially replaced by the methylene blue, which, supposing them to be leprosy bacilli, accords with what is subsequently noticed as to the behaviour of undoubted leprosy bacilli in sections.

The sections were transferred to the stains either direct from absolute alcohol or from water. Those transferred from alcohol stained rather the more strongly, but the results were not substantially modified.

It was found that in sections both the Bacillus lepræ and B. tuberculosis were stained unmistakably by both Baumgarten's methods.

They were also both stained in six minutes in cold concentrated watery solutions of rubin or magenta, with subsequent treatment by acid alcohol. It was not possible to confirm this last result in the case of human tuberculosis.

It is obvious, then, that there is no essential microchemical difference in the behaviour of the two bacilli.

It is of interest to observe that in the two sets of sections similarly stained, though the leprosy specimens showed a far greater number of bacilli, yet on the whole the tubercle bacilli were the clearer and took a purer stain. There was a strong tendency for the leprosy bacilli to be purplish in colour—partially stained by the methylene blue. It was noticeable, too, that the general appearance of the section seen with a low power was quite different in these rapidly stained specimens from that presented by more fully stained sections. In these

latter the most striking features were the large strongly stained lepra cells and globi. In the former, on the contrary, it is the intercellular bacilli and those contained in the minute cells which are stained well, while the large lepra cells are inconspicuous, being, for the most part, stained blue with a few scattered red-stained organisms. This would seem to indicate that there are considerable differences in the age and probably also in the activity of the bacilli. From what we know of other bacilli, it would seem probable that the scattered intercellular organisms are the youngest and most active, while the large masses are composed of older and possibly degenerated bacilli, or those which have developed a considerable capsule.

The resistance to decolourising agents has also served as a basis of distinction between the two varieties of bacilli, but is not reliable as an absolute criterion. The B. lepræ apparently resists decoloration more vigorously than B. tuberculosis. The remarks made above as to the reaction between number and rapidity of staining apply equally to decolorisation.

Babes states that B. lepræ in cover-glass preparations will resist decolorisation by strong nitric acid for one hour, while the tubercle bacilli seldom resist for more than half an hour. Not having any cover-glass preparations of undoubted leprosy material, it was impossible to confirm this statement; but the B. tuberculosis is certainly decolourised in the time. The method is, however, a very severe one, often resulting in the detachment of the film, and is obviously inapplicable to sections.

Lustgarten proposed to distinguish between the bacilli by the greater resistance of B. lepræ to the decolourising action of 1 per cent. hypochlorite of sodium. Wesener and Bonomé, however, have examined this method and rejected it as useless.

Voltolini states that if, before staining, a cover-glass preparation of B. tuberculosis be exposed to the action of fuming nitric acid, the bacilli appear when stained as a row of

points—a streptococcus form.   Bacillus lepræ does not give
this appearance.

It is a method inapplicable to sections, and, bearing in
mind the well-known action of strong acids in facilitating the
production of a streptococcus appearance in B. lepræ, does not
appear to be a reliable guide.

Unna and Lutz, by a modification of Gram's method, sub-
stituting nitric acid for alcohol as the decolourising agent,
concluded that the true form of B. lepræ is a coccothrix.
It does not appear to have been suggested as a method of
distinction.

The conclusions arrived at from an analysis of the work
previously done and my own observations are—

1. That any colouring agent which will stain the leprosy
bacillus will also stain B. tuberculosis.

2. That the methods proposed to stain B. lepræ, while
leaving B. tuberculosis unstained, are unreliable.   There is
no essential difference between the two bacilli in their relation
to stains or decolourising agents.

3. That the apparent differences in respect to rapidity of
staining and resistance to decolorisation are due to difference
in numbers of bacilli present.

It has frequently been suggested that leprosy and tubercu-
losis are closely allied, and that the bacilli present in the two
diseases only differ in their physiological activity.   It seems
certain that no distinctions can be based on morphological
grounds or microchemical reactions.

As a rule, the pictures presented by the lesions of leprosy
and tubercle are entirely different, and the nature of the
affection can be certainly affirmed by a consideration of the
anatomical nature of the lesion and the number and dis-
tribution of the bacilli.   In the lung, however, we have scanty
data for these conclusions, and any help that could have been
derived from the individual characters of the bacilli would
have been useful.

It may also happen that a diagnosis of the nature of a lung
affection may be wanting during the life of the patient, and in

this case the staining reactions and morphology are almost the only data for a conclusion, as the distribution is less helpful here than in sections.

It must be borne in mind that it is the lack of difference in the reactions of the bacilli, and not in the lesions produced, which is insisted upon in the above paper. It is possible to argue from the character of the lesion as to the nature of the bacilli, but not from the morphology or staining of the bacilli as to the nature of the disease.

Note.—Stains coming from different manufacturers, and different samples from the same maker, are very variable in their staining properties. The two red dyes made use of were a magenta obtained from Messrs. Martindale, and a rubin-fuchsine from König, of Berlin.

Baumgarten's Methods.—a. Sections taken from dis-tilled water, and stained for 12—15 minutes, at the most, in dilute alcoholic fuchsine, made by adding 5—6 drops con-centrated alcoholic stain to a small watch-glass of water. Decolourised by acid alcohol (absolute alcohol, 10; nitric acid, 1) for ½ minute, washed in distilled water. Counter-stained by methylene blue for 2—3 minutes. Dehydrated by absolute alcohol for 3—4 minutes. Cleared and mounted in xylol balsam. Cover-glasses stained for 5—6 minutes.

b. Stain in Ehrlich fuchsine for 2—3 minutes, and treat as in a.

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On a Specimen of the True Teeth of Ornithorhynchus.

By

Professor Charles Stewart, P.L.S.

With Plate VIII.

THE interesting discovery by Mr. Poulton of the existence of true teeth beneath the epithelium of the jaw of a young Ornithorhynchus, of which an account was given by him in this Journal, vol. xxix, 1889, followed by a further description given by Mr. Oldfield Thomas in the same year (' Proc. Roy. Soc.'), in which he pointed out that the teeth were not absorbed whilst still beneath the gum, but were functionally active until the animal had attained about half its adult size, made me anxious to obtain a specimen for the museum of the Royal College of Surgeons which would illustrate so remarkable and important an anatomical fact. A search amongst the rich stores of the College was fortunately rewarded by the discovery of a young male, 316 mm. in length, which showed these teeth in an extremely perfect state.

They were all provided with well-marked, slightly divergent fangs, mostly of a flattened form, which arose from some distance within the margin of the crown, to which their planes were parallel (the fangs had been absorbed in Mr. Thomas's specimen).

The upper jaws had two teeth on either side ; they were very firmly attached by their fangs, and were surrounded by a thick epithelial ridge, which was continued beneath the crown

as far as the origin of the fangs. In front of the teeth was a small oval soft papilliform elevation, which apparently corresponded with the most anterior of the three pairs of teeth found by Mr. Poulton in the upper jaw. These in his specimen were most developed, and would probably be the first to be shed. In old specimens the site of this elevation is occupied by a small triangular epithelial cup, continuous with the two main ones situated immediately behind.

The crowns of the teeth in the upper jaw were of an irregular rhombic outline, with crenulate margin; cusps of varied size roughened their free surface, each tooth bearing two of larger dimensions near its inner border.

The teeth in the lower jaw were three in number on either side; they presented much the same general features as those in the upper jaw, but the hindermost tooth was of small size, and had a single large cusp in the middle of its free surface, with a single fang corresponding to it. The two large teeth in front had their two chief cusps situated one in front of the other, and rather nearer the external than the internal border of the crown.

The complete dental formula would, as far as at present known, be $\frac{3-3}{3-3}$, as surmised by Mr. Poulton.

The specimen now described and figured in Pl. VIII shows the teeth in a more complete condition than has yet been described. Mr. Hollick having prepared for me a very careful enlarged drawing of the crowns of the molars, Professor Lankester expressed the wish to publish the drawing in the 'Quart. Journ. Micr. Sci.,' so that an accurate representation of the very remarkable and definite character of these multituberculate teeth might be in the hands of zoologists and palæontologists. I complied the more readily with my friend's wish, since Mr. Poulton's original illustrated account of the first discovery of these teeth appeared in this Journal.

EXPLANATION OF PLATE VIII,

Illustrating Professor Charles Stewart's paper "On a Specimen of the True Teeth of Ornithorhynchus."

a. Soft papilliform structure in front of true teeth of the right side of the upper jaw, the first or most anterior tooth having been shed.

b. Second tooth of the right side, upper jaw.

c. Third or posterior tooth of the right side, upper jaw.

d. Papilla of left side, upper jaw.

e. Socket for second tooth of left side, upper jaw.

f. Socket for third tooth, left side, upper jaw.

g. Deep surface of second tooth of left side, upper jaw, showing fangs.

h. Deep surface of third tooth, left side, upper jaw.

i. Second tooth of left side, upper jaw, viewed from posterior aspect.

k. Socket for first or most anterior tooth of left side of lower jaw.

l. Socket for second tooth, left side, lower jaw.

m. Socket for third tooth, left side, lower jaw.

n. First or most anterior tooth of right side of lower jaw in situ.

o. Second tooth of right side, lower jaw, in situ.

p. Third or most posterior tooth, right side, lower jaw.

q, r, s. Deep surface of first (*q*), second (*r*), and third (*s*) teeth of left side.

The crowns of the teeth of the right and left sides correspond more closely in size and shape than might be supposed from an examination of the drawing.

On Onchnesoma Steenstrupii.

By

Arthur E. Shipley, M.A., F.L.S.,

Fellow and Lecturer of Christ's College, Cambridge, and Demonstrator of
Comparative Anatomy in the University.

With Plate IX.

THE genus Onchnesoma was established in the year 1877 by
Koren and Danielssen,[1] its name being derived from ὄγχνη = a
pear, and σῶμα = body. The genus is characterised as follows:
"The body small, pear-shaped. The proboscis long. The
anal aperture a little in front of the base of the proboscis.
No tentacles; no vascular system. One retractor."

The genus consisted of two species: O. Steenstrupii,
which the authors regard as synonymous with Sipunculus
pyriformis of Danielssen [2] and Phascolosoma pusillum
of Sars; [3] and O. Sarsii, synonymous with the Phascolosoma
lævissimum of Sars.[3] These two species, with their charac-
teristics, are mentioned in Selenka's monograph of the Sipun-
culidæ. In 1881 a third species, O. glaciale, was described
by the Norwegian authors [4] from amongst the material col-
lected by the Norwegian North Atlantic Expedition, so that

[1] "Contribution to the Natural History of the Norwegian Gephyrea," by
J. Koren and D. C. Danielssen, 'Fauna Littoralis Norvegiæ,' Bergen, 1877.

[2] Danielssen, 'Videnskabsselskabet Forhandlinger i. Christiania,' Aaret,
1859.

[3] Sars, 'Videnskabsselskabet Forhandlinger i. Christiania,' Aaret, 1868.

[4] 'The Norwegian North Atlantic Expedition, 1876—1878, Gephyrea,' by
D. C. Danielssen and Johan Koren, Christiania, 1881.

the genus at present comprises three species, all found on the north-west coast of the Scandinavian peninsula.

The species differ considerably in size. O. Steenstrupii, whose body measures but 3 mm. in length, is the smallest Gephyrean hitherto described, and, corresponding with its minute size, the structure of the body is very much simplified. O. Sarsii attains a body length of 8 mm., whilst the body of O. glaciale is 35 mm. long. I have hitherto been unable to obtain specimens of the two last-mentioned species; but, thanks to the kindness of Professor E. Ray Lankester and Canon Norman, I have been enabled to investigate the structure of the smallest species, of the minute anatomy of which the following is an account.

The External Appearance.

In the better preserved specimens the body was about 3 mm. long, pointed behind, and in front passing abruptly into the introvert (figs. 1 and 2); some, however, which were not well preserved, and which did not appear to be normal, had a longer and more slender body, which passed gradually into the introvert. The length of the latter structure varied in accordance with the amount of its protrusion : it was, when fully extended, almost invariably coiled up, and consequently difficult to measure, but in no specimen was it 34 mm. long, the length described by Koren and Danielssen.

The skin is of a French grey, almost greenish colour, and is divided into small areas by numerous crinkles, which at the posterior end of the body cross one another almost at right angles; in some cases such folds of the skin occurred at more or less regular intervals round the proboscis, giving it a superficial appearance of being segmented (fig. 1).

The introvert is covered with papillæ, which, according to Koren and Danielssen, are disposed in regular rows. The nature of these organs, which correspond with the papillæ of the larger Sipunculids, will be described below; they open to the exterior, but the opening is not always situated on an eminence, but may be found anywhere on the wrinkled surface of

the body. The skin of the introvert, when extended, is transparent, so that the œsophagus and nervous system may be seen through it.

There is a marked thickening of the skin where the introvert joins the body; the anus is situated a little anterior to this. The external opening of the kidney is a little behind, just to the side of the ventral nerve-cord.

The Structure of the Skin.

The layers which compose the skin of Onchnesoma have been described by the Norwegian writers; there are, however, one or two details which may be added to their account. As in the skin of other Sipunculids, we meet with six layers. The state of preservation of my specimens did not allow the epidermal cells to be made out. But this outermost layer of cells probably forms part of the deeply stained external layer seen in fig. 6.

In section this layer appears ridged, the ridges corresponding with the wrinkles on the surface of the animal. It is obscured by a number of granules, which stain very deeply with hæmatoxylin; these granules are apparently produced by certain structures which correspond with the epidermal glands of other forms. Between the darkly stained external layer and the circular muscles is a thick gelatinous connective-tissue layer, or cutis, in which hardly any trace of cells could be detected. At the base of this the skin glands are situated.

The state of the preservation of my material did not allow me to see this point very clearly, but I have no doubt that the epidermal glands are composed of specialised epidermal cells. Each gland is of a spherical shape; from the outer edge of this a duct with sharply defined outline leads through the cutis to the surface of the body. Within the glands lie numerous darkly stained granules, similar to those which cover the outside of the body; and there is little doubt that the latter have their origin in these structures, and pass out with the mucus which

has in some cases been seen to exude from the pores of the glands.

The external circular layer of muscles is well developed in the introvert, and in the anterior half of the body; but about the middle of the body it fades away, so that the posterior end is provided only with longitudinal muscles (fig. 7). The circular muscles are arranged in bundles, but the longitudinal are in a continuous sheet.

Both the cutis and the external and internal layers of muscles take part in the thickening of the skin which exists at the junction of the proboscis and the body.

The body-wall is lined by an endothelium, which extends over the internal organs. In the living specimens, according to Koren and Danielssen, it can be distinctly seen that this endo-thelium is ciliated, and that the cilia, by their action, keep the perivisceral fluid in motion.

The General Anatomy.

If a longitudinal incision be made in the body-wall of Onchnesoma, and the sides reflected, the arrangement of the internal organs and their relation to one another become at once evident without further dissecting. These relations are clearly shown in fig. 3, which I have borrowed from Koren and Danielssen's 'Fauna Littoralis Norvegiæ.' It will be seen that the œsophagus is very long and loosely coiled, in order to allow for the extension of the introvert. The intestine, whose diameter is larger than the œsophagus or rectum, is also much coiled. The anus is situated rather too far forward to the right of the ventral nerve-cord.

There is only a single retractor muscle, which has its origin at the extreme posterior end of the body, where the skin is thickened and produced into a blunt point (fig. 7). The other end of this muscle is inserted into the wall of the œsophagus immediately below the brain. The muscle-fibres which compose this retractor muscle are bigger than those of the muscular sheaths in the skin. They are fusiform, with a rather flattened transverse section and a faint longitudinal striation.

There is no closed vascular system such as exists in the larger Sipunculids. The perivisceral fluid which bathes the internal organs is crowded with nucleated corpuscles and generative cells.

The single kidney varies in position; in some of my specimens it was situated to the left of the ventral nerve-cord, in others to the right. Both its internal and external openings are too small to be made out except by section. The ventral nerve-cord may be seen as a very fine strand running just inside the skin (fig. 8).

THE HEAD.

The head of Onchnesoma is of a remarkably simplified nature compared with that of the larger Gephyrea, but whether the simplification is primitive or the result of degeneration is not an easy matter to decide. The hooks which are so common in the group, arranged in rings round the proboscis, are entirely absent in this genus. This is a point of some interest taken in connection with the absence of several other structures which are usually met with in the group, but too much stress must not be laid on it, as with one exception, S. australis, the whole genus Sipunculus is devoid of these structures, and in other genera several species are without hooks; they are also apt to drop off as the animal grows old.

A more important feature is the entire absence of any tentacles. There is no trace whatever of the lophophoral ring of tentacles such as occurs in Phymosoma, and the crumpled pigmented tissue which occupied the hollow of the horseshoe is also entirely absent. The place of these structures, in the dorsal side of the mouth, is occupied by a slight elevation or blunt process which contains the brain. This process has a slight resemblance to a Doge's cap, but it is really nothing more than an extension of the body-wall on the dorsal side of the thickened lip which surrounds the mouth. The skin covering this process is not pigmented, but the whole of it is uniformly ciliated, the cilia being continuous with those which

line the œsophagus. The cilia also cover the ventral lip. The lobe is more or less solid (fig. 12), and contains the brain, the rest of the space being filled up with connective tissue. The brain gives off a median nerve (figs. 10 and 11), which passes into the lobe, and is distributed, I believe, to the epidermal cells, so that doubtless the lobe has a tactile and sensory function.

Just beneath the brain, on the dorsal surface of the œso-phagus, the retractor muscle is inserted; it wraps round about two thirds of the circumference of that tube (fig. 12).

Corresponding with the absence of the tentacular crown there is a total absence of any vascular system, a peculiarity which Onchnesoma shares with Petalostoma and Tylosoma. There can be no doubt that in those forms which possess ten-tacles they have both a tactile and sensory function, and that they serve, by the currents their cilia give rise to, to bring food to the mouth. It is also believed that they have a respi-ratory function; and though this is probably the case, it must not be overlooked that the above-mentioned genera manage to respire without tentacles. Where the exchange of gases takes place is not so easy to state. The skin of Onchnesoma is relatively to the size of the animal at least as thick as that of the larger Sipunculids, and is covered by a thick cuticle. It has occurred to me that the cœlomic fluid may possibly obtain the oxygen it requires from the water which passes through the intestine of the animal. The coiled nature of this tube exposes a very considerable area to the fluid in the cœlom, and the extreme thinness and delicacy of its walls would favour a ready exchange of gas. If such a function were exercised by the alimentary canal, it would possibly explain the thinness of the digestive walls, which in other respects seems ill adapted to a diet of sand.

In Onchnesoma there is only one kind of corpuscle in the cœlomic fluid; this is spherical or nearly so, with granular protoplasm and a well-defined nucleus (figs. 7 and 8). The cœlomic fluid must be kept in very constant motion, both by the ciliated cells of the peritoneal epithe-

lium, and by the alternate protrusion and retraction of the introvert.

In the two species of Phymosoma which I have described [1] there is a very extensile fold of skin or collar which surrounds the base of the head, and which, when the introvert is retracted, usually completely encloses the head. The function of this collar is perhaps to shield and protect the delicate ciliated tentacles and lips from contact with the indurated surface of the introvert, provided as it often is with horny hooks. No such collar is found in Onchnesoma.

The Nervous System.

The brain is an elongated mass situated dorsal to the mouth, at the base of the median dorsal ciliated lobe (fig. 11). It shows no trace of being bilobed. Like that of Phymosoma, the brain of Onchnesoma consists of a cap of ganglion-cells which cover in a fibrous portion on all sides except that nearest the œsophagus, the ventral (fig. 12). There are no giant ganglion-cells to be seen. The nerve-cells are all of one size, with nuclei which stain deeply. On the dorsal surface the brain is continuous with the epidermis; but in this region, just at the base of the median dorsal process, the epidermal cells are not in any way modified. The pigment which accumulates in similarly placed cells in other Sipunculids is absent. There are also no eyes.

The brain gives off three nerves; a median nerve to the median dorsal lobe, and one on each side, which pass round the œsophagus and fuse together to form the ventral nerve-cord (figs. 9, 10, and 11). The median nerve is doubtless the equivalent of the pair of nerves which supply the pigmented pre-oral lobe in Phymosoma. The median lobe is probably sensory and tactile, and is therefore supplied with a stout nerve. The second pair of nerves in Phymosoma, which supply

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[1] "On Phymosoma varians," 'Quart. Journ. Micr. Sci.,' April, 1890. "On a New Species of Phymosoma, with a Synopsis of the Genus," 'Quart. Journ. Micr. Sci.,' March, 1891.

the tentacular lophophore, is naturally not represented in Onchnesoma, as the tentacles are absent.

At the sides the brain is continued into two nerves which pass round the mouth embedded in the tissue, just where the retractor muscle is attached to the œsophagus (fig. 9) ; they fuse together on the ventral surface, and form the ventral nerve-cord, which shows no sign of its double origin (fig. 8). The portion of this cord which lies in the introvert is oval in cross section ; that which lies in the body is round. In Phymosoma and in Sipunculus the ventral nerve-cord is supported by numerous strands of muscle continuous with the skin, which permitted the introvert to be extended or withdrawn without any strain being placed on the cord; but in Onchnesoma the cord is closely attached to the skin, and in the region of the introvert is almost embedded in the muscular layer.

As is the case in other Sipunculids, the ganglion-cells are arranged on the ventral surface, the fibres on the dorsal. The nerve-cord gives off numerous branches into the body-wall, whose course I was not able to follow; but Koren and Danielssen have traced them into a fine ganglionated network amongst the muscles, &c.

The nerve-cord extends to the posterior end of the body.

THE NEPHRIDIUM.

There is only a single nephridium in Onchnesoma, and its position is not very constant ; it may lie either to the right or to the left of the nerve-cord, but its external orifice is always a little below the ring-like thickening which marks the junction of the proboscis and the body.

In its main features the nephridium resembles the same organ in Phymosoma varians, with the exception that there is no distinction between glandular and non-glandular regions. The external orifice leads straight into the lumen of the gland, which is as a rule somewhat pear-shaped. The internal opening is close to the external; it has a flattened, funnel-shaped border, and is ciliated.

The walls of the nephridium are lined throughout, with the exception of the small area between the external and internal opening, with glandular cells of a considerable size; with the exception of the ova they are the largest cells in the body (fig. 4). The lumen of the kidney in Onchnesoma is not split up into a series of crypts communicating with a central cavity, as was the case in Phymosoma; and the cells do not get rid of the product of their secretion by breaking off a bubble from their free end. Each of the large columnar cells has a very definite outline; their protoplasm is very clear and does not stain well, but scattered through it are a great number of granules which stain deeply. These concretions differ in size; they are always spherical, and the larger ones have a double contour. These latter are often found in the lumen of the nephridium, having doubtless passed out of the glandular cells, and being on their way out of the body.

I have never seen ova or spermatozoa in the lumen of the kidney, though I have no doubt that they leave the body through this channel.

The muscular layer is not so well developed in the nephridium of Onchnesoma as in that of some other Sipunculids, and the size of the kidney was more constant. Covering the outside of the organ is a layer of peritoneal epithelium.

With regard to the number of nephridia, two is undoubtedly the normal number in the Sipunculids; the genera Phascolion, Tylosoma, and Onchnesoma being singular in having but one. There are, however, exceptions to this rule: thus Phascolosoma squamatum has but one, and Aspidosiphon tortus also retains but one; and in both these cases it is the left that persists. Some species of Phascolion, on the other hand, retain the kidney of the right side only; and in Onchnesoma sometimes the left and sometimes the right persists, but never both together.

The Alimentary Canal.

The cilia which cover the dome-shaped dorsal process of the head and the lower lip are continued without break into the

alimentary canal (fig. 12). When the introvert is extended, the first part of the digestive tube or the œsophagus forms a straight tube with smooth walls; when, however, the introvert is retracted, the walls of the œsophagus are thrown into a number of circular folds with intervening depressions. The cells lining this part of the alimentary canal are cubical, and thickly beset with cilia.

Throughout the intestine the lining epithelium is surrounded by a layer of connective tissue, which is in its turn covered by the peritoneal epithelium ; the connective tissue varies in thickness in different parts of the tube, but it is especially thick on the dorsal surface of the anterior end of the œsophagus : it is just here that the single retractor muscle is inserted.

The œsophagus passes into the descending intestine, whose walls are lined by large glandular cells : these have, when the intestine is comparatively empty, a columnar shape ; but if the intestine is full of food its walls are stretched, and the lining cells become cubical, or even depressed. Owing to the small size of the animal it is not possible to wash the food out of the alimentary canal, and the nature of the food rendered it very difficult to cut satisfactory sections of the walls of the alimentary canal. These were in most cases torn ; hence I have not been able to settle quite definitely whether the cells lining the descending intestine are ciliated or not, but I am inclined to think they are.

The ascending intestine is certainly lined with ciliated cells. It is distinguished by the possession of a longitudinal groove, which is lined by cells bearing especially long and large cilia. A similar groove is described by Mr. E. A. Andrews in Sipunculus Gouldii.[1] He states that "in it a current of liquid passes from the action of cilia, and possibly also of the radiating fibres, towards the anus during life." The absence of this groove is the only thing which distinguishes the short rectum from the descending intestine.

[1] "Notes on the Anatomy of Sipunculus Gouldii, Pourtalès," E. A. Andrews, 'Studies from the Biological Laboratory,' Johns Hopkins University, Oct., 1890.

The whole alimentary canal is attached to the body-wall by a few fibrous strands, but there appears to be no spindle muscle running up the axis of the spirally coiled intestine.

The food of Onchnesoma, judging by the contents of the intestine, consists of vegetable débris ; mixed with this is a considerable amount of sand and a number of spicules, whose precise nature I was not able to make out.

The enormous amount of sand and mud which passes through the body of the Sipunculids shows that these animals must take a considerable share in the reducing action to which the mineral substances at the bottom of the sea are subjected. Mr. J. Y. Buchanan has recently published an interesting paper " On the Occurrence of Sulphur in Marine Muds,"[1] in which he has drawn attention to the fact that most silicates are to some extent soluble when pulverised under water, and the sand is to some extent crusted in passing through the body of most mud-eating animals, and this solubility is increased by the sulphates in the sea water which passes through the intestine of the animals. The sulphates are reduced by the organic products of the body to sulphides, and these unite with the iron or manganese of the silicates, and leave the body as sulphides of iron or manganese. These sulphides are then oxidised by the oxygen which exists in sea water, and form the red clays and chocolate muds which cover a considerable extent of the bottom of the sea. Thus the constitution of the mud at the bottom of the sea is to a very large extent artificial, and the Sipunculids play a considerable rôle in bringing this about.

These processes must be mainly effected by Holothurians, Echinids, Polychætes, and Sipunculids ; and to arrive at some sort of an estimate of the amount of sand taken into the body of the latter animals, I recently weighed five specimens, chosen at random, of S. nudus from Naples, and then weighed the sand in their intestines. The average weight of their body

[1] "On the Occurrence of Sulphur in Marine Muds and Nodules, and its Bearing on their Mode of Formation," J. Y. Buchanan, 'Proc. of the Royal Soc. of Edinburgh,' Dec., 1890.

was 19·08 grms., that of the sand 10·03 grms. In two of them the sand weighed more than one half the total weight, the body being in one case 24·4 grms. and the sand 13·72, and in the other 15·05 grms. and 9·45. The contents of the intestine consisted of blackish sand with a few Foraminifera mixed with it. In spite of the considerable amount of sand which these figures show to be contained in the intestine, the wall of this tube in all the Sipunculids with which I am acquainted is excessively thin, and apparently but poorly adapted to retain the sharp and jagged pieces of sand which lie within it. A similar tenuity of the wall of the alimentary canal also occurs in Echinids and Holothurians. Although this wall is so thin I have never found a Sipunculid with its intestine ruptured, so that in spite of appearances it seems to serve its purpose well.

I have mentioned above that I am of opinion that the respiration of Onchnesoma is carried on through the walls of the intestine. The seat of the process of respiration is still a debatable point in the anatomy of the unarmed Gephyrea. Of the two recent authors who have written on the anatomy of Sipunculus, Mr. Andrews[1] is convinced that the tentacles act as branchiæ, whilst Mr. Ward[2] is of opinion that they do not. In Onchnesoma, at any rate, there cannot be any question as to the respiratory action of the tentacles, as the latter are entirely absent. In other Sipunculids the tentacles may to a slight extent serve as the organs of respiration, but the closed vascular system which supplies them with blood is of such a very limited extent that it would only suffice for a small portion of the body ; on the other hand, it seems to me quite possible that the brain, which is almost entirely surrounded by this system, may obtain its oxygen from it.

The chief circulating medium in the body of the unarmed Gephyrea is undoubtedly the corpusculated cœlomic fluid, and

[1] Loc cit., p. 419.

[2] "On some Points in the Anatomy of Sipunculus nudus, L.," Henry B. Ward, 'Bull. of the Museum of Comp. Anat., Harvard College,' vol. xxi, No. 3, May, 1891.

in the case of Onchnesoma this forms the only circulating fluid. All the organs of the body, the alimentary canal, the nerve-cord, the nephridia, the chief muscles, and the generative organs, are suspended in this fluid, and bathed by it on all sides. The cœlomic fluid is kept in constant movement by the protrusion and retraction of the introvert, and by the action of the ciliated peritoneal epithelium which lines the body-wall and covers the internal organs. Thus the corpusculated cœlomic fluid is continually flowing over and circulating around all the organs suspended in it, and there is not much doubt that it acts as a carrier of oxygen to them.

The problem next arises, where does it effect the exchange of gas which constitutes respiration? This seems capable of two solutions: the cœlomic fluid takes its oxygen either from the corpusculated fluid of the closed vascular space, or through the walls of the alimentary canal. I am inclined to think that the latter alternative is responsible for the chief supply of oxygen to the body.

The walls of the vascular system are not very thin, and they do not present a very large surface to the cœlomic fluid; and although I think it possible that this fluid acts to a certain extent as a carrier of oxygen, more particularly to the brain, which, except where it is continuous with the epidermis, is surrounded on all sides by it, I still think that the primary function of the closed vascular system is to extend the tentacles by the contraction of its muscular walls forcing fluid into them, and that the primary function of the tentacles is to bring food to the mouth by the action of their cilia. For these reasons I think it, both on morphological and physiological grounds, inexpedient to speak of the tentacles as branchiæ.

The alimentary canal, on the other hand, has very thin walls, and owing to its looped and coiled disposition presents a very large surface to the cœlomic fluid. A considerable amount of water must be continually passing through the alimentary canal, since the food of the animal is brought into the œsophagus in a current of water set up by the cilia. This current is set up by the cilia lining the lips and œsophagus,

and is, I believe, maintained as a constant flow by the action of the cilia lining the ciliated groove which runs along one side of the ascending intestine. This groove is lined by cells bearing strong cilia. I have never seen any trace of food in it; and its chief function is, I think, to maintain the current of water which passes through the alimentary canal.

Professor Semper, in his 'Animal Life,' has drawn attention to those animals which breathe through their intestine. He has described certain foliated processes on the stomach of a Holothurian—S t i c h o p u s   v a r i e g a t u s—which function as gills; he also mentions the common loach, C o b i t i s   f o s s i l i s, which breathes through its stomach, but in this case it swallows air from the surface of the water. This air "is deprived of a portion of its oxygen in the intestine." Certain Brazilian fish, of the genera Calichthys, Doras, and Hypostomus, which also swallow air, have curious processes or folds of the lining of the intestine, which have been regarded as especially adapted for respiration. The anal respiration, which Professor Hartog has described in so many Crustacea and insect larvæ, is but another example of the alimentary canal being used as a respiratory organ. These instances are sufficient to show that in ascribing a respiratory function to the alimentary canal of Sipunculids one is supported by numerous analogous cases.

The Generative Organs.

Onchnesoma, like other Sipunculids, is diœcious. The testes are formed by the growth of a small clump of cells lining the cœlomic cavity in the neighbourhood of the point of origin of the single retractor muscle (fig. 7). I have not been able to find any ovary, though I suspect that when mature it is to be found in the same situation. Numerous ova were found floating in the cœlomic fluid of the females; but, as Koren and Danielssen have remarked, "while the ova continue their development in the perivisceral cavity, the last vestiges of the ovary disappear entirely, so that no trace of it remains."

Like the ova, the spermatozoa undergo a considerable de-

velopment whilst floating in the cœlomic fluid. They leave
the testis in the condition of the mother-cells of the sperma-
tozoa ; these segment, and the sperm morulæ result. The
spermatozoa keep together in sperm morulæ till they have
passed through the nephridium and out of the body.

Conclusions.

Onchnesoma is the smallest Sipunculid with which we are
acquainted, and its anatomy is to a considerable extent more
simple than that of other members of the group.

The head is much simplified ; the lip which surrounds the
mouth bears no tentacles, but is produced dorsally into a blunt
process covered with cilia. The simplicity of structure is
shown by the absence of any tentacles, hooks, collar, pig-
mented skin, and eyes ; there is also no vascular system,
no spindle muscle, and no giant-cells are found in the brain.
The retractor muscle is single and arises from the extreme
posterior end of the body, and is, therefore, symmetrical ; the
nephridium is also single, and may lie to the right or left of
the body. The brain is not bilobed.

Until we know something of the development of Onchne-
soma it would be hazardous to express an opinion as to whether
the absence of the above-mentioned organs is due to degenera-
tion, or whether they are primitive. On the one hand, the
small size of the animal and the presence of one nephridium,
which occurs on either side of the median line, points to
degeneration ; whilst, on the other, the structure of the head
indicates a primitive condition, which might admit of modifi-
cation in various directions.

The absence of any closed vascular system, correlated with
the absence of tentacles, may throw some light upon the vexed
question of the seat of the respiratory processes in Sipunculids.
Since there are no tentacles, there is one Sipunculid at least
which does not breathe by them ; and although I think, when
they are present, some respiration may be carried on by them
and the closed vascular system, especially in reference to the

brain, I am disposed to think that the main function of the tentacles is to create a current, and thus bring food to the mouth ; and the chief use of the vascular system is to extend the tentacles.

I am inclined to look for the chief respiratory organ in the intestine ; this has very thin and extensive walls, and exposes a large surface to the cœlomic fluid, which in its turn bathes all the organs of the body except the brain. A considerable volume of water passes through the alimentary canal, enough to supply the oxygen required, and this current is maintained by the ciliated cells of the groove in the ascending intestine.

THE MORPHOLOGICAL LABORATORY,
CAMBRIDGE; August, 1891.

EXPLANATION OF PLATE IX,

Illustrating Mr. Arthur E. Shipley's paper on " Onchne - soma Steenstrupii."

FIG. 1.—An enlarged view of O. Steenstrupii, with the introvert partially retracted.

FIG. 2.—The same, life size.

FIG. 3.—A view of the arrangement of the internal organs, shown by opening the body-wall along the right side and reflecting the sides. Copied from Koren and Danielssen.

FIG. 4.—Section through a portion of the glandular wall of the nephridium, showing the glandular cells and their concretions.

FIG. 5.—Section through the ascending intestine to show the ciliated groove.

FIG. 6.—Section through the skin, parallel with the long axis of the body, showing cutis, epidermal glands and their secretions, circular and longitudinal muscle layers, and lining peritoneal cells.

FIG. 7.—Longitudinal section through the posterior end of the body, showing origin of single retractor, and the group of peritoneal cells which form the testis.

FIG. 8.—Transverse section through the introvert, showing the layers of the skin, the œsophagus, retractor muscle, and ventral nerve-cord. The body-cavity contains corpuscles and sperm morulæ.

FIG. 9.—Longitudinal lateral section of the extended introvert, showing mouth, œsophagus, and thickened ciliated lip, with the circum-œsophageal nerve of each side.

FIG. 10.—A section of the brain, parallel to the preceding (fig. 9), but more dorsal. It shows the distribution of the ganglion-cells and fibres in the brain, and the three main nerves given off from it ; also the insertion of the retractor muscle, and one of the recesses formed by the crumpled nature of the œsophagus.

FIG. 11.—Diagram representing the arrangement of parts in the head, which is supposed to be divided medianly. The right half only is shown.

FIG. 12.—Section through the head and brain. The introvert is retracted. The section is not quite in the middle line, and does not show the connection of the brain with the epidermis.

Note on a Sieve-like Membrane across the Oscula of a Species of Leucosolenia, with some Observations on the Histology of the Sponge.

By

Edward A. Minchin,

Assistant to the Linacre Professor of Human and Comparative Anatomy in the University of Oxford.

With Plates X and XI.

At Plymouth, on the rocks immediately beneath the Marine Biological Laboratory, there are to be found at low tide a great number of calcareous and other sponges. Among them occur at least two quite distinct species of Leucosolenia, which can even be distinguished at sight by their mode of growth. The first species, which has only triradiate spicules (genus Ascetta, Haeckel), forms a network of anastomosing tubes, which at first creep close round the seaweeds and other objects, but finally, in large specimens, form great white masses of as much as two inches or more in height. From the network of tubes arise here and there the chimney-like oscula, which are simply continuations of the tubes, and not marked out by their greater diameter from them. The diameter of both the oscula and the ordinary tubes may vary within limits, but the diameter of the oscula is, if anything, less (fig. 10), at any rate not markedly greater than that of the tubes. On the other hand, in the second species of Leucosolenia, which has tri- and quadri-radiate spicules (genus Ascaltis, Haeckel), the mode of growth is essentially the same, but the oscular tubes are at once marked off from the remainder of the sponge by their very much larger size

and greater diameter.   They arise at intervals from the com⟨
paratively minute basal tubes, as if from a creeping stolon.
Both species are of a pure white colour.   The second of the
two species is, without doubt, Leucosolenia botryoides
(Ellis and Sol.), Bwk., the Ascaltis botryoides, var. Ellisii,
of Haeckel.   The first, or Ascetta species, I have more diffi-
culty in identifying.   Fig. 14, *a, b, c,* represents some of its
spicules.   As may be seen, they are precisely similar to the
spicule of Leucosolenia coriacea, figured by Bowerbank.[1]
On the other hand, they differ somewhat from the figures of
the spicules of Ascetta coriacea given by Haeckel[2] in having
much sharper points, in which they resemble his figures of the
spicules of A. primordialis.[3]   In his description of the
spicules of A. coriacea Haeckel states that the spicules are
"gar nicht, oder nur wenig gegen die Spitze hin verdünnt.
Die Spitze ist stets stumpf, niemals scharf, meistens glatt
abgerundet," &c.[4]   This description does not apply well to the
spicules here under consideration ; still less, however, does the
description "schlank conisch," applied to the spicules of
Ascetta primordialis, suit them.   They have much more
cylindrical rays than the spicules of A. primordialis.   Since,
moreover, A. primordialis is said to be wanting on the
Atlantic coasts, and to be replaced there by A. coriacea,[5]
this sponge may stand, for the present at any rate, as Leu-
cosolenia coriacea (Montague), Bwk., the Ascetta cori-
acea (Tarropsis form ?) of Haeckel.   It is in Leucosolenia
coriacea, as here identified, that the sieve membrane occurs
which I am about to describe.   In the summer of 1890 I
collected a quantity of this sponge in order to make prepara-
tions for teaching purposes.   In vertical sections of the sponge
passing through an osculum I was at once struck by the
appearance of a thin perforated membrane stretched across the

[1] 'Mon. Brit. Spongiadæ,' vol. iii, pl. iii, fig. 14.
[2] 'Die Kalkschwämme,' Bd. iii. Taf. v, figs. 2 *a—c.*
[3] 'Die Kalkschwämme,' T. c., Taf. v, figs. 1 *a—h.*
[4] Ibid., Bd. ii, p. 30.
[5] Ibid., Bd. ii, p. 27.

opening just above where the collar cells end (figs. 1, 2, 3, 5, 13). Having found no mention of such a membrane in the sponge literature[1] I proceeded to investigate it further, intend-

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[1] Haeckel, in his classical monograph ' Die Kalkschwämme,' Bd. i, p. 267, describes, under the name of " Mundhaut oder Oscular-membran," a structure in Sycons and Leucons which resembles somewhat from his description (there are unfortunately no figures) the membrane here described. In the Ascons " ist mir ihre Existenz überhaupt noch zweifelhaft." He writes, " Die Oscular-Membran ist eine dünne, keine Spicula enthaltende Lamelle des Syncytium, welche inwendig von der Basis (dem aboralen oder unteren Rande) des Rüssels oder des Peristom-Kranzes ausgeht. Bei weit geöffnetem Mundcanal wird sie (durch Retraction in das Exoderm) entweder ganz unsichtbar, oder bleibt bloss als ein ganz schmaler Ring stehen. Bei völlig geschlossenem Mund-canal hingegen bildet sie eine sehr zarte transversale Scheidewand, welche senkrecht auf der Längsachse des Magen steht." This membrane of Haeckel's has therefore quite a different structure from that which I describe here, but it has precisely the same relations to the osculum, and may well be homo-logous with it. It is to be hoped someone will give us before long a fuller description (with figures) of this " Oscular-membran." My friend, Mr. G. P. Bidder, has directed my attention to a passage in the works of Dr. Grant (' Edinburgh Philosophical Journal,' xiii, 1825, p. 381), quoted in Johnston's ' British Sponges and Corallines,' p. 51. " When we cut," says Dr. Grant, " a thin piece off the surface of a living sponge and look down through one of its pores with the reflecting microscope, we perceive, immediately beneath the projecting spiculæ which defend the pore, a very delicate network of gelatinous threads thrown over the entrance of the tube. This piece of structure is so fine as to be perfectly invisible to the naked eye; it consists of five or six threads, which pass in from the sides of the tubes to be con-nected with a central mesh, so that there are six or seven meshes thus formed; and while this soft apparatus is beautifully defended by the pro-jecting spicula of the pore, it serves still further to guard the interior of the animal from the smallest particles of sand, or the minutest visible animalcules." Since Grant distinguished clearly, both in this work and in others, between " pores " and " fæcal orifices," this network of his can have nothing to do with the membrane I describe here. But Johnston seems to have taken this description as applying to the oscula (T. c., p. 53, foot-note), and reminds his readers that Grant's description can apply to the oscula of one or two species only. He adds that " in general the oscula are merely simple or com-pound outlets, without any protective net over the orifice or in the funnel; and indeed it can rarely be seen except in newly formed oscula before the fibres of the sponge have been broken away by the effluent current "—a most noteworthy statement.

ing at the same time to thoroughly work out the anatomy and histology of this and other Plymouth species of Leucosolenia. As, however, I was obliged to leave England for Naples at an early stage of my investigations, I thought it best to publish an account of this membrane at once, together with a few scattered observations on the histology of the sponge, hoping at some future time to make a more complete study of this interesting sponge genus.

In a typical osculum the interior of the chimney-like tube is seen in sections to be lined by a layer of collared epithelium, only interrupted at intervals by the openings of pores (fig. 1). At a certain height the layer of the collar cells stops abruptly, but the wall of the oscular tube is continued on for a short distance as a funnel-like expansion ("rüsselformige Mund-öffnung") consisting of jelly containing spicules and lined by ectoderm. Immediately above the layer of collar cells the sieve membrane stretches across the opening. It is thus some distance below the actual margin of the oscular opening. Figs. 1, 2, 3, 12 a and b, and 13 show the membrane in section. Figs. 6 and 9 represent portions of it macerated out in glycerine, the portion in fig. 1 having been previously treated with weak acetic, and that in fig. 9 stained in picrocarmine. Fig. 5 shows a side view of a whole osculum (the one from which fig. 6 was dissected out) mounted in glycerine after fixation with osmic and removal of the spicules by dilute acetic. Fig. 10 shows a view from above of a whole osculum mounted in Canada balsam, after having been fixed with absolute alcohol and stained in hæmatoxylin, while 10 a represents the entire sieve membrane of the same osculum, drawn with a somewhat low magnification. Finally, fig. 11 a and b represent two consecutive sections from a series taken across an osculum transversely but slightly obliquely, so that portions of the membrane are obtained flat.

The sieve membrane varies in size, naturally, with the diameter of the osculum. The smallest open osculum I have seen was about 175 μ in diameter, the largest about 465 μ, or nearly half a millimetre. The osculum without any opening

shown in fig. 3, which we shall consider again below, was only
about 116 μ in diameter.   The sieve membrane is composed
of two layers of cells in apposition, but separated by a thin
layer of jelly (figs. 2, 3, 12, 13).   These cells have a central
portion containing the nucleus, and are continued out into
three, four, five, or even six processes, which unite with the
processes of other cells, thus forming a network with compa-
ratively wide meshes.   The body of the cell forms a node or
part of one, but not all the nodes of the network are formed
thus.   Many nodes are formed simply by the union of three
cell processes.   Thus larger and smaller nodes can be distin-
guished.   The former contain usually (not always) one, two,
or even three (fig. 9, c) nuclei, and are often of considerable
thickness.   In a side view (9, b) or section (12 a, 12 b, 13) of
such a node the two cells with the jelly between can be easily
seen.   Round the nuclei are a great number of granules,
sometimes large, more often very small, which turn black in
osmic, and make the nucleus hard to distinguish in surface
views. The best preparations are obtained by fixing with osmic,
which preserves the shape of the network, and then staining
with picrocarmine, which removes to a great extent the blacken-
ing.   The nuclei can then be readily seen as small spherical
clear bodies (fig. 9, a, b, c), usually with a nucleolus, which is
not, however, always visible.   In osmic preparations not
cleared with picrocarmine, the opacity of the cell makes it
almost impossible to see the nucleus (fig. 11, a and b).   In pre-
parations fixed in absolute alcohol the nuclei show up well
after staining, but the network seems to shrink a little.   From
these larger nodes radiate out the fine strands composing the
network.   Each strand is composed of a very fine core of jelly
coated by a delicate prolongation from a granular nodal cell.
The smaller, usually triangular nodes are, as already stated,
simply formed by the confluence of the fine strand making up
the network, and have the same structure.   The meshes of
the network are approximately equal in size in different mem-
branes; in large oscula there are more openings, in smaller
ones fewer.

To resume, then, this membrane may be described as a delicate network composed of two layers of cells with a minute quantity of jelly between them. The inner layer becomes directly continuous with the layer of collared epithelium composing the endoderm of the sponge. I have not observed with certainty any form of cell intermediate between the flattened cell of the sieve membrane and the columnar collared endoderm cell. The round cells often seen at the junction of the two, as in fig. 13, appear to me to be ordinary collared cells cut obliquely. Similar appearances can be seen in any spot where the section is not accurately radial to the wall of the tube. The outer layer of the sieve membrane becomes similarly continuous with the ectoderm. To discuss the morphology of this sieve membrane it is necessary to know the homologies of the layer composing it. Three alternatives are possible; either the inner layer is endoderm and the outer ectoderm; or both layers are ectoderm; or both are endoderm. I think the third hypothesis may be dismissed at once, and that it lies between the first two. The question could only be solved satisfactorily by a study of the development of the membrane, which I have not been able to make, but I believe certain facts point very strongly to the first hypothesis being true, i. e. to the inner layer of the membrane being composed of endoderm, the outer of ectoderm. In the first place, in a growing colony of this sponge, there are two ways in which a new osculum may be formed. The first way is by actual division of an osculum into two. Fig. 2 represents two oscula, recently formed, I have no doubt in this way. It is a process similar to that described and figured by Schulze in Farrea occa,[1] a sponge which grows in a manner very similar to this Ascetta. The second way in which an osculum could arise would be by a cæcal diverticulum growing out from the side of one of the sponge tubes, which after growing to a certain length, becomes perforated distally to form an osculum, very much in the manner in which new individuals are budded in a

[1] "Monograph of the Hexactinellida," ' "Challenger" Rep. Zool.,' vol. xxi, pl. lxxii, figs. 1—3.

Hydroid colony. This is, I believe, the commonest method of the formation of new oscula. In fact, I believe that in the other species of Leucosolenia mentioned above, it is the only way in which new oscula are formed, and explains the difference in the mode of growth between the two sponges. Blind diverticula of the tube composing the sponge occur very commonly, and I have observed many such. This method of formation of the osculum is essentially similar to the formation of the primitive osculum in the young sponge after the metamorphosis from the larval condition, when the osculum always arises as a breaking through of the gastral cavity to the exterior. Now in this mode of oscular formation a sieve membrane similar to that here described might be formed in one of two ways. The simplest method would be by the gastral cavity breaking through to the exterior in not one, but several places. The result would be the formation of a sieve-like membrane of two layers, in which the inner layer was endoderm, the outer ectoderm. Or secondly, after a simple wide opening was formed, a ring-like ingrowth of the margin of the osculum might take place towards the centre of the aperture, forming a kind of diaphragm, which, after becoming secondarily perforated, would form a sieve membrane in which both layers of cells might be ectoderm. I strongly believe myself, though I have no direct observations to support my views, that the sieve membrane here described arises in the first method suggested above, as a breaking through in several places of the gastral cavity to the exterior. In fig. 3 is represented one of a series of sections through an osculum, which, besides being of very small size (116 μ in diameter, vide supra), is further marked out by the fact that its membrane has no opening, either in this or in any of the sections, of which my series is perfectly complete. I have also another series of sections through a precisely similar osculum, in which there is no trace of an opening in the membrane. In the osculum represented in fig. 3, of which my sections are very satisfactorily preserved and stained, I noticed three other points. First, I could see no pores at all in the wall of the oscular tube.

Secondly, the collars of the endoderm-cells were compara-
tively low, not more than one third the height of the cell, while
in cells from other parts of the sponge in the same section the
collars were more than half the height of the cell, (fig. 4).
Thirdly, in the membrane itself, the cells composing it were
less granular and opaque, appearing more protoplasmic, with
very distinct nuclei. The first two of these points makes it
probable that the osculum was not in full functional activity;
the third point shows that the cells were in a more primitive
and less differentiated condition. Here then is just such an
osculum as one would expect to find on the hypothesis that the
sieve membrane arises as a breaking through of the gastric
cavity to the exterior in several places and that the inner layer
of cells composing it is endoderm, derived by flattening out
of the collared endoderm-cells, while the outer layer is simi-
larly ectoderm. Unfortunately I have observed no other inter-
mediate stage. A curious point is the projection above the
membrane of the wall of the sponge, forming the funnel-shaped
expansion mentioned above. Here I may refer to Schulze's
well-known figures of the young Sycondra raphanus.[1]
These figures represent the young Sycon in an Ascon stage, and
one might say that here we had a transitory Leucosolenia, with
an osculum covered by a sieve membrane with only a single
perforation. Round the edge of the osculum a fringe of
spicules projects up. As one knows that projecting spicules
in sponges are not really naked, one can easily imagine how
from such a condition a rim like that in Ascetta could be
formed.

The conclusion is, then, that the sieve membrane we are here
concerned with is formed by the gastral cavity breaking
through to the exterior in several places during development,
and that its inner layer of cells is endoderm, the outer layer

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[1] 'Zeitschr. f. wiss. Zool.,' xxxi (1878), Taf. xix, figs. 12, 13; or see
Vosmaer, 'Porifera' (Bronn's Thierreich), Taf. xxxi, figs. 9, 10; Balfour,
'Comp. Embr.'

From the condition figured by Schulze might easily arise either a sieve
membrane or an oscular sphincter, or an " Oscular-membran."

ectoderm—a conclusion which, it must be confessed, is still in need of further developmental facts to raise it above the rank of a probable hypothesis. If now we go further afield, and try to find something with which to compare it, we are at once struck by the great resemblance it presents, in many points, to the sieve plates of Euplectella, Holascus, and Hyalonema, among Hexactinellida. In fact, Schulze's figure[1] of Euplectella suberea, Wyv. Thomson, looks at first sight almost as if it had been drawn from a preparation of my Ascetta. But of course there is an enormous difference between the two, not only in size, but in structure, since the membrane of Euplectella is mostly made up of spicules. In the sieve membrane of Ascetta, however, there is a thin layer of jelly between the two layers, and it is not very difficult to imagine how this layer might be invaded by scleroblasts, and come to contain spicules. It is evident that, if the gastral cavity and osculum of Ascetta were to grow to the size of that of Euplectella, a support of spicules would be necessary for the sieve membrane, and doubtless would be acquired. If this homology between the oscular sieve membrane and plate of Ascetta and Euplectella respectively be true, it would show that the osculum of Euplectella is a true osculum, and its gastral cavity a true gastral cavity,[2] since it can hardly be

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[1] Schulze, 'Monograph of Hexactinellida,' pl. v, fig. 1.

[2] Apart from any considerations about the oscular sieve plate there can hardly be any doubt that the internal cavity of a sac-like Hexactinellid is a true gastral cavity, especially if one considers the young forms figured by Schulze on pl. liii, and also on pl. lxii, fig. 5, of his beautiful monograph. Schulze has further shown in the clearest manner how this simple gastral cavity may become modified. "By the expansion of the upper oscular margin many species acquire a funnel-like shape. A further widening and flattening leads to the formation of a flat saucer-like body, while a more unilateral growth results in an ear or shell-like form, . . . or even in certain circumstances in a simple perpendicular plate-like form." "If the outer margin of a stalked or originally cup-shaped sponge becomes folded outwards and downwards through great development of the median portion, a fungoid form arises. . . . In this way, then, as the gastral cavity and osculum have thus been lost, what was originally the internal gastral has become the upper and outer surface, so that the water enters the body from below and escapes

doubted that the osculum and gastral cavity of Ascetta are such. But it is a far cry from Leucosolenia to Euplectella, and no amount of similarity in structural relations will

again from the upper surface," and so on (Schulze, ' Mon. of Hexact.,' pp. 21, 22). What then must be our astonishment, after reading these words, to find Von Lendenfeld, in his ' Monograph of the Horny Sponges,' quietly putting down the gastral cavities of Hexactinellids as preoscular spaces, or even as pseudoscular, a name which he applies to entrances to the inhalent system (l. c., p. 739)! "In the tubular Euplectella aspergillum and in allied forms the central cavity, considering the wall of such a tube homologous to the lamella of the cup-shaped or irregular flattened forms, appears as a preoscular tube, so that here also there are no proper exhalent canals [I must confess to finding some difficulty in understanding this conclusion]. Thus, roughly speaking, all Hexactinellids are lamellæ, and the exhalent canal system is represented by a continuous cavity pervaded by lamellæ. The chambers open into one side of it, and the oscula are situated in the opposite wall " (l. c., p. 717). And this from an author who can only have obtained such knowledge of the Hexactinellids as he possesses from reading Schulze's monograph, and who has not a particle of evidence to support his view! nor, indeed, does he attempt to give us any. One more instance of Dr. von Lendenfeld's homologies may well be discussed here, since it concerns the oscular sieve-plate. On p. 720 of his monograph of the ' Horny Sponges ' we find it stated in the description of Dendrilla cavernosa that "the terminal parts—pseudoscula—are covered over by fine sieves with circular pores 0·2 mm. wide, which can be entirely closed at the will of the sponge;" and on p. 758 of the same work we find that Dr. von Lendenfeld " does not hesitate to compare it [" the cribriform membrane which is stretched over the wide terminal pseudoscula of Dendrilla cavernosa "] directly to the terminal sieve of Euplectella aspergillum." This sounds very well; but if we now turn to the more detailed description of this sieve in Dendrilla cavernosa given in Dr. von Lendenfeld's " Studies on Sponges and the Vestibule of Dendrilla cavernosa " (' Proc. Linn. Soc. New South Wales,' vol. x, 1886, pp. 557—561), we learn that "the cavity covered by the pore sieve is a pseudogaster, no oscula are found in its surface; it is a vestibule belonging to the inhalent system " (p. 557). Consequently the gastral cavity of Euplectella belongs to the inhalent canal system ; but the chambers of Euplectella open, directly or indirectly, towards this gastral cavity, which leads to the astounding result that the chambers of Euplectella are turned the wrong way ! A new kind of inversion of the layers ! I am sure it is quite unnecessary to comment further on these fantastic and utterly groundless theories, or to point out the flagrant contradictions in which this author lands himself.

prove absolutely a true homology between the two structures. I should prefer to look upon them as " homoplastic " merely ; as agreeing in anatomical relations, and perhaps also in their development and mode of origin, but not as genetically connected. In Calcarea I know of no structure which can be strictly homologised with this membrane, though I think it probable that the " Mundhaut" described by Haeckel, as well as the oscular sphincters, not unfrequent in Calcarea, may be so homologous. Here in Naples I have examined very carefully, by various methods, numerous oscula of Leucosolenia primordialis, but can find no trace of anything resembling the sieve membrane of Leucosolenia coriacea; neither could I in Leucosolenia botryoides.

There is another point about this membrane which is not without importance. Leucosolenia coriacea is a sponge which usually occurs totally devoid of oscula.[1] In Bowerbank's ' Monograph of British Spongiadæ,' vol. ii, p. 35, we read, " Dr. Johnston in treating of this sponge says, ' There are no fæcal orifices.' I have carefully examined a considerable number of specimens with a microscopic power of 160, but have been unable to detect any of the mouths of the cloaca, and attribute this failure to the habit of the animal of closing these orifices at the approach of danger, or while in a state of inaction ; and the total absence of internal defensive spicula would seem to indicate the existence of such a power for its protection from its enemies." Haeckel (' Kalkschwämme,' vol. ii, p. 25) writes, " Die bisherigen Beobachter dieses Kalkschwammes haben allerdings fast ausnahmslos nur eine Hauptform derselben beschrieben, nämlich den mundlosen Stock (Auloplegma coriaceum, figs. 27—33)." This " lipostomy " is of common occurrence in many sponges, and according to Haeckel is always found in some Calcarea, e. g.

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[1] Ciard found at Wimereux Tarrus, Auloplegma, and Ascometra forms; see ' Bull. Scient. de France et de la Belgique, xxii (1890), part 1, p. 70. But Topsent ("Contributions à l'étude des Clionides," ' Arch. de Zool. expér. et gén.,' v bis, mém. iv) found at Luc only Auloplegma forms.

Ascetta clathrus, Ascaltis cerebrum, &c. Haeckel discusses it at some length, and comes to the conclusion—rightly, I think—that it is "durch Anpassung erworben" ('Kalkschwämme,' Bd. i, pp. 209, 270). He also brings into the discussion the sieve-plate of Euplectella speciosa. I think it is not without interest to find that, in a form most commonly lipostomous, the oscula when present are partially closed by a sieve membrane. Were the opening in this membrane to close up, and the inner layer of cells to become collared cells like the remaining endoderm, lipostomy would be at once attained. The osculum in fig. 3 might be an osculum thus closed; but I believe not, on account of its extremely small size.

In its biological or physiological aspect, I believe this membrane is of use for keeping intruding animals out of the gastral cavity. We can distinguish three ways in which sponges do this. One way is by a fringe of projecting spicules round the osculum, which effectually prevents the ingress of animals into the gastral cavity, though not their exit; e. g. Sycon ciliatum. Other sponges have an iris-like membrane over the osculum, which is sensitive, and can be closed by a sphincter (see Haeckel, 'Kalkschwämme,' Bd. i, p. 266). A third method is by a sieve-plate, as here and in Euplectella, &c. Whether the sieves of Euplectella and of my Ascetta are homologous or not, I have little doubt they are analogous.

On the remaining histology of this sponge I have a few observations to record, though they are not as complete as I could wish. First of all as to the—

Endoderm.—In sections of the sponge hardened in osmic immediately after plucking it from its native haunts, the collar-cells present the appearance of a columnar epithelium composed of cells quite distinct from one another, each with a rounded slightly enlarged base stuck into the jelly and containing the nucleus, while the upper part of the cell is prolonged into a collar about half or two thirds the height of the cell. In the centre of the collar the flagellum is usually

distinct.   These cells are best studied either by maceration of carefully fixed portions of the sponge, or by tearing up and examining living pieces.   My macerations were done by fixing fresh pieces of the sponge in ½ per cent. osmic acid, and then soaking them in dilute glycerine, with or without previous treatment with ½ per cent. acetic acid or picro-carmine.   After a time the cells may easily be separated by taps on the cover-glass with a needle.

The shape of the cell in fresh specimens well hardened in osmic is elongated, as described above, but when teased up alive the cells become rounded (fig. 16), as is also the case in specimens hardened in Flemming's fluid (fig. 22).   In the living condition they may be observed to vary in shape, becoming, in fact, amoeboid, as has been often observed.   In living cells thus treated I have never been able to observe a trace of the collar.   It appears to become completely retracted. Here I am in partial agreement with Topsent, who says of the collar-cells of Cliona, "Collerettes et cils sont retractiles comme les pseudopods de cellules améboides."[1]   I have always found the flagella quite distinct, but never the collar, though I have no doubt the former are also retractile.   This retraction of the collar may be compared to that known to occur in Choanoflagellata.[2]   In macerations the collar is sometimes very low (fig. 17), but more often of considerable height (fig. 18).   It is usually somewhat crumpled, and appears as two fine lines on each side of the flagellum.   It is very seldom that the rim of the collar can be distinctly seen.   I never found the collars of neighbouring cells joined together to form a "Sollas's membrane;" I do not say this, however, as a disbeliever in the existence of this structure in some other sponges.   I have sections of H a l i c h o n d r i a   p a n i c e a in which I can see it

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[1] "Contributions à l'étude des Clionides," 'Arch. de Zool. expér. et gén.,' tome v bis (1887—1890), mém. iv, p. 27.

[2] Bütschli, "Protozoa" in Bronn's 'Thierreich,' Bd. ii, Mastigophora, p. 881: "Wie bekannt, ist der Kragen, wenigstens bei den Craspedomonadinen, ein gestaltsveränderliches Organ, ja er kann unter Umständen ganz eingezogen, und wiederum neugebildet werden."

distinctly, as described by Dendy.[1] The flagellum is usually
about twice the height of the cell, or more. In the cell in fig.
16, which I had under continuous observation for a long time,
I noticed that the flagellum made a somewhat slow stroke to
the right, followed immediately by a quick stroke to the left,
after which there was a pause, and then the two strokes were
repeated, and so on. Similar rhythmic pulsations were ob-
served in other cells, and doubtless have to do with causing a
current in a definite direction. I observed, also, that when a
foreign body came into contact with the end of the flagellum
so as to hinder its movements, it stimulated it to greater
activity, and caused it to lash about violently so as to become
almost invisible, until the foreign body was thrust away, or the
cell itself removed from it. When the flagellum was in full
swing it appeared thicker at the base than at the tip. But
during its pauses it was easy to see that this apparent disparity
in thickness was an optical delusion (owing, presumably, to
the tip moving more quickly than the base), and the flagellum
was really of precisely the same thickness throughout. Similar
observations have been made by Clark and Bütschli[2] on the
flagella of Flagellata, and these authors have shown that in
many species the flagellum, though depicted by earlier writers
as tapering towards the tip, was really quite cylindrical. Von
Lendenfeld, in his ' Monograph of the Horny Sponges'
(p. 777), draws the flagellum of S pongelia dis tans tapering
towards the tip, and at its base sending roots into the proto-
plasm. I do not wish, however, to cast any doubts (however
much I may feel them) on von Lendenfeld's picture, as it was
made from a sponge very far removed from Leucosolenia.
I never observed any continuation of the flagellum into the
interior of the cell. As to cell-contents, I was unable to
observe with certainty in the living condition the contractile
vacuoles often described. I saw a vacuole frequently, but could

[1] " Studies on the Comparative Anatomy of Sponges:" iv, " On the Flagel-
lated Chambers and Ova of Halichondria panicea," 'Quart. Journ.
Micr. Sci.,' Jan., 1891.

[2] Bütschli, " Protozoa " in Bronn's 'Thierreich,' Bd. ii, Mastigophora, p. 673.

not see it contract. But my observations are far from con-
clusive on this point. The nucleus was best seen in prepara-
tions stained with picro-carmine. In other preparations it was
often not visible on account of the opacity of the cell. It was
always spherical, lodged in the base of the cell, with a distinct
cell membrane and nucleolus. In the living condition it was
not visible. Besides vacuoles and a nucleus, the cells usually
contained pigment granules, which appeared as black specks,
both in the living condition and after osmic. They were
especially distinct in sections made from sponges hardened in
Flemming's fluid and stained with safranin (fig. 22), which
tinges them slightly. Sometimes there were very many granules,
sometimes none at all. In the living cell these granules were
observed to alter in position, but no regular direction of move-
ment was noticed. Only two instances were found of what
appeared to be collar-cells in division. In one of these (fig.
18, e) no collars could be seen ; the flagella were very short, and
no nuclei were visible (after staining with picro-carmine), but
the whole cell appeared pinkish—from which, perhaps, it might
be inferred that the nuclei were undergoing karyokinesis. In
the other instance (fig. 18, d) two normal collar-cells were
found joined at their base. The only noteworthy point about
them was that each nucleus had two nucleoli, not in its centre
but at opposite ends, as shown in the figure. I have never
found, either in sections or maceration, any process connect-
ing neighbouring collar-cells to one another, though I feel sure
they must exist.

Mesoderm.—In macerations I found the spicule to have a
nucleus at the extremity of each ray, and a fourth at the con-
fluence of the rays (figs. 15 a, b). In some sections, especially
those prepared with Flemming's fluid followed by safranin,
numerous irregular cells of a yellowish colour, and containing
a number of black granules but no distinct nuclei, were to
be found close under the collar-cells (fig. 22). These are
perhaps identical with Topsent's " cellules digestives." I
found no trace of muscular, elastic, or other special cells ; nor
does there appear to be much occasion for them.

Ectoderm.—I am more in the dark about this layer than about any other part of the sponge. All that was made out with certainty was as follows :—In surface views, after fixing with osmic and removal of the spicules with acid, one sees at intervals patches of black granules (figs. 7 and 8). Some of these are isolated, but most are continuous with the wall of a pore, which also appears granular, either all round or only on one side (see fig. 8 ; in fig. 7 there are no pores). In maceration the cells appear as seen in fig. 20, where the clear space doubtless represents the nucleus. Fig. 21 represents a single pore macerated out. Each pore appears to be formed of a single ectoderm-cell. In sections the superficial ectoderm-cells sometimes appear as little heaps of granules (fig. 22), but are in general very hard to make out. On the other hand, the granular walls of the pores are easily seen. It is possible that in the fully formed sponge wall the ectoderm-cells may to a certain extent degenerate into a cuticle-like structure.[1] I may

[1] Topsent, in his most important memoir on the Clionidæ already cited, finds that in these forms, the most contractile sponges known, and also in the genera Reniera and Halichondria, it is the flattened epithelial cells of the ectoderm and endoderm clothing the canals, sphincters, &c., that are the real contractile elements (pp. 24—27, 96, &c., and p. 122). He terms them " cellules de revêtement," and states the following important fact: " Sur ces points [i. e. on the papillæ of Cliona] les cellules de revêtement n'auraient pas de raison d'exercer leur contractilité; aussi y sont elles remplacées constamment par une cuticule incolore d'apparence anhiste " (p. 26). The author then cites the observations of Kölliker and Schulze as to a similar cuticle in Cacospongia and Euspongia, &c. Now in our Ascetta the only parts where contraction could take place is round the pores and round the openings of the sieve membrane, and it is precisely in these places that I find the cellular nature of the " cellules de revêtement " most distinct. In other places it has, I believe, degenerated, as Topsent finds. This author's work was not known to me when I made my observations. Metschnikoff, in his "Anatomisches über Ascetta " ('Spongiologische Studien ii, Zeitschr. f. wiss. Zool.,' xxxii, pp. 358—362, Taf. xxii), finds the ectoderm very distinct in A. blanca, primordialis, and clathrus; and is astonished that earlier writers (Haeckel, Oscar, Schmidt, and Keller) could not see it. He figures distinct epithelial cells (figs. 9—11), but these are from Olynthus forms, in which he finds the ectoderm " noch stärker und auffallender ausgebildet als bei den obenerwähnten Tarrusformen." It seems to me probable that

add that neither in the living nor preserved condition could I observe any trace of the flagella described by von Lendenfeld as present in all calcareous sponges (' Descriptive Catalogue of Sponges in the Australian Museum,' Introduction, p. vi), and for my part I feel very sceptical as to their existence.

A few words in conclusion as to the mode of life of this sponge. I have only found it between tide-marks in rock pools. So delicate a creature appears to shelter itself from the violence of the waves by creeping down in amongst the stems of the calcareous algæ, &c. I have frequently pulled up a colony of Styela and found a rich ramification of the sponge tubes round the bases of the Ascidians. Hence it is a very inconspicuous form, and one may look into a pool full of it without observing any until one pulls up and examines the seaweeds closely. In one pool I found it specially abundant. This was a pothole about a foot and a half across, and about two feet deep; the side towards the sea was concave and over-hanging, the other sides more or less straight, but all round there was a thick growth of weeds. Here, well sheltered from the waves which must beat over the spot four times in the twenty-four hours, the sponge attained the greatest develop-ment I have seen. Nearly every bit of weed was clothed with it, while under the overhanging seaward edge the sponge came out, as it were, from the seaweeds and formed the large masses mentioned at the commencement of this paper.[1] This pool

Metschnikoff had before him a young and undifferentiated form of the ecto-derm. It would be of great interest to trace the modifications of the ectoderm during the growth of a single form.

[1] In Sorrento my friend Dr. Otto Maas and I observed a very similar pothole at the entrance to one of the grottes, situated just at the water's edge where the waves were constantly beating into it. This pothole also had the side towards the sea deeply concave, and what made it specially interesting was the fact that under the overhanging edge were growing a large number of specimens of a calcareous sponge (probably Sycandra raphanus; at any rate a Sycon) of various sizes, while the other sides of the pool were bare, except for a few weeds. Dr. Maas has observed, in his valuable paper on the development of Spongilla (' Zeitschr. f. wiss. Zool.,' Bd. l, 1890), that the larvæ avoid the light; and other sponge embryologists have observed the same thing so often, that one is justified, I think, in putting down this habit of

was most convenient to me, as I was able at low tide to go down with a bottle of hardening reagents and preserve pieces of the sponge perfectly fresh from its native habitat, or in five minutes I could have fresh living pieces on the laboratory table. It was remarkable that I found no specimens of Leucosolenia botryoides in this pool, though it occurred in the very next pool to it, both species growing side by side.

Before concluding it is my pleasant duty to express my best thanks to the committee of the British Association for appointing me to one of their tables in the Marine Biological Association Laboratory during three months of the summer of 1890, when I made the bulk of these observations ; to my friend Mr. Walter Garstang, then Assistant to the Director of the Marine Biological Association, for a great deal of help and advice ; and, finally, to the delegates of the Common University Fund, Oxford, for appointing me to the Oxford table in the Naples Zoological Station, where I have been able to add a few observations to those made at Plymouth.

ADDENDUM.

Since the above was written two works have appeared by Dendy which I must notice. In his ' Organisation and Classification of the Calcarea Homocœla, with Descriptions of the Victorian Species ' (" A Monograph of the Victorian Sponges," part 1, ' Transactions of the Royal Society of Victoria,' vol. iii, part 1, 1891), Dendy finds " that the ectoderm of the Homocœla agrees precisely with what Schulze has described for Sycandra raphanus, and what he himself found and described for Grantia labyrinthica. Except in very well-

avoiding the light and seeking the darkest places as a well-marked characteristic of sponge larvæ. Now, in potholes like the two here mentioned, the larvæ by avoiding the light would either settle under the overhanging edge, or down amongst the seaweeds. On the other hand, those that did not do so would inevitably be smashed by the waves. Hence it is probable, I think, that with more extended observations one could give a simple explanation of this habit, as well as a beautiful instance of the power of natural selection in producing it.

preserved specimens it is a matter of great difficulty to make out satisfactorily the structure of the ectodermal epithelium." In carefully prepared sections "the ectoderm generally appears . . . as a delicate but sharp outline, with a moniliform or beaded appearance, due to the swelling caused by the presence of the nucleus in the centre of each cell." The author then figures and describes the ectoderm in Leucosolenia Wilsoni, n. sp. I never saw such distinct cells in L. coriacea, but I have already expressed my opinion above that the structure of the ectoderm varies at different ages. The collar-cells figured by the author from Leucosolenia proxima on pl. viii, figs. 3 and 4, are unlike any I have seen in L. coriacea, but it is possible that they vary in shape in different species. The mesodermal network described by the author in the gastral cavities of L. proxima and Wilsoni (p. 13, pl. viii, figs. 1 and 2) is very remarkable, and reminds one at first sight of the sieve membrane described here ; but from the description of the network there can be no real comparison between it and my sieve membrane.

In his " Preliminary Account of Synute pulchella, a New Genus and Species of Calcareous Sponges " (Dendy, ' Proceedings of the Royal Society of Victoria,' March 12th, 1891), the author mentions that " each gastral cavity has also a single large well-developed diaphragm situate just within the osculum " (p. 3). As far as one can judge from the description, this diaphragm may well be homologous with my sieve membrane in Leucosolenia coriacea.

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EXPLANATION OF PLATES X and XI.

Illustrating Mr. Edward A. Minchin's "Note on a Sieve-like Membrane across the Oscula of a Species of Leucosolenia, with some Observations on the Histology of the Sponge."

ect. Ectoderm. *end.* Collared endoderm. *s. m.* Sieve membrane. *G. c.* Gastral cavity. *p.* Pores. *n.* Nuclei. *sp. sh.* spicule sheath.

PLATE X.

Fig. 1.—Vertical section of an oscular tube. Osmic and hæmatoxylin. Zeiss, oc. 2, obj. B.

Fig. 2.—Vertical section of an osculum which has just divided into two. Osmic, picro-carmine, and hæmatoxylin. Zeiss, oc. 4, obj. D. $\frac{152}{1}$.

Fig. 3.—Vertical section of a small oscular tube, in which the sieve membrane was without any opening. There are no pores to be seen in the walls of the tube, and the collars of the endoderm-cells are very low. Osmic, picro-carmine, and hæmatoxylin. Zeiss, oc. 4, obj. D. $\frac{210}{1}$.

Fig. 4.—Collar-cells from another part of the same section as Fig. 3, to show the normal height of the collars relatively to the cells. Zeiss, oc. 8, obj. F, apochrom. $\frac{285}{1}$.

Fig. 5.—Side view of an oscular tube, seen as a transparent object. Osmic half per cent., acetic acid half per cent., glycerine. Zeiss, oc. 2, obj. B.

Fig. 6.—A portion of the sieve membrane of the osculum drawn in Fig. 5, dissected out. Zeiss, oc. 8, obj. F, apochrom. $\frac{400}{1}$.

Fig. 7.—A portion of the wall of the oscular tube above the sieve membrane, from the osculum drawn in Fig. 5. Zeiss, oc. 8, obj. F, apochrom.

Fig. 8.—A portion of the wall of the sponge from below the sieve membrane. From the osculum drawn in Fig. 5. The spicule sheaths are barely visible on account of the opacity of the preparation. Zeiss, oc. 8, obj. F, apochrom.

Fig. 9.—Portions of the sieve membrane of an osculum, dissected out to show the nuclei in the nodes. Osmic half per cent., picro-carmine, glycerine. Zeiss, oc. 8, obj. F, apochrom.

Fig. 10.—View of an osculum looked at from above, showing the sieve membrane, partly hidden by the edge of the aperture, stretched out over the oscular opening. Absolute alcohol, hæmatoxylin. Zeiss, oc. 2, obj. B. $\frac{90}{1}$.

Fig. 10a.—The whole of the sieve membrane of the same specimen. Zeiss oc. 4, obj. C.

PLATE XI.

FIGS. 11*a* and *b*.—Two consecutive sections taken across an osculum transversely but slightly obliquely. Osmic 1 per cent., hæmatoxylin. Zeiss, oc. 8, obj. F, apochrom. $\frac{370}{1}$.

FIGS. 12*a* and *b* (Plate X).—Two sections of nodes of the sieve membrane, showing nuclei. $\frac{258}{1}$.

FIG. 13 (Plate X).—Section of sieve membrane, passing throughi ts origin from the wall of the osculum and four nodes. Zeiss, oc. 8, obj. F, apochrom, $\frac{487}{1}$.

FIGS. 14*a*, *b*, and *c*.—Three spicules. $\frac{310}{1}$.

FIGS. 15*a* and *b*.—Two spicules macerated out in glycerine, showing nuclei. Osmic half per cent, picro-carmine, glycerine.

FIG. 16.—Two drawings of an isolated living collar-cell. Zeiss, oc. 8, obj. F, apochrom.

FIG. 17.—A collar-cell from a preparation made with half per cent. osmic, and macerated out in glycerine.

FIG. 18.—Collar-cells from a preparation fixed with half per cent. osmic, stained in picro-carmine, and macerated out with weak glycerine. Two of them are dividing. Zeiss, oc. 8, obj. F, apochrom.

FIG. 19.—Collar-cells macerated out from the same preparation as Figs. 5—8. Zeiss, oc. 8, obj. F, apochrom. $\frac{365}{1}$.

FIG. 20.—An ectoderm-cell. Osmic half per cent, glycerine.

FIG. 21.—A pore macerated out, from the same preparation as Figs. 9*a*, *b*, *c*.

FIG. 22.—Section of the wall of the sponge. Flemming's fluid, hæmatoxylin, safranin. Zeiss, oc. 4, obj. D.

The Development of the Oviduct in the Frog.

By

Ernest W. MacBride, B.A.(Cantab.), B.Sc.(Lond.),
Scholar of St. John's College, and Scholar of the University of London.

With Plates XII and XIII.

THE investigation which forms the subject of the present memoir was commenced in July, 1890, at Mr. Sedgwick's suggestion. A collection of tadpoles, which were preserved in the Morphological Laboratory, Cambridge, was first examined without result. None of them showed any trace of the oviduct, though in some cases both pairs of limbs were well developed. Through the kindness of Mr. Sedgwick I was enabled to procure a considerable number of Frogs, in which the tail was being absorbed or had just vanished; and amongst these I found all the necessary stages for my work.

The material was treated in some cases with corrosive sublimate, and in other cases with Perenyi's fluid, and preserved in alcohol. Both methods seemed to yield equally good results.

The stain employed was borax carmine; twenty-four hours in this I found to be quite sufficient, a longer period produced too deep a coloration. It was found in all cases necessary to decalcify the specimens; this was effected by HNO_3 of strengths varying from 1 to 10 per cent. Strange to say no difference in the result was observable, whatever the strength employed. I should recommend about 3 per cent. for twenty-four hours.

Material preserved in picric acid proved to be utterly unsuitable.

The method employed was exclusively that of series of transverse sections. The air contained in the lungs proved a great nuisance; and it was found advisable to open the frogs and remove the greater part of the gut, almost all the liver, the heart, and most of the lungs. Care had, however, to be exercised not to disturb the proximal parts of the liver and lungs, owing to the peculiar relation of the abdominal opening of the oviduct to them. (Vide infra.)

It will be best to commence the account of the development with a short summary of previous work on the subject. Für-brenger's classic research on the Salamander ('Entwicke-lungsgeschichte der Amphibien-Niere,' Heidelberg, 1877), is well known. He asserts that the oviduct arises as an involution of the peritoneal epithelium behind the pronephros, which applies itself to the Wolffian duct, and grows back as a rod of cells split off from the latter (quoted in 'Balfour's Comparative Embryology,' p. 710). Hoffmann, in the 'Zeitschrift für wissenschaftliche Zoologie,' Bd. xliv, 1886, gives a somewhat similar account for the Frog. He finds that the oviduct arises from a patch of modified peritoneum just ventral to the third and now only remaining nephrostome of the pronephros. This patch, he says, is dorsally involuted to form a groove, open below. Ventrally it is prolonged downwards and outwards over the surface of the pronephros, and even beyond it for a short distance. It is distinguished from the unmodified epithelium by being highly columnar. The part of the Wolffian duct in front of the mesonephros, the lumen of which is at this stage much reduced, then separates itself from the degenerating pronephros, and splits into two rods of cells. The dorsal of these is continuous with the Wolffian duct behind, and the ventral one applies itself in front to this involuted patch of peritoneum, and forms the first rudiment of the oviduct. But the oviduct does not grow back in continuity with the Wolffian duct. On the contrary, it enters into close connection with a longitudinal strip of peritoneum which lies to the outer border of the kidney, and consists of columnar epithelium. Hoffmann states that the hinder portion of the duct

is formed from these cells, though whether by involution to form a groove, or by proliferation he could not determine. In the meantime the groove which formed the ostium of the duct, and which was originally dorsal, has become prolonged ventrally round the base of the lung. It closes, forming a canal, which now opens ventrally. Later this ventral extension to some extent atrophies leaving the ostium in a dorsal position.

Marshall and Bles in a paper dealing chiefly with the development of the Frog's kidney, published in the second volume of the 'Studies from the Biological Laboratories of the Owens College,' incidentally mention the oviduct. They confirm Hoffmann in his account of the ventral displacement of the ostium; but fail to observe any splitting of the front part of the Wolffian duct. They state that the hind end of the duct is, in the first year, a solid rod of cells, but fail to notice any relation of this rod to the peritoneum.

My own observations differ from those of Hoffmann in several important particulars. It will be convenient to describe first the origin and fate of the abdominal opening, and then that of the rest of the duct.

The Abdominal Funnel.—In a tadpole in which the hind limbs alone are visible, I find three nephrostomes in the head kidney, the cells of which bear long flagella pointing inwards, as Hoffmann has pointed out. The first of these is situated some way in front of the glomerulus, the second immediately in front of the attachment of the glomerulus, and the third immediately behind it. Before proceeding to describe subsequent stages it will be convenient to say a word as to the criteria of age employed in the case of these animals. The determination of their age is no easy matter; they vary a good deal in size, and just before absorbing their tails are considerably larger than when their metamorphosis is quite complete. I have used concurrent measurements of the whole length and the length of the tail, and obtained from them indications which are approximately true; but even so one is often disap-

pointed in expecting a stage of development corresponding to these external marks.

My next stage is the earliest in which any trace of the duct is visible. The animal is truly a frog, having lost the peculiar tadpole mouth, and having both pairs of limbs well developed, but the tail is just commencing to be absorbed (total length 38 mm., tail 21 mm., in one specimen ; in another, total length, 19 mm., tail 4 mm.; both show same features). One nephrostome only remains (*pn. f.*, fig. 1) ; but, as this is situated some considerable distance in front of the glomerulus, it must be regarded as the first, and not the third, of the preceding stage. Immediately ventral to it there is a groove in the peritoneum open below, the epithelium of which is highly columnar, and stains much more deeply than that of the nephrostome (*mf.*, fig. 1). This columnar epithelium is continued out over the surface of the pronephros and beyond it, as Hoffmann has described (*C. p. e.*, fig. 1). This groove, traced backwards for fifteen sections, becomes a canal, which, after two sections, ends in a solid thickening of the peritoneum (*Md.*, fig. 2). In the next stage only a rudiment of the tail remains, but the condition of the funnel is exactly the same, except that there is an indication of the groove running ventrally, described by Hoffmann (*Vg.*, fig. 3). In both the preceding stages traces of the almost atrophied pronephric tubules are still visible (*pn. t.*, figs. 2 and 3).

In the next two stages the tail has completely vanished. In the first of these the groove formed from the columnar epithelium is very distinct, but still open (fig. 5, *b*). In the second it has closed to form a canal, so that the abdominal opening is carried ventralwards round the base of the lung, and at the same time backwards. It does not, therefore, appear as before in the same section as the groove, which latter is now an additional piece of the oviduct ; consequently it appears in the same section as a more posterior part of the duct (*Md.* and *mf.*, fig. 6 *b*). It is closely attached to the ventral surface of the liver, and runs back along it, gradually shallowing out. Traces also of the fimbriæ of the adult orifice can be seen. I have examined

the opening in the adult with great care, but I have not been able to detect any important difference between its position and that of the funnel in the stage just described.   The abdominal opening in a fully-developed frog is not situated dorsally but at the base of the lung.   It is fimbriated, and these fimbriæ are continued lying in a groove over the ventral surface of the liver round the base of the lung.   It is true that the orifice appears to be extended more dorso-ventrally and less antero-posteriorly than before, but that is all.

The Duct behind the funnel.—In the stage to which figs. 1 and 2 belong we can trace a distinct strip of columnar epithelium on the outer border of the kidney, the whole way to the cloaca, just as Hoffmann describes ($C. p. e.$, figs. 7 and 9). This is really more distinct in sections than appears in the figures, owing to its staining properties.   It is continuous in front with the epithelium, from which the funnel is formed.   In the next stage, viz. that in which there is still a slight rudiment of a tail, a good part of the groove which forms the oviducal funnel has been converted into a canal, which ends in a thickening of the peritoneum as before.   Continuous with the latter a slight protuberance (fig. 8, $Md.$) may now be traced running back along the outer side of a peculiar cord of lymphoid tissue, which joins pro- and meso-nephros, and which later extends at the expense of both.   This protuberance occurs on the line of the modified epithelium referred to.   The Wolffian duct takes no part in the formation; this can be clearly seen from an inspection of figs. 7 and 8.   The Wolffian duct in front of the kidney is now reduced to a cord of brown degenerate cells, from which no new development such as Hoffmann describes could be expected.   This thickening or protuberance above described seems to me to originate entirely from the peritoneum; the cells, or rather closely-packed nuclei composing it, have the shape of, and stain exactly similarly to, those forming the modified strip of columnar epithelium.   However, it is clearly impossible to speak with absolute certainty on such a point as the origin of cells in a compact cellular mass.   In

later stages this protuberance travels back along the line marked out for it by the columnar cells. It nowhere comes into contact with the Wolffian duct, nor do the cells of the latter exhibit the least sign of proliferation, except in one point. This is behind the kidney. A representation is given of it in fig. 13. But the facts that it appears early, and before the front part of the protuberance is formed, and that it is strongly developed in young males (of which I have two series of sections), seem to show that it is connected with the formation of the vesicula seminalis. Fig. 13 (which is from a female) shows, indeed, that a distinction can be clearly drawn between the cells from the proliferating Wolffian duct and those forming the protuberance. If my supposition as to the meaning of these appearances be correct, it will be a new case of a characteristically male organ being formed in a rudimentary condition in the female. The two males I examined gave me the impression that in them only the funnel of the oviduct, and a small solid rudiment joining the cloaca, extending but a short distance forward, are formed, but they were too young to allow one to speak with any certainty on the point.

The lumen of the oviduct appears only in my latest stages, viz. in frogs of 15—17 mm. long (figs. 10 and 11). It appears here and there in patches, first in front of, and then behind, the kidney—sometimes one cell, sometimes two deep beneath the surface. In fig. 10 it appears certainly to be bounded towards the outside only by peritoneum. Once formed it sinks in, and the whole protuberance bearing it enlarges, and the indifferent tissue constituting the apparent forward prolongation of the kidney all passes in. There is a certain amount of this lymphoid tissue also along the edge of the kidney, as may be seen from figs. 10 and 11. Finally, the attachment of the whole rod of tissue is drawn out into a mesentery. Excessive growth and folding are all that are required to bring about the adult condition.

Fig. 14 shows the manner in which the oviduct, whilst still solid, joins the cloaca. It is noteworthy that it is separated from the Wolffian duct by one or two layers of flattened cells.

Summary and Conclusions.

The principal new points contained in this memoir are as follows:

(1). The fact that the oviduct arises opposite the first and not the third nephrostome of the pronephros.   (2). The fact that the whole of the duct, and not merely the posterior half as Hoffmann supposed, arises for connection with a strip of modified peritoneum, apparently by proliferation from it, and entirely independently of the Wolffian duct.   (3). The fact that the lumen appears quite close to the peritoneum, and in patches.   To these ought to be added that an examination of the series from which fig. 5 and fig. 10 are taken, leads to the conclusion that the formation of the duct along the outside of the kidney takes place not regularly from before backwards, but more or less simultaneously, often being better marked behind than in front.

The idea which this investigation has suggested to me is, that the whole oviduct is in the Frog a production of the peritoneum.   It is not a little remarkable that Wiedersheim has published a paper on the development of the Crocodile and Turtle ('Uber die Entwickelung des Urinogenitalappartes bei Krokodilen and Schildkröten.   Anatomischer Anzeiger, 1890'), in which he says with regard to these animals, " Der Müllersche gang ist nichts anderes als ein Derivat des Cölom-epithels sowie des subperitoneal Bildungsgewebes."

The view which is still taught in text books is that the oviduct is to be regarded as a part of the primitive pronepheric duct, and its funnel as one of the pronepheric funnels.   This view is founded on its development in Elasmobranchs, where the anterior knob of the segmental duct is, or was, regarded as a rudiment of the pronephros, its knob afterwards becoming the funnel of the oviduct.   Now in only three groups of animals is a true pronephros known, viz. Marsipobranchs, Tectibranchs (Ganoids and Teleostei), and Amphibia.   To these, according to Wiedersheim, must be added Crocodiles,

in which he has described a pronephros. Marsipobranchs have no oviducts. Lepidosteus is the only Tectibranch in which anything is known of the development of the generative duct, and in it Balfour found reason to believe that the oviduct was formed by a prolongation of the same fold which enclosed the cavity of the ovary, whilst in Amphibians and Crocodiles we have already seen that the pronephros is quite independent of the duct. So that we are at any rate justified in saying that whatever the oviduct may be, it has nothing to do with the pronephros.

If one might be allowed to found phylogenetic conclusions on the facts above related, I would be inclined to say that in the phyla of Ganoids, Amphibia, and Reptiles, which groups probably approximately represent stages in the actual line of descent of Vertebrates, the oviduct is derived from a dorsal groove in the peritoneum, just as the portion of it extending round the base of the lung is shown to be by actual development in Amphibia.

I have, in conclusion, to express my warmest thanks to Mr. Sedgwick for his advice and assistance through the whole course of my work.

EXPLANATION OF PLATES XII and XIII.

Illustrating Mr. Ernest W. MacBride's paper on "The Development of the Oviduct in the Frog."

List of Reference Letters.

Br. Brain. *C. Musc.* Circular muscles of the cloaca. *C. p. e.* Columnar peritoneal epithelium. *f. p. e.* Flat peritoneal epithelium. *gl.* Glomerulus. *In.* Integument. *K.* Kidney. *K. t.* Kidney tubule. *L.* Lung. *Liv.* Liver. *L. Musc.* Longitudinal muscle of cloaca. *lt.* Quasi-embryonic tissue of degenerate pronephros, and front part of mesonephros. *Ly.* Lymph space. *M.* Cut end of mesentery. *Md.* oviduct. *mf.* Oviducal funnel. *Nch.* Notochord. *œ.* Œsophagus. *Ov.* Ovary. *pn. f.* Pronephric funnel. *pn. t.* Pronephric tubule. *R.* Cloaca. *Sp. ch.* Spinal cord. *V. g.* Ventral groove

running ventralwards from primitive funnel of the oviduct. *W. d.* Wolffian duct (ureter). *Wds.* Thickening of Wolffian duct.

Fig. 1.—Transverse section of Frog 38 mm. long, including a tail of 17 mm. Section is through level of hind brain. *pn. f.* Pronephric funnel. *m. f.* Oviducal funnel.

Fig. 2.—Transverse section of the same series as Fig. 1 a little further back. *md.* Solid rudiment of oviduct.

Fig. 3.—Transverse section of a Frog 13 mm. long, including a tail of 1 mm. at same level as Fig. 1. *pn. t.* degenerate pronephric tubules. *v. g.* Groove which runs ventralwards from primitive dorsal funnel of the oviduct.

Fig. 4 is Fig. 3 under a lower power, showing the whole section. *In.* Integument. *Ly.* subintegumentary lymph space.

Figs. 5 *a, b, c.*—Three sections from a series cut from a Frog 13·5 mm. long with no tail. Sections about same level as all preceding. *Ly.* is lymphatic space above lung.

Figs. 6 *a* and *b.*—Two sections from a series cut from a Frog 14·5 mm. long with no tail. Same level as Fig. 5. *Liv.* Liver.

Fig. 7.—Transverse section through the same Frog as Fig. 1 behind pronephros, but in front of kidney. *Wd.* Wolffian duct.

Fig. 8.—Transverse section through the same Frog as Fig. 3 in same place as Fig. 7.

Fig. 9.—Transverse section through the kidney of same Frog as Fig. 5. *kt.* One of the kidney tubules. *Ly.* Lymphatic space above kidney.

Fig. 10.—Transverse section through the kidney of a Frog 16 mm. long with no tail.

Fig. 11.—Transverse section through the kidney of a Frog 16·5 mm. long with no tail.

Fig. 12 is a section of the same Frog as Fig. 10 under a lower power. *M.* Mesentery. *Ov.* Ovary. *Ly.* Lymphatic dorsal to the kidney.

Fig. 13.—Transverse section behind kidney of same Frog as Fig. 11. *Wds.* Thickening of the wall of the Wolffian duct apparently a rudiment of vesicula seminalis.

Fig. 14.—Transverse section through the cloaca of same Frog as Fig. 11. *Md.* Oviducts, solid rudiments, one touching the cloacal wall.

On the Nauplius Eye persisting in some Decapods.

By

Margaret Robinson,
University College, London.

With Plate XIV.

THE nauplius eye has been described as persisting in Schizo-pods, but I have heard of no description of it in Decapods, though Dr. Paul Mayer mentions having seen it in an adult Palæmonætes ('Carcinologische Mitt. Naples Mitt.,' vol. ii). Professor Weldon noticed it in Palæmon, and suggested that I should work it out either in that or in some other member of the Carididæ. This is the only family in which I know of it as persisting. I have used Palæmon serratus, Virbius varians, and Pandalus annulicornis.

External Appearance.—On removing the rostrum from a fresh specimen the median eye can be seen as a black speck lying in the centre of the triangle formed by the brain and the stalks of the lateral eyes. In preserved specimens it is not quite so obvious. I have made a dissection of the brain of Pandalus annulicornis (fig. 13), in order to confirm the results obtained from sections.

On removing the rostrum one sees the speck lying in the centre of the above-mentioned triangle. There is chitin covering the brain, and this chitin is connected by means of a

membrane with that over the eye-stalks.   At the anterior apex
of the triangle there is a dorsal elevation, which is also covered
by chitin.   This elevation, looked at from above, makes the
triangle appear to have a knob for its anterior apex.

On removing the chitin it is found to be lined by a thin
layer of ectoderm.   On removing the dorsal part of this ecto-
derm and a small piece of the anterior portion of the brain,
the eye can be seen lying in a blood-space just dorsal to the
brain.   It has the appearance of a black χ, which is slung
on to the ectoderm by two slender threads which swell out in
the concavities formed by the arms of the χ, and then narrow
again as they approximate to each other.   Surrounding the
posterior ends of these threads is a semicircle of pigment
corpuscles lying in the brain.

Minute Anatomy.—I have cut transverse and horizontal
sections through the brain in Palæmon serratus and
Virbius varians, and have had at my disposal some made
by Professor Weldon through that of Pandalus.   The figures
were made from transverse sections through the brain of
Virbius (No. 3), being the most anterior section.   In cutting
the sections the chitin covering the ectoderm broke away, so
that it is not shown in the drawings except in the posterior
dorsal groove.

By means of sections it is seen that the χ consists of two
large pigment cells, and that the supporting strings consist in
their narrow anterior diverging parts of ectoderm, while their
swollen parts in the concavities of the χ and the narrow stalk
consist respectively of the thickened and narrow ends of
club-shaped nerve-end cells.

Ectoderm.—In the anterior sections the ectoderm lying
dorsal to the median eye is thickened.   I do not think that
this thickening is due to obliquity in the sections, as it is present
invariably, but rather that we are here cutting through the
knob-like prominence mentioned above.   Going further back
an inward prolongation of the ectoderm is seen on either side.
These prolongations are seen to converge ventrally as we trace
them backwards.   They join the thickened ends of the nerve-

end cells. This arrangement obtains also in Palæmon and Pandalus.

Pigment Cells.—These are two large cells placed side by side so as to form an Υ. In Pandalus and Virbius the divergence of the arms is slight, but in Palæmon it is considerable (thus Χ).

By partially depigmenting the sections with chlorine dissolved in alcohol it can be seen that the nuclei lie in the ventral halves of the cells and far forward.

Nerve-end Cells.—In transverse sections the nerve-end cells appear to be arranged in three groups, of which two are lateral and one ventral to the pigment cells.

On counting the nuclei I find that there are almost invariably six cells in each of the lateral groups, and two in the median ventral group, making fourteen in all. The cells in the ventral group are continuous on either side with those of the lateral groups, and through them are connected with the ectoderm anteriorly, while posteriorly all three groups are continuous with the "Punkt-substanz" of the brain. In all the genera which I have examined the cells are club-shaped in horizontal section.

Muscles.—On the outside of each band of supporting ectoderm, between that and the outermost ectoderm, is a band of muscles. I have traced these muscles back to where the circumœsophageal commissures are given off from the brain, and have seen that they slope inwards and ventralwards from the outside ectoderm to the centre of the brain. I think that their function must be to help in casting the chitin from the brain at the moult.

I can find no trace of a refractive body within the eye, but in the posterior sections where anteriorly the ectoderm is thickened there is a groove filled with chitin lying dorsal to the eye.

Comparison with the Eye of Branchipus.

As regards the shape of the pigment cells and the position of their nuclei and the arrangement of the nerve-end cells in three groups, this eye exactly resembles the median eye of Branchipus as described by Claus ('Wiener Arb.,' vi, p. 59), but I cannot find any trace of a connective-tissue sheath like the one he describes as investing the eye. In his description he says nothing about a blood-space, nor does he mention any connection between the nerve-end cells and the ectoderm.

This eye has been observed in adult specimens of the following members of the Carididæ :

Palæmon serratus.	Crangon fasciatus.
Palæmon squilla.	Pandalus annulicornis.
Hippolyte Cranchii.	Pandalus brevirostris by Pro-
Virbius varians.	fessor Weldon, and in Palæmo-
Crangon vulgaris.	nætes varians by Dr. Paul Mayer.

Before concluding, I must express my thanks to Professor Weldon for the assistance and advice he has most generously given me.

EXPLANATION OF PLATE XIV,

Illustrating Miss Robinson's paper "On the Nauplius Eye persisting in some Decapods."

ec. Ectoderm. *p.* Pigment cells. *n. c.* Nerve-end cells. *m.* Muscle. *g. br.* Ganglion cells of brain. *p. s.* Punkt-substanz of brain. *bl. c.* Blood-corpuscles. *bl. s.* Blood-space. *ch.* Chitin. *l. e.* Lateral eye. *pig. corp.* Pigment corpuscles in brain. *comm.* Circumœsophageal commissures. *ant.* 1. First antenna. *ant.* 2. Second antenna. *sq.* Square of second antenna. *r.* Rostrum. *c. t.* Connective tissue.

Fig. 1.—Head of Hippolyte Cranchii with rostrum removed.

Fig. 2.—Dissection of brain of Pandalus annulicornis.

Fig. 3.—First transverse section through region of median eye in Virbius varians showing ingrowth of ectoderm.

Fig. 4.—Second ditto.

Fig. 5.—Third ditto.

Fig. 6.—Fourth ditto.

Fig. 7.—Fifth ditto.

Fig. 8.—Eighth ditto.

Fig. 9.—Tenth ditto.

Fig. 10.—Twelfth ditto.

Fig. 11.—Sixteenth ditto.

Fig. 12.—Nineteenth ditto.

Fig. 13.—Twenty-first ditto.

Fig. 14.—Diagrammatic horizontal section through brain of Virbius varians.

Notes on Two Acanthodriloid Earthworms from New Zealand.

By

W. Blaxland Benham, D.Sc.Lond.,

Aldrichian Demonstrator of Anatomy in the University of Oxford.

With Plates XV and XVI.

In the summer of last year (1890) I received, through the kindness of Professor Jeffrey Bell, of the British Museum, some earthworms for identification. They were collected by Mr. Vaughan Jennings in New Zealand, at Manngatua, south of Dunedin, who generously allowed me to retain them for further examination. I regret that so much delay has occurred in preparing my report for publication, for I almost completed my observations on them during last summer vacation, but continuous work since September last prevented me putting certain finishing touches to the descriptions and figures till August of the present year (1891).

I found in the bottle three worms and some fragments. One of these worms turns out to be Beddard's N e o d r i l u s m o n o - c y s t i s; the other two belong to a new genus.

N e o d r i l u s m o n o c y s t i s, Beddard.

A single specimen[1] of a worm, found by Mr. Beddard amongst species of A c a n t h o d r i l u s from New Zealand, differed from

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[1] It is a curious thing that in so many cases only one specimen of a worm is found, which serves as a type of a new genus ; and it has occurred to me, sometimes, that some of these may be hybrids or abnormalities, which from some weakness have failed to reach their burrows, just as we find the common earthworm on the surface of the ground after rains, &c., in a weakly or dying condition.

this genus mainly in the possession of only one pair of spermathecæ, and one pair of male pores (atriopores) on Segment xvii, instead of the usual two pairs of each of these structures.   Beddard[1] himself appears to have had some doubts as to the generic value of these features, for he states that " it is possible that this supposed new genus Neodrilus is really an Acanthodrilus in which the posterior pair of male generative pores (atriopores), together with their glands, have not yet been developed." In my recent contribution in this Journal, " An Attempt to classify Earthworms," I placed Neodrilus as a " doubtful genus."

I am now, however, in a position to confirm and extend Mr. Beddard's brief description, although, like him, I had but one specimen, the anterior end of which after dissection I cut into a series of sections.

The general colour of the worm, in spirit, is sienna brown ; its length about two and three quarter inches, with an average diameter of a quarter of an inch, but rather wider anteriorly to the clitellum.   In shape it appears cylindrical, but is nearly square in transverse section, owing to the position of the four bundles of chætæ.   There are 155 segments.   The chætæ are in the usual four couples, which are equidistant, so that the outer couples lie on the dorsal surface (fig. 1).   The clitellum, which is rather lighter in colour than the neighbouring region, occupies the Somites xiv to xix.   In addition, the dorsal surface of Somite xiii is also glandular, as is also the ventral surface of Somites xviii and xix where the glandular epidermis extends across the middle line.[2]

In Somite xvii, in a line with the ventral couple of chætæ on each side, is a conspicuous rounded papilla, the apex of which is distinctly lighter than the base.   At the apex of each of these papillæ is the pore of the prostate (fig. 2, *prst. p.*).

On Somite xviii, immediately behind those of Somite xvii, are two other papillæ, much less conspicuous and more depressed.   These bear the apertures of the sperm-ducts.   The

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[1] 'Proc. Roy. Soc. Edinb.,' 1887, vol. xiv, p. 158.
[2] Beddard states that the clitellum occupies Somites xiii to xvii.

lips are tumid, and the pores are connected with those on Segment XVII by grooves (fig. 2).

Beddard describes much the same sort of arrangement, but states that the grooves from the pores on Somite XVII extend on to Somite XIX. I do not find this extension of the grooves backwards. The chætæ appear to be absent on Segments XVII and XVIII.

The prostomium is completely dovetailed into the buccal segment (fig. 1).

The most noticeable external feature, besides the position of the male pores, is the alternation of the nephridiopores (figs. 1 and 2), a fact which Beddard mentioned, and which is known in Acanthrodrilus dissimilis, and other species. This "alternation" is not quite regular, as will be seen from the following table, the pore being in front of sometimes the 4th, sometimes the 3rd or 2nd chæta, counting the most ventral chæta as the 1st :

Somite.					Left side.		Right side.
II.	Nephridiopore in front of		.	.	4th chæta	. .	4th chæta.
III.	,,	,,	.	.	3rd ,,	. .	Worm injured.
IV.	,,	,,	.	.	3rd ,,	. .	injured.
V.	,,	,,	.	.	3rd ,,	. .	3rd chæta.
VI.	,,	,,	.	.	3rd ,,	. .	3rd ,,
VII.	,,	,,	.	.	3rd ,,	. .	2nd ,,
VIII.	,,	,,	.	.	2nd ,,	. .	?
IX.	,,	,,	.	.	2nd ,,	. .	2nd ,,
X.	,,	,,	.	.	3rd ,,	. .	3rd ,,
XI.	,,	,,	.	.	? ,,	. .	2nd ,,
XII.	,,	,,	.	.	3rd ,,	. .	3rd ,,
XIII.	,,	,,	.	.	2nd ,,	. .	2nd ,,
XIV.	,,	,,	.	.	3rd ,,	. .	3rd ,,

And so on in regular alternation. A similar alternation in the position of the nephridiopores exists in the new genus described below.

INTERNAL ANATOMY.

There are no specially strong septa (fig. 3). The pharynx is covered by masses of glandular cells, as in the common earthworm, and resembling those which I described and figured

in Eminodrilus.[1] The gizzard lies in Somite VI. There are no calciferous glands, no typhlosole, nor intestinal cæca. The tubular intestine is slightly dilated in Somites VII, VIII, and enters the sacculated intestine in Somite XV.

Lateral hearts occur in Somites XI, XII, XIII, XIV, the first three being moniliform.

In accordance with the variations in positions of their pores, the nephridia lie latero-ventrally or latero-dorsally (figs. 4, 5), and exhibit slight differences in the mode of convolution of the tube and in other points. The inner (ventral) nephridium has a large cæcal portion to the bladder directed dorsally, while that of the dorsal nephridium is but feebly developed, the nephridiopore being placed near the end of the bladder (fig. 5). There is no nephridial network nor communication between the nephridia.

THE GENITAL SYSTEM.

The testes and ovaries have the usual position. There are three pairs of sperm-sacs in Somites X, XI, XII, that of the left side in Somite XII being much smaller than the right one (fig. 3).

The prostates are enormous, and there is but one pair of them. Each consists of two parts (figs. 3 and 6),—a soft, more or less cylindrical portion, which is white, glandular, constricted, and convoluted (*prst.*); and a short, narrow, shining duct (*pe. d.*). The latter opens externally in Somite XVII, and passes backwards into Somite XIX, where it joins the glandular region; this passes still further back to the 24th somite, and then bends forwards on itself, its free end (*a*) lying in Somite XVII, at the side of the duct.[2]

Lying above the prostate is a sac containing a bundle of penial chætæ (*pe. s.*) nearly as long as the prostate—that is about a quarter of an inch (figs. 6, 7). This penial sac opens

[1] "Report on an Earthworm from Equatorial Africa," 'Journ. Roy. Micr. Soc.,' 1891, Pl. 1V, fig. 12.

[2] The shading in fig. 3 does not indicate satisfactorily the convolutions of the prostate.

externally on Somite XVII (fig. 8), by a separate pore from that of the prostate duct. Its wall is muscular, and its posterior extremity appears to be fixed to the body-wall by muscles. The sac contains four long, delicate penial chætæ, of which two are much longer than the others (fig. 9). The longer chætæ are sharply bent near their free ends, and this bent part is gracefully curved to a very fine point (fig. 9, b.). The shorter chætæ are not bent. The ends of all the chætæ are beset by numerous minute spines or asperities (fig. 10), and there appears to be a groove along one side, so that they are somewhat triangular in section.

When the prostate is removed or turned aside the sperm-duct can be traced along the body-wall to its pore in Somite XVIII (fig. 7). There is no trace of a prostate in Segment XIX.

The microscopic structure of the prostate is similar to that in Acanthodrilus, Deinodrilus, and other genera. The wall is formed of pyriform "clitellar" cells, amongst which a few blood-vessels occur. The whole is surrounded by a flat cœlomic epithelium (fig. 11, a). The penial duct is lined by short columnar cells, surrounded by a very thick muscular coat (fig. 12), around which are blood-vessels and cœlomic epithelium.

There is only one pair of spermathecæ in Somite VIII; at first sight, however, there appears to be two pairs of these organs (fig. 3) ; but the structures lying in Somite VII are in reality the appendices or diverticula of the spermathecæ, so common amongst earthworms. Mr. Beddard has already remarked upon the large size of the diverticulum in this species. On one side it is actually larger than the body of the spermatheca itself.

The spermatheca (fig. 13, s) is ovoid, with rather a thick wall, as can be seen in the left sac in fig. 3, where its dorsal wall has been removed. The sac communicates with the exterior by a short, thick, muscular duct, which resembles in its structure that of the prostate. This duct opens in the anterior region of the segment, and is joined close to the body-wall by the diverticulum. This is a sac of smaller or larger size than the main sac, roughly cylindrical and truncated, and contains spermatozoa.

Such is the coarse anatomy of Neodrilus monocystis; and though I have done little more than confirm Mr. Beddard, I have thought it worth while to illustrate its anatomy.

The present specimen was found by Mr. Jennings among " rotten wood and mould."

Plagiochæta punctata, n. gen., n. sp.

The other worms have a very distinctive shape and colouring, and have numerous chætæ arranged in a nearly complete circle.[1] The generic name[2] refers to the fact that the chætæ are always seen crossing the body, whether this is looked at above, below, or laterally. At a first glance, indeed, one might mistake them for species of Perichæta (or Megascolex); but the possession of two pairs of tubular prostates, amongst other anatomical characters, removes them from that genus.

Of this worm there were two complete and mature (?) specimens, and two halves of a third, but immature, worm.

The size and shape of the worm are rather characteristic (fig. 14); it is 1·6 inches long by 0·5 inch across its widest part, which is posterior to the clitellum, so that it is, compared to its length, very broad; it is composed of eighty-nine segments.

As will be seen from the sketch, which is twice the size of a spirit specimen, the anterior and posterior extremities are nearly similar, tapering gradually to the mouth and anus respectively. The clitellum forms, so to speak, a waist; but that it is in life narrower than the neighbouring anterior and posterior regions is, I think, doubtful.

The body is depressed, so that the dorso-ventral diameter is only about half the lateral breadth (Pl. XVI, fig. 17). A groove runs along the dorsal and ventral mid-line, which is probably due to shrinkage.

The general colour reminds one somewhat of Michaelsen's

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[1] This suggested the name Cyclochæta for the genus, but Mr. Hatchett Jackson has employed the term for a genus of ciliate Protozoa.

[2] 'Jahrb. d. Hamburg wissensch. Anstalten,' vi, 1889.

Acanthodrilus (Mandane) pictus,[1] but is a richer choco-
late-brown, with rows of white spots[2] (hence the specific name),
which are more noticeable dorsally than ventrally.

A narrow dark band (fig. 14), due to absence of spots, extends
along the whole length of the body in the middle line dorsally
and ventrally, but becomes rather less marked towards the
hinder end of the worm, and less noticeable ventrally.   This
dark band occupies the groove above mentioned.

The spots, which, though white in the spirit specimens, are
perhaps only "light" in life, are very regularly arranged,
forming transverse and longitudinal rows; and in each spot
is embedded a couple of chætæ (Pl. XVI, fig. 18).   On the
majority of segments there are twenty-five to twenty-seven of
these white spots, diminishing in the anterior segments to
fifteen.

The median unspotted grooves are about three times as
wide as the distance between any two white spots of a circle.
At the hinder end of the body, for about a quarter of the total
length of the animal, the spots are much closer together,
giving the appearance of a series of white rings.   The ventral
surface is much lighter than the dorsal and lateral surfaces,
and the spots less noticeable.

The clitellum, though apparently not quite fully deve-
loped, is perfectly distinct; it occupies Somites xiv to xvii
and part of xviii.   It is lighter in colour than the rest of the
body, both dorsally and ventrally; and the white spots are
quite distinct dorsally.   As already mentioned, the body in the
clitellar region is rather constricted.

The chætæ are numerous in each segment; they occur
always in couples, each couple being embedded in a white
spot (fig. 18).   There are usually fifty to fifty-four chætæ in
each somite, i. e. twenty-five to twenty-seven couples.   They
decrease in number in the more anterior somites, being forty-

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[1] Πλαγιος, transverse.

[2] In a letter to me Mr. Jennings states that "while living they are lighter
in colour than ordinary earthworms, and have a peculiar surface marking.
Except contraction, they seem to undergo little change in spirit."

eight in Somite IV, forty-four in Somite III, and thirty in Somite II.

The chætæ are simple and sigmoid, without markings, and each is ·036 mm. in length.

The prostomium is completely dovetailed (fig. 15) into Somite I. I can find no dorsal pores.

The pores of the two pairs of spermathecæ are fairly large, and provided with white lips. Each pair lies in a median ventral depression, which is situated on the anterior margin of Somite VIII and of Somite IX; each pore being in line with the ventralmost couple of chætæ (Pl. XVI, fig. 16, *spth.*).

The spermiducal pores are not to be seen externally, but by means of sections I find them situated in Somite XVIII, in front of the ventralmost couple of chætæ.

There is an oval depression on the ventral surface of Somites XVII, XVIII, and XIX, bounded laterally by a distinct ridge. Within this depression, on each of the Somites XVII and XIX, is a pair of small transverse slit-like pores—those of the pro-states—which lie in a line with the other genital pores (fig. 16, *prst.*).

The chætæ of these two segments appear to be absent, but in reality they are especially long, and are retracted into the body-cavity—in fact, have become modified for copulatory purposes.

The oviducal pores have the usual position on Segment XIV, though they are not visible externally.

The nephridiopores (*ne. o.*) are very distinct, even on the clitellum; they appear as light spots, which are larger than those around the couples of chætæ. These pores alternate in position (fig. 16), as in Neodrilus, &c.; this alternation being nearly but not quite regular, and sometimes not symmetrical. In the following table the position of these pores in regard to the chætiferous spots is shown. The first spot is the ventralmost, so that the fourth spot is latero-ventral (fig. 17^4), the tenth is latero-dorsal—in fact, lies on the dorsal surface, and is the fourth spot from the mid-dorsal line (fig. 17^{10}).

Specimen A.

No. of Somite.					Right side.			Left side.
III.	Nephridiopore in front of	. .	10th spot	. .	10th spot.
IV.	,,	,,	. .	10th	,,	. .	10th	,,
V.	,,	,,	. .	5th	,,	. .	10th	,,
VI.	,,	,,	. .	5th	,,	. .	10th	,,
VII.	,,	,,	. .	4th	,,	. .	10th	,,
VIII.	,,	,,	. .	10th	,,	. .	4th	,,
IX.	,,	,,	. .	11th	,,	. .	10th	,,
X.	,,	,,	. .	4th	,,	. .	4th	,,
XI.	,,	,,	. .	10th	,,	. .	10th	,,
XII.	,,	,,	. .	4th	,,	. .	4th	,,

And so on, in a regular way, with a few exceptions, i. e. on the even-numbered somites the nephridiopores are dorsal, on the odd-numbered they are ventral till the twenty-third somite, when there are the following divergences from the rule.

No. of Somite.					Right side.			Left side.
XXIII.	Nephridiopore in front of	. .	10th spot	. .	9th spot.
XXVIII.	,,	,,	. .	5th	,,	. .	4th	,,
XXX.	,,	,,	. .	4th	,,	. .	4th	,,
XXXI.	,,	,,	. .	9th	,,	. .	4th	,,
XXXII.	,,	,,	. .	5th	,,	. .	4th	,,
XXXIV.	,,	,,	. .	5th	,,	. .	4th	,,
XXXVI.	,,	,,	. .	5th	,,	. .	4th	,,
XXXVIII.	,,	,,	. .	5th	,,	. .	4th	,,
XL.	,,	,,	. .	5th	,,	. .	4th	,,
XLIII.	,,	,,	. .	10th	,,	. .	9th	,,
XLIV.	,,	,,	. .	5th	,,	. .	4th	,,
LIV.	,,	,,	. .	5th	,,	. .	4th	,,
LIX & LX.	,,	,,	. .	9th	,,	. .	9th	,,

In Specimen B.

No. of Somite.			Right.			Left.
III, IV, & V.	Nephridiopore in line with		10th spot	. .	10th spot.
VI.	„	„	9th „	. .	9th „
VII.	„	„	10th „	. .	10th „
VIII.	„	„	4th „	. .	4th „
IX.	„	„	9th „	. .	4th „
X.	„	„	4th „	. .	4th „
XI.	„	„	10th „	. .	4th „
XII. XIV, &c. }	„	„	10th „	. .	10th „
XIII.	„	„	9th „		
XV. XVII, &c. }	„	„	10th „		

In this specimen the variations from the normal position are fewer in the posterior region of the body than in specimen A.

The distances between the pores when normally situated are equal; that is to say, the space between right dorsal and left dorsal pores = space between dorsal and ventral pores = space between right ventral and left ventral pores. See fig. 17, where the position of the couples of chætæ are indicated by numerals.

It is so very generally taken for granted that the nephridiopores in any genus or species have a fixed relation to one of the couple of chætæ, that it is worth while to carefully note such deviations from the rule, as exhibited in this table, and in that for Neodrilus, and to bear in mind that even in Lumbricus and Allolotophora this pore is not invariably situated anteriorly to the ventral couple of chætæ. Claparède and Hering pointed this out some years back, and Borelli, in 1887, recurred to the matter,[1] and examined some half dozen of the common species of the family Lumbricidæ (*s. s.*) from this point of view. His results, which are not very detailed, show, however, that in some species, L. herculeus, for instance, nearly as many nephridiopores have not the "normal" position as have. In L. rubellus there are more

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[1] 'Boll. d. Mus. Zool. ed Anat. Comp. Torino.,' vol. ii, No. 27.

pores in an "abnormal" position than in the normal, i. e. in front of the ventral chætæ. The "abnormal" position is itself variable, the pore sometimes occurring in front of the dorsal chætæ, sometimes dorsal of these, and even near the mid-dorsal line. Borelli does not state whether there is any approach to an "alternation," or whether the variability is more marked posteriorly than anteriorly.

There is little doubt that this sort of irregularity is of more frequent occurrence in other genera than we are apt to think. We have, amongst recorded cases (besides Plutellus), Beddard's description of Acanthodrilus dissimilis[1] and Ac. rosæ,[2] &c., where the alternation is not regular; Bourne's Perionyx saltans,[3] where there are two rows of nephridiopores, usually regularly alternating, though he mentions certain variations; Fletcher's[4] "Cupptodrilus" mediterreus, with an irregular alternation, and Megascolides tasmanicus, where the pores are in a sinuous series.

The deviation from any fixed positions is especially interesting in these genera, such as Acanthodrilus, Megascolides, and Cryptodrilus, in which some species are in a plecto-nephric condition, for it seems to support the theory of Beddard and Baldwin Spencer that this condition is primitive, and that the network communicated with extensively many scattered pores, as in Perichæta, &c.; when the meganephric condition supervened, by aggregation and enlargement of some portion of the network, the pores enlarged, and the nephridia now opened, not at some fixed point, as has usually been presumed to be the case, but at any point on the surface of the segment, the relatively fixed portions being probably a later phenomenon, and its relation to the bundles of chætæ being due to some secondary causes, with which at present we are unacquainted.

[1] 'Proc. Zool. Soc.,' 1885, p. 822.
[2] This Journal, vol. xxx, p. 434.
[3] 'Proc. Zool. Soc.,' 1886.
[4] 'Proc. Linn. Soc.,' N.S.W., 1887, p. 601.

INTERNAL ANATOMY, Pl. XVI, fig. 19.

There are no especially strong septa (though those of xiii/xiv, xiv/xv, and xv/xvi are slightly thicker than the rest), nor any great displacement of organs.

The Alimentary Canal.—The pharynx, which occupies the Somites iii, iv, and part of v, is partially concealed by two pairs of lobulated masses, or " salivary glands" (*Sal.*), lying dorsally and laterally in Somites iv and v, the gland in the latter being bilobed. These are not modified nephridia, but consist of groups of large granular cells, deeply stained in borax carmine, and closely resembling the " salivary glands" of Eminodrilus,[1] &c.

The pharynx is internally divisible into a ventral cuticulated region, and a dorsally placed flattened diverticulum, laterally produced, and lined by tall ciliated cells, as in many other, perhaps all earthworms.

The œsophagus, which is not ciliated, is narrow and tubular : it passes backwards to the large sacculated intestine, which commences in Somite xvi.

The hinder region of the œsophagus in Somites x to xiii is highly vascular, and its wall thrown into folds; these become more definite in Somite xiv and give rise to a well-marked pair of calciferous glands. Each is a large sac, laterally placed, but extending dorsally and ventrally, so that the pair almost surround the œsophagus (Pl. XVI, fig. 20, *cal.*). Its structure calls for no remark.

There is no gizzard to be detected on dissection, but in Somite vi the wall of the gut is slightly more muscular than elsewhere, the coat being about twice the thickness of that of the neighbouring tract, instead of eight to ten times as thick, as in the case of the functional gizzard in most earthworms. The sacculated intestine commences in Somite xvi, where it suddenly dilates, to become about three times the width of the œsophagus. This character it retains in Somites xvii, xviii, and xix, beyond which it is spirally coiled (Pl. XVI, figs. 19, 21). This condition has been described in Didy-

[1] Benham, 'Journ. R. Micr. Soc.,' 1891, pl. iv, fig. 12.

mogaster by Fletcher;[1] and when I first examined the
present worm I believed that the spiral appearance was
merely due to contraction, causing the gut to bulge, first on
one side and then on the other.   But it is a true spiral, as
can be seen by dividing the worm either longitudinally or
transversely (Pl. XVI, figs. 17, 21).   There is no typhlosole,
nor are there any cæca, such as are found in most species of
Perichæta.

The nephridia are large, conspicuous (Pl. XVI, fig. 19),
and alternate in position in correspondence with the position
of the nephridiopores; the funnels, however, are always placed
below the gut, and in line; they do not share in the alter-
nation.   The nephridium (Pl. XVI, fig. 22) presents the usual
regions described by me for Lumbricus and other genera;[2]
the coils are arranged somewhat differently in the dorsal and
ventral series, and there is a dilated cæcal bladder, which is
longer in the dorsal nephridia than in those of the ventral
series.

The funnel has the same general structure as in Perichæta
malamaniensis—that is to say, there are no centripetal
marginal cells; but whereas in that species all the eight
cells are of the same size, in the present worm there is a
difference in size, and an increase in number of the marginals,
as in P. aspergillum.   I append two figures (fig. 23, a, b)
representing two consecutive sections through a funnel; and
from it there appears to be about sixteen marginals, which
decrease in size on each side of the funnel.   I am doubtful as
to the presence here of a "central cell,"[2] and can readily
believe that this is absent, as is the case in some Perichæta.

The first full-sized nephridium in the second segment is in
Somite III; but there is a pair of rudimentary structures which
are, I believe, degenerate nephridia.

That on the left is a fairly typical though very small
nephridium; the one on the right is much less developed.

This very minute organ only occupies some four or five

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[1] 'Proc. Linn. Soc.,' N.S.W., 1886.
[2] This Journal, vol. xxxii, p. 293.

sections in a longitudinal series; these are represented in Pl.
XVI, fig. 24, *a, b, c, d.*

A small pore on the anterior margin of Somite II, just out-
side the peripharyngeal nerve commissure, leads by a short
duct with a narrow lumen into a dilated portion (fig. 24, *b.*),
which is filled with a coagulum (*coag.*) ; from this " bladder "
a tract of cells (fig. 24, *c.*) leads backwards to a small coiled
tubule which appears to end blindly (fig. 24, *d.*).   I am unable
to detect a lumen in the tract of cells lying between this coil
and the " bladder."

On the opposite side of the body, occupying the same posi-
tion as this minute structure, which is absent on this side, I
find the small nephridium above mentioned ; so that I think
there can be little doubt as to the correctness of my interpre-
tation of this minute organ as a " vestigial" nephridium.   I
expected that these two small nephridial pores and ducts
would lead to " peptonephridia," but such is not the case;
there is no modification of the anterior nephridia other than
degeneration.

Genital System.

The two pairs of testes, and of spermiducal funnels, the
pair of ovaries and oviducts, are placed in the usual segments.
The ovary (Pl. XVI, fig. 25) is rather remarkable, in that
the ripe ova are not confined to the tip of the organ, but are
present along the whole of one side.   The ovary is attached to
the septum by only a small peduncle ; it is not pear-shaped, as
in Lumbricus, but slightly lobed, and more closely resem-
bling the testes in shape than is usually the case.   I am not
acquainted with any ovary amongst the Oligochæta with
exactly this form, though Horst has figured some curiously
shaped ones in Benhamia Schegelii,[1] where it is rosette-
shaped; B. Buttikiferi,[2] where it is somewhat like the
parent ovary.   The oviducts open close to ventralmost chætæ
of their respective sides, at the tip of very slight papillæ.

[1] 'Notes from the Leyden Museum,' 1887, pl. iv, fig. 7.
[2] Ditto, pl. v, fig. 6.

There are four pairs of lateral sperm-sacs (fig. 19, *sps.*), almost filling the Somites IX, X, XI, and XII, and hiding the œsophagus and the two median sperm-sacs which lie below the gut in Somites X, XI, and enclose the testes and ciliated rosettes.

There are two pairs of prostates, in Somites XVII and XIX respectively. Each prostate (figs. 19 and 26, *pro.*) is a soft, irregular body, but roughly cylindrical, terminating in a free, blunt point, placed dorsally. The free end is sharply curled upon itself; the prostate then gradually widens, and is slightly convoluted as it passes downwards at the side of the intestine towards the ventral wall of the body, just before reaching which its character suddenly changes, both in appearance and in structure. This "penial duct" (fig. 26), as I will call it, is much narrower than the glandular part or prostate, and is slightly curved as it penetrates the body-wall to open by the pores seen externally. In appearance, instead of the dull, whitish, uneven character presented by the prostate, the penial duct is smooth and glistening, due to the fairly thick layer of circular muscles which forms its wall (fig. 28). The histological structure of the prostate is similar to that of Neodrilus. In fact, these prostates are closely similar to those of Acanthodrilus and its allies, but are less compact in appearance.

In front of each prostate is a sac containing long penial chætæ (figs. 26, 29). Each sac enters the body-wall immediately in front of the penial duct, but, as can be seen in section (Pl. XVI, fig. 29), crosses the duct in the thickness of the body-wall, and opens independently to the exterior just behind, and rather to the outer side of it. Each sac is in reality double, and each subdivision contains a couple of chætæ, of which one is small (*b*) and no doubt in reserve, the other being longer (*a*). There appears to be no particular marking or other peculiarity about these penial chætæ. The ordinary chetæ are absent in these somites, and the penial chætus occupy their places, as is usually the case in Acanthodrilidæ.

There are two pairs of spermathecæ (figs. 19, 30), each consisting of two parts, as is so generally the case in the

prostatiferous worms, viz. (1) a subglobular sac with a short,
thick, muscular duct opening externally on the anterior edge
of the segment; and (2) a conspicuous, curved, cylindrical,
glistening diverticulum or appendix, which lies in the segment
in front of that containing the main sac, with the neck of
which it communicates. The spermathecæ lie in Somites VIII
and IX, so that the appendices are in Somites VII and VIII
respectively.

Repeated observations have shown that in spermathecæ
formed on this plan the sac does not contain spermatozoa,
which are, as Beddard was the first to point out, found only
in the diverticulum. This is, on the whole, true in this case
too, although in sections through the organs I detected a few
spermatozoa in the neck of one of the spermathecæ (Pl. XVI,
fig. 31).

The microscopic structure of the spermatheca is illustrated
by figs. 31 and 32. The former, which is combined from two
or three of a series of longitudinal sections, represents the
general relation of sac to appendix, and the position of the
cells which are shown enlarged in fig. 32. The lining cells of
the sac itself are very much taller than those of the appendix,
and of slightly different structure when fully developed.

The appendix has, outside the epithelium, a thick muscular
coat (mus.). This is absent in the wall of sac, except around
the neck. The muscle fibres are continuous with the circular
coat (c. mus.) of the body-wall, which is here very strongly
developed.

The cavity of the sac contains some granular matter (x),
which is produced by the epithelial cells (fig. 32 b), together
with a few spermatozoa (spoa.). Each cell is columnar, with an
oval nucleus near its inner end. The rest of the cell is occupied
by a finely spherular or granular secretion (x). It appears that
when the cells have reached a maximum of activity the free
end projects into the lumen of the sac and discharges its
contents, leaving then a vacuole or space near this free end
(as at m.). It then probably shrinks, and gradually elongates
again as renewed activity commences. The nucleus is only

feebly stained, and the contents of the cell remain unstained in borax carmine.

The epithelium of the appendix (figs. 31 and 32 *c*) consists of tall columnar cells, not quite so high as those of the sac. The outer free end of each cell is flat, and the inner end of all the cells in my sections is bent at a slight angle to the body of the cells, and at this point lies the nucleus. This is smaller than that in the epithelium of the sac, and stains much more deeply : it is surrounded by a deepy staining almost homogeneous protoplasm. The rest of the cell is occupied by similar granules to those found in the epithelial cells of the sac.

The appendix is filled with spermatozoa, which are to some extent regularly arranged, with the heads towards the walls, and the tails directed centrally. The heads appear embedded in or attached to the epithelium (figs. 31, 32, *d*). The cells in these regions are much shorter, less than half the length of those just described ; and have no definite outer surface : otherwise they resemble the above-mentioned cells. Intermediate conditions between *c* and *d* can be seen in the sections.

It is probable that the cells of the appendix do not simply discharge their product, but disintegrate, leaving only the nucleus and homogeneous protoplasm behind, which will come into renewed activity, and give rise to more secretion during the next breeding season.

The spermatozoa, as seen in the section, are arranged as if they formed a sperm-rope, but the heads and tails are reversed when compared with sperm-rope of Tubificidæ, &c., which may perhaps be due to the fact that there would be no opportunity for the use of the tails for locomotion of the sperm-rope in earthworms.

We are totally ignorant of the purpose of this sperm-rope in earthworms, and it is a matter of some uncertainty as to where they are formed in Lumbricus, &c. ; but it seems, from the above observations and others made by Beddard that, in those genera which have " an appendix " to the spermatheca, the sperm-ropes or spermatophores are formed in that portion of the organ.

Among the bases of these cells are a few roundish nuclei without any stained protoplasm. These perhaps help in the renewal of the epithelium.

The duct (at *a.*, fig. 31), common to the sac and the appendix, is lined by a layer containing two sorts of cells, the nuclei of which differ in shape, size, and capacity for stains, and in position in the cell. In sections two rows of nuclei are evident, a deeper and a more superficial series. The nuclei nearer the base of the epithelium are more deeply stained, and about half the length of those of the outer row, which themselves are so placed that their outer ends are at about the middle of the cells (see fig. 32 *a*). The latter, longer nuclei belong to narrow cells filled with granules similar to those in other parts of the spermatheca. The shorter nuclei, which alternate with and lie more deeply than the others, belong to clear cells without any very definite contents. The alternation of the cells is quite evident with a high power, and is very regular. The spaces between the granular cells are not artificial, as the figure might suggest, but are occupied by excessively fine protoplasmic (?) contents. These two sorts of cells are, no doubt, modifications of the ordinary columnar and goblet cells of the epidermis.

Locality.—These worms were found, with the specimen of Neodrilus, amongst mould and decaying wood at Manngatua, south of Dunedin.

AFFINITIES OF PLAGIOCHÆTA.

This new genus apparently has affinities in two directions viz. with Perichæta and with Acanthodrilus. The circle of chætæ is known in Perionyx and Perichæta (including Megascolex and Beddard's sub-genera); but in no form do I find any mention of the chætæ being in couples, in the way in which they are arranged in the present genus. Again, the position of the genital pores marks it off from Perichæta, where both spermiducal and spermathecal pores are much further removed from the ventral mid-line than is here the case, while the male pores are usually on papillæ.

These pores in Perionyx are, relatively to the chætæ, in about the same position as in Plagiochæta, but the male pores and oviducal pores are in depressions.

The position of the clitellum and its appearance are more Perichætous than Acanthodriloid.

With Perichæta Stuarti it is in agreement in the position of the genital pores and in the character of the prostates ; but this species has no appendix to its spermatheca, and differs from Plagiochæta in other points.

The alternation of the nephridiopores calls to mind Perionyx saltans of Bourne, Acanthodrilus novæ-zealandiæ, and other species, and Plutellus of Perrier; but with none of these does it agree in the essential generic characters.

The genus differs considerably in its internal anatomy from Perichæta, viz. in the character of the nephridium, in the shape of the prostates, in the position of these, and the possession of two pairs of them; in the presence of calciferous glands, in the absence of gizzard and of intestinal cæca ; in the arrangement of sperm-sacs, in the shape of the ovary, &c.[1]

With Acanthodrilus it agrees in the position of the male pores and in the character of the prostates, and with some species in the arrangement of the nephridia, and in the presence of sacs containing penial chætæ.   But Plagiochæta differs from Acanthodrilus, and its allies Trigaster, Benhamia, and Deinodrilus, in the form of the clitellum, in the arrangement of the chætæ, in the absence of a gizzard (cf., however, Ac. Georgianus, Mich.).

Mr. Beddard has pointed out that the interesting genus Deinodrilus presents a somewhat similar mixture of Perichætoid and Acanthodriloid characters; and it seems to me that Plagiochæta more closely resembles in certain points each of these genera.   In its locomotor organs it is closely

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[1] P. intermedia, Beddard, and P. Bakeri, Fletcher, however, possess two pairs of prostates, which are cylindrical or " acanthodriloid " in the former species.

connected apparently with Perichæta, while its genital and alimentary systems are on the Acanthodriloid plan.

The genus appears to support the view as to a Perichætous condition which I have put forward in my recent paper on the "Classification of Earthworms" (p. 275), viz. that it is not primitive, but secondary, and hence may be developed in any family.

It seems evident, too, that the meganephric and plecto-nephric condition may occur within the same genus, as we see to be the case in Acanthodrilus, and that this condition of the nephridium is not of itself sufficient to decide to which series of families a genus belongs. The sum of the characters of Plagiochæta places it amongst the Plectonephrica, mihi, or Acanthodrilini of Beddard.

The general features of its internal anatomy point to its close affinity with the family Acanthodrilidæ, mihi, rather than with the Perichætidæ.

Beddard forms two families, Deinodrilidæ and Acan-thodrilidæ, to contain the genera which I have grouped in one family. I think that we shall do well to wait awhile before we subdivide the families too much; and I would still retain Deinodrilus in the same family as Acanthodrilus, Tri-gaster, and Benhamia, for it differs from these only in the possession of twelve chætæ; the extent of clitellum not being even a generic distinction, since it varies in different species of Acanthodrilus.

I therefore place Plagiochæta as a fifth genus in the family Acanthodrilidæ, mihi, with the following characters :— The chætæ are numerous, and arranged in couples, so as to form nearly a complete circle in each somite. The clitellum is short. There are four sperm-sacs ; the spermathecæ have diverticula; penial chætæ replace the ventral chætæ of Somites XVII, XIX; a pair of calciferous glands is present. There are no peptonephridia ; no dorsal pores. Some of these characters are shared by some one or more of the other genera of the family.

It is possible that Bourne's P. Stuarti may belong to this

family, but it is to be regretted that we have received no detailed account of the anatomy of this and the other interesting forms which have been briefly described by him.[1]

[1] 'Proc. Zool. Soc.,' 1886.

EXPLANATION OF PLATES XV and XVI,

Illustrating Mr. W. Blaxland Benham's "Notes on Two Acanthodriloid Earthworms from New Zealand."

Neodrilus monocystis.—FIGS. 1 to 13.

FIG. 1.—Dorsal view of anterior end of body, showing the character of the prostomium (*pr.*), arrangement of chætæ (*d. ch.*), and position of the nephridiopores (*ne. o.*) in these somites.

FIG. 2.—Ventral view of the somites in the neighbourhood of the genital pores. *ne. o.* Nephridiopores. *prst. p.* Pore of the prostate. *sp. p.* Spermiducal pore. *v. ch.* Ventral chætæ.

FIG. 3.—General anatomy, as seen when the worm is opened in the usual way; from the dorsal surface. *app.* Appendix of spermatheca. *d. v.* Dorsal vessel. *giz.* Gizzard. *l. h.* One of the lateral hearts. *ne.* Nephridium. *pe. d.* Penial duct. *pe. s.* Sac with penial chætæ. *prst.* Prostate. *sp. s¹.* Anterior sperm-sac. *sp. s³.* The most posterior (third) sperm-sac. *spth.* The main sac of the spermatheca.

FIG. 4.—A transverse section of the worm behind Somite xxx, showing the general squareness of the worm, the position of chætæ, and of the nephridia. It would be unusual to find the asymmetry of the last structures as represented in the figure. *a.* Separation of dorsal muscle bundle into two lateral halves. *b.* Lateral muscle bundle. *c.* Ventral muscle bundle. *d.* Accessory muscle bundle, separated from *c* by penetration of a nerve into the body-wall. *d. ch.* Dorsal chætæ. *d. n.* Dorsal nephridium. *d. v.* Dorsal blood-vessel. *m.* Band of muscle passing from the dorsal to the ventral bundle of chætæ, and acting as their retractor. *N.* Nerve-cord. *v. ch.* Ventral chætæ. *v. n.* Ventral nephridium. *v. 'v.* Ventral blood-vessel.

FIG. 5.—The inner surface of body-wall (rather more than half the worm is represented), to show position of the nephridia in consecutive segments. Letters as in Fig. 4.

FIG. 6.—A portion of Fig. 3, more enlarged (× 8). It represents the prostate of the eighth side; the numbers are placed nearly in the mid-ventral line. *Prst.* Prostate. *a.* Its free extremity. *pe. d.* Penial duct. *pe. s.* Sac with penial chætæ.

FIG. 7.—The sac of the penial chætæ, which protrude from its cut end which is turned forwards, and the prostate are cut away to show the sperm-duct (*sp. d.*) entering the body-wall in Somite XVIII.

FIG. 8.—A diagrammatic longitudinal section through this region of the body-wall, to show the pores of the penial duct of the sac with penial chætæ, and of the sperm-duct. [It is compiled from a series of sections.] *ci.* Circular muscles of the body-wall. *ep.* Epidermis. *lg.* Longitudinal muscles of the body-wall. *pe. ch.* One penial chæta in the sac (*pe. s.*). *pe. d.* Penial duct opening to the exterior, independently of the sac of penial chætæ. *pores.* Aperture common to the prostate duct and penial sac. *Prst.* Prostate. *pr. pap.* Papilla carrying the pore of the prostate and penial sac. *sp. d.* Sperm-ducts. *sp. p.* Spermiducal pore. *v. ch.* Ventral chætæ.

FIG. 9.—The sac with penial chætæ; it was broken in removing it from the body, and the two portions *a* and *b* are really continuous at *x*.

FIG. 10.—A portion of one of the long penial chætæ, very highly magnified to show the notched ridges and the triangular section. It is surrounded by the cuticular membrane (*m.*) which lines the sac.

FIG. 11.—Transverse section through the prostate. *a* is the cœlomic epithelium surrounding it. *bv.* Blood-vessels in the wall. Only a few cells are represented in detail, the rest in outline.

FIG. 12.—Transverse section of the penial duct. *ep.* Its epithelium. *m.* Its muscular coat. *v.* Blood-vessels.

FIG. 13.—The spermatheca of the right side. *s.* The chief sac. *app.* The appendix or diverticulum. *d.* The duct penetrating the body-wall.

Plagiochæta punctata.—FIGS. 14 to 32.

FIG. 14.—Dorsal view of the worm (× 2), to show the characteristic marking (from a spirit specimen). *cl.* Clitellum. *pr.* Prostomium.

FIG. 15.—Dorsal view of anterior end of worm, showing the shape of the prostomium.

FIG. 16.—Ventral view of the anterior 23 somites, showing the characteristic arrangement of the chætæ (represented by dots) in couples, the position of the nephridiopores (*ne. o.*), the pores of spermathecæ (*spth.*) in depressions, the prostate pores (*prst.*[1], *prst.*[2]) also in a depression. *clit.* Clitellum. *m.* Mouth. *pro.* Prostomium.

FIG. 17.—A transverse section of the worm behind the clitellum, showing the way in which the longitudinal muscular coat (*lg.*) of the body-wall is

broken up by the chætæ ; these are not represeuted, but occur in couples at the spots marked with numerals ; the position of the nephridia (*n. v., n. d.*) is also shown. *D. v.* Dorsal blood-vessel. *Int.* Intestine, spirally coiled, a part of it showing through the septum (*sep.*). *N.* Nerve-cord.

FIG. 18.—About half of a segment (drawn to scale), showing the couples o chætæ inserted in light areas, the pigmented part being represented diagrammatically.

FIG. 19.—A general view of the internal anatomy, as seen when the dorsal portion of the body-wall is cut away. *app.* Appendix of the anterior spermatheca. *cal. gl.* Calciferous gland. *d. v.* Dorsal vessel. *int.* Intestine. *ne. ne.* Nephridia. *pro.*[1], *pro.*[2] The two prostates. *sal.* Salivary glands. *sp. s.*[1], *sp. s.*[4] The most anterior and most posteroir sperm-sacs. *spth.* The main sac of the spermatheca.

FIG. 20.—Transverse section of œsophagus and calciferous gland, represented diagrammatically.

FIG. 21.—A longitudinal section of the intestine, to show its spiral coils. *sep.* Septa.

FIG. 22.—One of the ventral nephridia (the funnel is not shown). *a.* The convoluted large and middle tubes. *b.* The coil of small tubes. [These are only represented diagrammatically ; it is not intended to show the exact arrangement of *a, b.*] *c.* The cæcal portion of the bladder. *p.* Portion leading to the nephridiopore.

FIG. 23.—Two consecutive sections (*a, b*) through a nephridial funnel (from a series of transverse sections). *c. ep.* Cœlomic epithelium. *m.* Marginal cells.

FIGS. 24, *a, b, c, d.*—Four consecutive sections through the degenerate nephridium of the second segment. In *a* one edge of the nephridiopore and a part of the dilated bladder is seen. *b* goes through the nephridiopore. *coag.* Coagulum in bladder. *m.* Muscle. I, II. Portion of wall of the 1st and 2nd segments : *c* shows the tract of cells leading to the convoluted tube, a portion of which (*t*) is present. *m* is the position occupied in the next section by the coil *d. l* shows the position occupied by the tract of cells represented in *c.*- *b. v.* Blood-vessels.

FIG. 25.—An ovary attached to septum (*spt.*).

FIG. 26.—View of the prostates, &c., after removal of the gut. *pe. s*[1]., *pe. s*[2]. The two sacs containing penial chætæ. *pro*[1]., *pro*[2]. The two prostates. *sp. d.* Sperm-duct. *pe. d.* Penial duct.

FIG. 27.—Transverse section of a sperm-duct, showing the oval nuclei with long axis at right angles to the axis of the duct. *b.* Blood-vessel.

FIG. 28.—Transverse section of penial duct. Here the nuclei of the epithelium have their long axes radially placed. *c. m.* Circular muscular coat. *lg. m.* Longitudinal muscles.

FIG. 29.—Diagrammatic obliquely longitudinal section through the aperture of prostate, combined from a series of sections. *Pe. d.* Penial duct. *pr.p.* Prostate pore. *pe. s.* Sac with penial chætæ. *a.* The longer ; *b*, the reserve penial chæta. *p. s. p.* Pore of this sac.

FIG. 30.—A spermatheca, showing its various parts.

FIG. 31.—A longitudinal section through a spermatheca (combined from a series of sections). *a, b, c, d* point to the regions which are represented more highly magnified in Fig. 32. *a.* The duct of spermatheca. *app.* The diverticulum. *b.* Wall of the sac. *c. m.* Circular muscle of the body-wall. *c. d* Epithelium of appendix. *lg. m.* Longitudinal muscle of the body-wall. *mus.* Muscular coat of the diverticulum. *sept.* Septum VIII/IX. *spoa.* Spermatozoa in the diverticulum, and a few in the sac. *v. ch.* Ventral chætæ. *x.* Granular secretion in the sac.

FIG. 32.—Portions of epithelium of the spermatheca from different regions. *a.* From duct. *g.* Glandular cells. *p.* Nuclei of non-glandular cells. *b.* From the wall of the sac. *m.* Cells which have probably just discharged their contents (*x*). *n.* Cells not quite fully developed. *c.* From the diverticulum. *k.* Nucleus of the interstitial cell. *d.* Also from the diverticulum, showing spermatozoa attached to the cells by (*h.*) their heads. *t.* Tails of spermatozoa.

On a New Genus of Synascidians from Japan.

By

Asajiro Oka,
Of the Imperial University, Tokio ;

AND

Arthur Willey, B.Sc.Lond.

With Plates XVII and XVIII.

THE remarkable compound Ascidian which we[1] are about to describe was collected in quantity by one of us at Moroiso, a place which stands at the head of a small bay to the north of Misaki, some fifty miles south of Tokio. It was found in the months of July and August, hanging downwards from the lower surface of the shelving rocks between the tide-marks, and is therefore exposed at low water at certain times of the year. It was soon regarded as probably a new form, and such has since proved to be the case.

As will be seen below, the new genus belongs to the family of the Didemnidæ, and the name we propose for it is S a r c o - d i d e m n o i d e s m i s a k i e n s e.   It may be considered as standing in a similar relation to the genus Didemnoides that Sarco-botrylloides does to Botrylloides.

The following description will be found incomplete in one or two points, partly owing to the time of the year at which

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[1] The study of this Ascidian colony was commenced by one of us at Misaki, continued by both of us in Freiburg-i.-Br., and concluded by the other of us at Plymouth and Naples.

the material was obtained (there being no male genital organ in any of the colonies examined), and partly owing to the fact that while the specimens which reached Europe were in a sufficiently good condition to establish the systematic position of the new genus, yet they were not suitable for a very minute examination, especially with regard to the details of budding. In spite of these gaps, the most serious of which is the failure to observe the testis and vas deferens, the general appearance of the colony is so singular and characteristic, and the canal system so interesting, that we thought it worth while to send in this account.

1. Manner of Growth and Outer Form.

On Pl. XVII is given a reproduction of a coloured drawing of a living colony according to the natural size, which was executed at the behest of one of us by a Japanese artist.

The portrait is singularly striking and accurate. The colour is a brilliant red, and the surface of the colony is smooth and glistening.

At the tips of the round knoll-like lobes, which are a very characteristic feature of the genus, are seen the small but distinct excurrent orifices. The lips of the pores are slightly raised above the level of the surrounding surface. The incurrent orifices are, of course, the mouths of the Ascidiozooids, but they are too small to be seen with the naked eye on a living colony, which, indeed, is not transparent.

The shape of a colony in the transverse direction is shown in fig. 2 on Pl. XVIII. From this figure we note the unusual circumstance that the base or surface of attachment is considerably narrower than the free portion.

According to the observations of one of us in Japan, the direction of the length of a colony is always parallel to the coast-line; and further, colonies growing end to end frequently undergo concrescence, and in this way produce a compound aggregation which often attains to a great length, namely, two or three feet.

This Didemnid presents a great contrast to most if not all

other Didemnids in the fact that its height or thickness in the vertical direction exceeds two- or three-fold the width, while in other forms the thickness or vertical height of a colony is usually very small. Our genus agrees with all other Didemnidæ in being sessile, but differs from most of them in not forming encrusting or straggling masses.

Beyond the characteristic lobes and the upraised margins of the excurrent orifices there are no processes of any kind on the external surface of the colony.

2. Canal-System.

The canal-system of the new genus bears a general resemblance to that found in other forms of the Didemnidæ, but in its extent and elaboration stands alone.

A schematic representation of the system of canals is given in fig. 1, while an actual transverse section is shown in fig. 2.

It will be at once evident that the canal-system consists of a peripheral portion and a central portion. The peripheral portion consists of much-branched canaliculi, whose ultimate extremities lead from the atrial apertures of the Ascidiozooids (fig. 3), and then open into larger canals, which again discharge into the large central spaces from which water and excrement are conducted through the excurrent orifices to the outer world. It will of course be borne in mind that these so-called "canals" are only irregular spaces excavated in the mass of the common test, and having nothing in the shape of an epithelial lining, although the cells in the test are very numerous, and often are so arranged as to present a deceptive resemblance to a flat epithelium.

As already mentioned, the incurrent orifices consist of the pores leading to the branchial apertures of the Ascidiozooids (fig. 3). These (i. e. the Ascidiozooids) are some little distance removed from the surface of the colony, so as to give rise to a veritable incurrent canal.

It is thus seen what a justifiable analogy may be drawn between these Ascidiozooids and the ciliated chambers of a sponge; in other words, between individuals on the one hand

and organs on the other. It is, however, necessary not to push this analogy too far.

It may have been noticed that so far we have not applied the term " common cloacal apertures" to the excurrent orifices, and we have intentionally abstained from doing so. In the Botryllidæ and Polyclinidæ, as has been long ago pointed out by Savigny, the cloacal apertures of the individuals belonging to a system which may be more or less regular, conspire together, sometimes in a very close manner, as in Botryllus or Circinalium, in other cases less closely, to form a true common cloacal aperture, which is capable of being opened and closed by muscular action.

In the Didemnidæ as a whole, and par excellence in our new Didemnid, this association of cloacal apertures does not occur. Here the cloacal apertures of the individual Ascidiozooids are perfectly distinct, and each opens independently into the canal-system in the way above described (see fig. 3).

Thus, while in the Botryllidæ, for example, the excurrent orifices are true common cloacal apertures, in the Didemnidæ they are distinctly not the same thing in the same sense, although they are possibly homologous structures.

This is why we have preferred to use the term "excurrent orifices."

3. Ascidiozooids.

As already indicated, the Ascidiozooids cannot be seen from the outside in a living colony; but in preserved specimens they appear as very numerous white specks, which are studded evenly all over the colony without any reference to a system or systems of distribution. In fig. 2 the black points immediately beneath the surface represent the Ascidiozooids, while the round bodies which lie deeper in the test are the larvæ.

On the structure of the individual Ascidiozooids we have nothing special to say, since they conform very closely to the Didemnid type. The most characteristic feature of all for the Ascidiozooids of the Didemnidæ is the single unpaired testis, round which the vas deferens is wound spirally.

This remarkable condition was first described and figured by Della Valle (5), and then insisted upon as being of classificatory importance by von Drasche (6).

As we have before said, we have not had the testis of our new genus under observation, so that it must be left doubtful at present whether the male gonad of this genus departs in any way from the usual condition of things in the other members of the family of the Didemnidæ.

The remaining important characters which the new genus possesses in common with the other Didemnidæ are—(a) the simple branchial sac, possessing only four rows of small oval stigmata; (b) long muscular processes of the mantle, which penetrate deeply into the test, and which are regarded as retractor muscles by von Drasche, who calls them "Ectodermfortsätze;"[1] (c) the absence of an oviduct. The ova float about in the body-cavity along with the blood-corpuscles, and as they grow in size they push the body-wall of the Ascidiozooid before them, and so give rise to a sac which gradually separates itself by constriction from the Ascidiozooid, and finally comes to lie freely in the test (fig. 4). This process has also been described by Della Valle (5, pp. 38, 39).

Tangential sections reveal the fact that the branchial apertures have an hexagonal form, corresponding to what von Drasche calls "Sechszähnig."

The budding of the Ascidiozooids takes place on the same plan as that called by Giard pyloric budding, and which has been well described by Della Valle (loc. cit., pp. 48—56). The detailed discussion of this process must, for reasons stated above, be left to a future occasion. By this method of budding, two different buds arise from the parent individual, and ultimately fuse together to form a new individual. One bud arises as an outgrowth from the œsophagus, and the other as

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[1] The muscles in these processes are prolongations of the two strong dorsal muscles, which converge together at the base of the pharynx, and are then continued into the test as a single muscular band. A short hollow vascular ectodermic process can often be seen apparently springing from the body-cavity of the intestinal region of an Ascidiozooid.

an outgrowth of the parietal wall of the abdomen. We can see indications of this curious process in our preparations, more especially plainly the buds from the œsophageal region, but we have not been able to trace the further development of them.

The branchial tentacles of the Ascidiozooids are alternately long and short.

4. Larvæ.

The embryos having separated from the parent Ascidiozooid sink into the deeper parts of the test, and there develop into large tailed larvæ. This is also very characteristic for the Didemnidæ.

We wish to draw particular attention to the ring of twelve tentacle-like processes which surround the anterior end of the body of the larva, from which project the three adhering discs. These processes are hollow and communicate with the body-cavity, and contain very numerous blood-corpuscles.

They seem to be of very general occurrence in the tadpoles of the Didemnidæ. Ganin (2) called them " pelottenför-migen Anhänge," and states that among six species of Di-demnum examined by him the number of these processes varied from four to fifteen or sixteen. Giard says (3, p. 610) that the Didemnidæ " ont des embryons pourvus de tubes stolo-niaux généralement au nombre de 8, très-développés et prêts à former des blastozoïtes quand le jeune oozoïte sera fixé." In different places of his work Giard calls these processes indifferently " tubes stoloniaux," " tubes embryonnaires," and "tubes gemmifères." He figures them in the case of Lepto-clinum gelatinosum. As a matter of fact, however, they have nothing to do with the budding, and Della Valle (loc. cit., p. 49) has rightly insisted that in the Didemnidæ the process of gemmation only commences after the larva has fixed itself. In spite of this von Drasche states wrongly that the embryos of the Didemnidæ show "rudimentäre Knospung." Herdman (7) has figured these processes in two species of Leptoclinum and in one species of Didemnum, but makes

not the slightest reference to them in the text, except in so
far that he follows von Drasche in giving the appearance of
" rudimentary embryonic blastogenesis " as a characteristic
for the group.   We will not deal with these tentacle-like pro-
cesses any further at present, as one of us has recently been
following their occurrence and relations in another group of
Ascidians, namely, in the Styelinæ; and we will therefore
defer the discussion of them.   Suffice it to say that far from
being connected with any budding process, they are, both in
Didemnidæ and in the Styelinæ, transient larval appendages,
which become reabsorbed in the course of development.

In the Botryllidæ, on the other hand, according to Metschni-
koff (' Bull. de l'Acad. Imp. des Sciences de St. Petersburg,'
t. xiii, 1869), they persist and give rise to the well-known
vascular processes with swollen extremities which traverse the
test of the adult colonies.

How the larvæ escape from their lodgment in the test is
not quite clear.   We may assume a process of absorption or
possibly rupture, but neither can be asserted.   The probability
is that both occur.   The larvæ can only sink into the depths
of the test accompanied by a process of absorption, and when
they are only separated from the central canal by a thin layer
of test their movements could easily rupture that, and they
could thus escape through the excurrent orifices.

5. Structure of Test—Spicules.

The deeper parts of the test contain numerous pigment
concretions, and in the more superficial region there is a thin
layer of extremely delicate calcareous spicules.

Although the presence of spicules is not an absolute feature
of the Didemnidæ—since von Drasche has described two
species of Didemnum, and Herdman one, in which spicules
were absent; and, again, von Drasche has described a species
of Diplosoma with spicules—yet combined with the other cha-
racters mentioned above, and especially with those belonging
to the larvæ, the presence of spicules may be taken as a
decisive feature in assigning the new genus to the family of

the Didemnidæ. The spicules form a distinct layer, and are fairly numerous. Their form is very various. A few varieties are shown in fig. 6, but the crenate form is perhaps the commonest.

They occur in special aggregations round the branchial apertures of the Ascidiozooids (fig. 5). They are seen to best advantage in sections which have been mounted unstained.[1]

At the surface the test consists to a very limited depth (fig. 3) almost entirely of large bladder-cells, which are usually rendered polygonal by mutual pressure, but when they contain crystals they are invariably perfectly round. Below the layer of bladder-cells the fusiform cells occur, and the bulk of the crystals are contained in a zone about equal in thickness to the layer of bladder-cells, but mainly scattered among the fusiform-cells. They occur more rarely in the bladder cells at the very surface.

But wherever the crystals—or, as they are usually called, calcareous spicules—occur, they are always found in large round bladder-cells; and, as Giard pointed out (p. 508), they are formed round the nucleus of the bladder-cell.

Lower down in the test the pigment concretions begin, and they seem also to be produced in similar large round cells of the test.

6. Systematic Position.

According to the classification of von Drasche (6), the Didemnidæ consist of two genera, namely, Didemnum and Leptoclinum,[2] the former having three and the latter four rows of branchial stigmata.[3] Leptoclinum, again, he splits up into

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[1] It may just be noted that sections so mounted in benzole Canada balsam were of no use for observing the crystals, as the latter were very soon destroyed, probably through the influence of the impurities which frequently occur in benzole.

[2] Herdman, however, with good reason insists on maintaining the Eucœlium hospitiolum of Savigny, which has six rows of branchial stigmata.

[3] In von Drasche's scheme of classification the numbers are accidentally transposed. In the description of genera and species they are given correctly.

two sub-genera, viz. Leptoclinum proper, characterised by its thin encrusting cormi ; and Didemnoides, which is qualified as follows :—" Fleischiger Cormus, in Polstern oder Knollen." Von Drasche describes and figures two species of Didemnoides, viz. D. macroophorum and D. resinaceum.

There can be very little doubt that we are right in allying our new genus with the Didemnoides of von Drasche, while the extraordinary habit of growth of our new form unquestionably calls for the formation of a new genus. As Della Valle (4) indicates, the thickness of 3 cm. is very great for a Didemnid, while our Didemnid attains a thickness of over 6 cm.[1] Perhaps the most striking feature of the new genus is the lateral compression which it exhibits (see fig. 2).

7. Summary.

Sarcodidemnoides misakiense, Oka and Willey.

Generic Characters.—Colony (or cormus) forming very thick lobose masses, laterally compressed ; sessile, but not encrusting.

Excurrent orifices placed on the tips of the knoll-like prominences.

Ascidiozooids very numerous, not arranged in systems ; branchial sac with four rows of stigmata ; canal system complicated, differentiated into peripheral and central portions.

Specific Characters.—Atrial apertures of Ascidiozooids simple pores without teeth or languet; spicules fairly abundant, extremely delicate, confined to a thin layer near surface of test.

Test gelatinous, containing numerous bladder-cells, crystals, fusiform cells, and pigment concretions.

Stomach of Ascidiozooids vertically placed; surface of attachment of colony narrower than the free portion.

Colour, brilliant red.

Habitat.—Moroiso, Japan, between the tide-marks.

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[1] Von Drasche (p. 32) mentions a species of Didemnum—D. tortuosum —which attains a thickness of 5 cm.

N.B.—Since the above was written I have seen for the first time the exhaustive work of Fernand Lahille, entitled ' Recherches sur les Tuniciers des côtes de France,' Toulouse, 1890. Lahille devotes considerable attention to what have been spoken of above as tentacle-like processes of the larva, figures them in many larvæ, and gives an excellent figure of the metamorphosing larva of Styela glomerata. He gives an opinion as to their significance which I cannot entirely endorse in the light of my own researches on the " Post-embryonic development of Styela," commenced last August at Plymouth. However, I hope to return to this question on a future occasion. Lahille raises an objection to von Drasche's genus Didemnoides on the ground that the thickness of the cormus is not an anatomical character, and that the distinction between thick and thin colonies is a purely subjective one. There is no doubt some truth in this ; but the difference between a compound Ascidian which possesses, say, a very few spicules, and one which possesses none at all, would appear to be no more fundamental than that between a colony whose mode of growth resulted in the production of a fleshy mass and one which grew in the form of a thin leathery crust.

As stated above, von Drasche intends by Didemnoides a fleshy form of Leptoclinum, the test containing spicules, and the Ascidiozooids having four rows of stigmata in the branchial sac. Lahille, on the contrary, applies the name Didemnoides to those Didemnidæ which are characterised by the absence of spicules, and the possession of three rows of stigmata in the branchial sac.

The compound Ascidian which we have described above has spicules in the test, and four rows of stigmata in the branchial sac. But as it would be too absurd to call the new form " Sarcoleptoclinum," we shall persist in regarding the genus Didemnoides from the point of view of von Drasche.—A. W.

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EXPLANATION OF PLATES XVII and XVIII,

Illustrating Mr. Asajiro Oka's and Mr. Arthur Willey's paper
" On a New Genus of Synascidians from Japan."

PLATE XVII.

Sarcodidemnoides misakiense, nov. gen. et spec.

View of colony, natural size and colour. Length 12 cm., height 6 cm.
width about 3 c.m.

PLATE XVIII.

Fig. 1.—Diagrammatic view of the canal system of S. misakiense,
showing peripheral and central portions of the system.

Fig. 2.—Transverse section through a whole colony to show the lateral
compression and the narrow base. Dark points round margin are Ascidio-
zooids ; the round bodies lying deeper in test are embryos and larvæ.

Fig. 3.—Horizontal section through the branchial sac of an Ascidiozooid to
show the position of the openings. The atrial opening is placed far back,
a characteristic feature for the Didemnidæ. The surface-layer of bladder-cells
in the test is shown on the right-hand side of the figure. Zeiss, DD, 2. cl.
Cloaca, opening by cloacal aperture into peripheral canal.

Fig. 4.—Section showing an egg-cell in the body-cavity surrounded by
blood-corpuscles.

Fig. 5.—Section through the branchial funnel of an Ascidiozooid to show
the collar-like aggregation of calcareous spicules. Zeiss, E, 2, cam. luc.

Fig. 6.—Isolated spicules from the test. Zeiss, E, 4, cam. luc.

Fig. 7.—Tadpole of S. misakiense to show the ring of tentacle-like pro-
cesses surrounding the three adhering papillæ. Zeiss, B, 2, cam. luc.

A New Branchiate Oligochæte (Branchiura Sowerbyi).

By

Frank E. Beddard, M.A.,

Prosector to the Zoological Society of London, Lecturer on Biology at Guy's Hospital.

With Plate XIX.

WE are acquainted at present with three genera of Oligochæta which render the Cuvierian name of "Annélides abranches sétigères" no longer applicable to the group. Dero has been known since the time of Rösel and Otto Friederich Müller, the latter of whom recognised the branchial function of the ciliated processes at the anal extremity of the body. Alma nilotica, originally described by Grube,[1] and lately reinvestigated by Levinsen,[2] is, according to Grube, much like a Rhynchelmis in general habit. It has paired sigmoid setæ; the posterior sixty segments or so are furnished with short dorsally placed processes, more numerous upon the anterior than the posterior of these segments. The name given by Levinsen is Digitibranchus niloticus; but this worm is, in all probability, generically and specifically identical with Alma nilotica. The third branchiate form has been quite lately described in this Journal[3] by Professor A. G. Bourne.

[1] "Beschreibungen neuer oder wenig bekannter Anneliden," 'Arch. f. Naturg.,' 1855, p. 129.

[2] "Om to Regnormslaegter fra Ægypten," 'Vidensk. Meddel. naturk. Foren.,' 1859, p. 321.

[3] "On Chætobranchus, a New Genus of Oligochætous Chætopoda," 'Quart. Journ. Micr. Sci.,' vol. xxxi, p. 83.

It is a Naid furnished with long processes on the anterior seg-
ments which lodge the dorsal setæ.   I shall refer more par-
ticularly to the structure of these processes in describing a
quite new type of branchiate Oligochætous Annelid which I
propose to name.

Branchiura Sowerbyi, nov. gen. et sp.

In looking over some mud from the " Victoria regia tank "
in the Royal Botanical Society's Gardens, Regent's Park, I
found three specimens of an Annelid which struck me at once
as probably new.   It was remarkable for the unusual con-
tractility of the body, which suggested a leech or a flat-worm
rather than a Chætopod.   For this reason it is impossible to
give any accurate measurement; half an inch to three quarters
is about its length.   It consists of about 120 segments (there
were 170 in the largest individual).   Examined with a lens,
the orange-coloured digestive tract traversed by the bright
blood-vessels could be seen ; and at the posterior end of
the body a series of delicate dorsal and ventral pro-
cesses.   The position of these organs, which I shall presently
give reasons for believing to be branchiæ, suggested the generic
name ; the species I have great pleasure in associating with
the name of Mr. Sowerby, of the Botanical Society.

After carefully noting and sketching the principal features of
the worm when examined alive under low and high powers of
the microscope, I preserved one specimen with corrosive subli-
mate and acetic acid, followed by gradually increasing strength
of alcohol; the others with Perenyi's solution—a much better
reagent—for histological study.

§ Branchiæ.

The principal structure of interest in this worm is of course
the series of branchial processes.   These are apparent, when
the worm is examined with a lens, as a delicate fringe which
decks the last one sixth or one seventh of the body, giving it a
feather-like appearance.   There are altogether about fifty
pairs of these processes, which are segmentally arranged, i. e.

one pair to each segment. In the largest of the three worms there were at least eighty of these processes on each side. Here, again, I am unable to be absolutely accurate; the processes first appear as mere mammillary outgrowths, which gradually increase in length upon successive segments; it is difficult, therefore, to say exactly when they begin. From being the slightest elevations, barely perceptible, of the integument, the processes become nearly as long or even longer than the diameter of the worm's body: they then gradually diminish in length towards the anus; but they are not so small at the finish as at the beginning, nor are there so many rudimentary branchiæ at the anal end of the series as there are at the oral end. In one specimen, with only twenty pairs of branchiæ, there were no rudimentary ones at the posterior end of the series.

By a remarkably fortunate accident, the water which produced the specimens of Branchiura Sowerbyi contained also three or four examples of Bourne's Chætobranchus. I have, therefore, been able to compare the two forms, detail for detail, though I have convinced myself that Bourne's excellent description really rendered an examination of the worm Chætobranchus itself unnecessary.

The first important difference, then, between the branchial processes is a difference in position. In Chætobranchus the branchiæ are anterior, gradually diminishing and finally disappearing posteriorly; in Branchiura they are developed upon the last sixty segments of the body or so.

In examining the living Chætobranchus it is easy to see that, as Bourne has described and figured, the setæ are contained within the branchiæ. The long capilliform seta of the dorsal bundles reaches nearly to the end of the branchia. In Branchiura there is no connection at all between the branchial processes and the setæ. The branchiæ are, in fact, not latero-dorsal in position, as in Chætobranchus; they arise from the body-wall dorsally and ventrally in the middle line.

In the figure which illustrates the living worm (fig. 2) the anus is shown to one side of the body apparently; the worms

were nearly always in this position when examined alive. I
have found, however, by transverse sections, that the bran-
chial process arises in the middle dorsal and middle
ventral lines of the body respectively. This is shown
in the sections represented in fig. 13. The anus is thus
really dorsal, as in Dero, and there is nothing especially ab-
normal about its position. But the position of the branchiæ
is very remarkable indeed; a fusion between the branchiæ of
successive segments would produce something like the un-
paired fins of Vertebrates. It has been suggested, by the
advocates of the Annelid origin of Vertebrates, that the ap-
proximation and fusion in the mid-dorsal line of the notopodia
of the parapodia may have produced the dorsal unpaired fin;
in Branchiura we have the unpaired character of the pro-
cesses of the body already established.

When Chætobranchus is in motion the branchial pro-
cesses naturally move with the setæ; there is, so far as I could
ascertain, no intrinsic movements of the branchiæ themselves,
which indeed do not appear to be provided with any muscles,
as Professor Bourne has rightly stated. They are kept rigid
by the setæ. In Branchiura the individual movement of
each branchia can be readily seen. The whole of the posterior
end of the worm's body was in continual movement, while the
anterior end remained quiet. The body itself oscillated to
and fro with a peculiar vibratory movement: this of itself
caused a fluttering of the branchiæ; but in addition to this
each branchia moved independently, writhing about like the
cirri and tentacles of some Polychæta. The branchiæ not only
moved from side to side, but became alternately extended and
retracted, so that their length was much greater at one time
than at another. Hence it is difficult to give any measure-
ment that would apply accurately to the branchiæ of the living
worm. After treatment with corrosive sublimate and alcohol
the length of the longest branchia was generally rather less
than the diameter of the body; during life it was frequently
the other way about. The branchiæ frequently present the
wrinkled appearance shown in the accompanying figure (Pl.

XIX, fig. 5); this is of course due to the contraction. The
movements of the branchiæ are effected by muscular fibres
which run across the cavity of the branchia from side to side;
these fibres are elongated, fusiform, with a central nucleus.
They are sometimes branched, forming star-shaped bodies with
the nucleus in the centre.

The structure of the branchiæ (see fig. 6) is very simple; they
are covered by a firm cuticle, beneath which lies the epidermis.
Bourne detected fine vibratile cilia upon the branchiæ of
Chætobranchus. I failed to discover any cilia upon the
branchiæ of Branchiura. The axis of the branchia is occu-
pied by a cavity evidently belonging to the cœlom; imme-
diately beneath the epidermis is a layer of muscles which
appears to be continuous with the circular layer of the body-
wall; beneath this is the peritoneum. In Chætobranchus
there is a capillary loop running up the branchiæ in the cœlom,
which in that worm, as in Branchiura, is prolonged into the
branchiæ. In Branchiura there is no such capillary loop
lying freely within the cavity of the branchia, but immediately
beneath the epidermis is a blood-vessel on each side which
gives a yellowish or even red colour to the branchia when
examined under the microscope. I shall speak further of the
blood-supply of the branchiæ in considering the course of the
blood-vessels.

Although the branchiæ have an axial cavity, traversed by
the muscular fibres which cause the contractions of the branchiæ
and lined with peritoneum, this cavity is not actually in com-
munication with the body-cavity; in the living worm the cavity
of the branchia can be easily seen to be shut off from the
cœlom by a diaphragm which moves synchronously with the
contractions of the dorsal vessel. It becomes alternately convex
and concave outwards : when convex, i. e. when forced forwards
by the liquid of the cœlom, it projects some way into the
interior of the branchia, and is very conspicuous; it appeared
to me to completely block all ingress of solid matter into the
lumen of the branchia. The cœlom of this worm contained no
freely floating corpuscles that I could discover; I am not able

to make a definite assertion upon the matter.   But in one specimen the cavity of the branchia contained a quantity of oval or fusiform bodies (fig. 11), of problematical nature.   These moved to and fro with the contractions of the branchiæ, but I found none in the body, nor did I ever see them pass the diaphragm.

In the above account of the branchial organs I have compared them with those of Chætobranchus.   The important differences lie in the position, the contractility, and the independence from the setæ of the branchiæ in Branchiura.   The fact that in Chætobranchus the setæ are embedded in the branchiæ suggests a comparison with the parapodia of Polychæte Annelids ; but it must be remembered that the enclosure of the setæ within the branchial processes of Chætobranchus appears to be secondary.   They are at first, partially at least, unenclosed by the branchiæ.   In Branchiura the branchiæ recall the simpler forms of gills in certain Chætopods, perhaps also the cirri.   I am not, however, disposed to láy any stress upon these resemblances, apart altogether from the wide separation between the existing Polychæta and Oligochæta ; it is quite intelligible that structures of this kind may have arisen independently.   Indeed, I am of opinion that there is no genetic connection between the branchiæ of even the four Oligochætous genera now known to possess these organs, viz. Dero, Alma, Chætobranchus, and Branchiura.   Though Dero and Chætobranchus are probably both Naids, their branchiæ are too different in structure and position to admit of a direct homologisation without further facts than those known.   Branchiura I believe to be a Tubificid, and its branchiæ are again different.   Alma cannot at present be certainly regarded as an Oligochæte at all ;[1] if it is, I am inclined to regard its branchiæ as more like those of Branchiura than those of either Dero or Chætobranchus.

[1] Its resemblances to such a Capitellid as Mastobranchus have been pointed out ; but Eisig ('Monograph der Capitelliden'), though admitting these resemblances, leans to the opinion that it is an Oligochæte, on account of the blood-vessels described by Grube.   There are, of course, no blood-vessels in the Capitellidæ.

The remaining external characters of Branchiura are of less importance. The prostomium is conical, short, and rounded in front (see fig. 4). Each of the anterior segments is biannulate, as in many Tubificidæ. The dorsal seta bundles alone contain capilliform setæ, and there are at the most two of these to a bundle. The other setæ of the dorsal bundles are "crotchet-shaped." Very frequently the upper fork is worn away, so that the setæ resemble those of certain Lumbriculidæ, and appear to be simple sigmoid setæ, unnotched at the extremity. The ventral setæ are of one kind only— "crotchet-shaped." There is no "cephalisation" shown by the setæ. The second segment of the body has both dorsal and ventral bundles. The first two pairs of bundles, however, sometimes have no capilliform setæ, but this is probably accidental or an individual peculiarity, since in a second specimen they were present. The number of short setæ in the dorsal bundles of the first twenty segments or so is four to six, generally five; but generally six or seven in the larger specimen. Further back the number of these setæ in each bundle is less, being two or three. In the dorsal bundles of the most posterior segments there seem to be no capilliform setæ. In this the worm resembles Tubifex.

§ Vascular System.

I studied the vascular system principally in the living worms, but also by following out the course of the vessels in transverse and longitudinal sections. The blood was distinctly red in the larger vessels. The only contractile trunks are the dorsal vessel and certain of the circumœsophageal rings which connect it with the ventral vessel. The pulsations of the dorsal vessel pass from behind forwards, of the "hearts" from above downwards. Both the dorsal and ventral bloodvessels run from end to end of the body. It is remarkable that the term "dorsal" vessel[1] is quite inapplicable to the longi-

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[1] It is curious to learn from Eisen ("Preliminary Report on Genera and Species of Tubificidæ," 'Bik. K. Svensk. Ak. Handl.,' Bd. iv, p. 7) that something of the same kind occurs in Telmatodrilus, though reversed;

tudinal contractile vascular trunk; it is only in the œsophageal region that it is upon the upper surface of the alimentary tract. Further back it runs in close contiguity to the ventral blood-vessel, so close that at first I was disposed to regard this worm as resembling certain Polychæta in having a double ventral blood-vessel. It is very easy to see, however, that the two trunks do not always run side by side (they do in the branchial region), and that one only is contractile. In addition to these there is a supra-intestinal vessel, which I was only able to recognise in the living worm, upon the œsophagus and just the beginning of the intestine, but in sections I traced it further back; it lies beneath the peritoneal covering of the alimentary tract, while both the dorsal and ventral vessels lie freely in the body-cavity, and the former, like the latter, has no covering of pigmented cells. The figure (fig. 10) illustrates the course of the principal blood-vessels so far as I was able to make them out. I am not certain about the connection of the dorsal and ventral trunks in the most anterior segments. In longitudinal sections the close proximity of the dorsal and ventral vessels could be easy made out; the latter rests upon the nerve-cord. In each segment of the body after the hearts there seem to be two peri-intestinal vessels; one runs in the septum, the other at about the level of the setæ; but I did not make out their connection in a satisfactory way.

In the section illustrated in fig. 13 the relations of the dorsal and ventral vessels in the branchial region of the body and the blood-supply of the branchiæ can be made out—at least partly. The body appears something like the fig. 8 in section, but this was not always the case. In those sections which exhibited the figure-of-eight outline the upper cavity was exclusively occupied by the intestine, the lower cavity by the nervous system and the principal blood-vessels. The intestine is attached to the parietes by numerous muscular strands, and there appears always to be a partition[1] (at *sp.* in the fig.) which shuts

the ventral trunk lies towards the dorsal side of the body. In Dero the dorsal vessel is ventral in position.

[1] Among the Capitellidæ and other Polychæta the cœlom is similarly divided.

off the upper part of the cœlom from the lower part, and which corresponds to the "waist" of the figure of eight. There is nothing particularly noteworthy about the structure of the body-wall. The longitudinal muscular layer (*l. m.*) consists of a single layer of flat plates, the interstices between which are occupied by a granular nucleated substance which also forms a thickish layer on the inside of the muscles. Where the branchiæ arise the muscular layer is interrupted. The lower compartment of the cœlom is occupied, as already stated, by the nerve-cord and the blood-vessels. The ventral blood-vessel can be easily distinguished from the dorsal, not by its position, for they both lie side by side, but by the structure. The pulsating dorsal vessel has thicker muscular walls and much less blood in the lumen; the blood in this vessel was never so darkly stained by the carmine as the blood in the ventral vessel. I do not understand this unless it be that the muscular walls are particularly impermeable to that fluid. The dorsal vessel lies on the left side just above the nerve-cord, and when fully expanded is of about the same calibre as the ventral vessel. In parts, however, its lumen was so contracted that the vessel could only with difficulty be recognised. The ventral vessel has very thin walls, and was gorged with blood. It gives off (see fig. 13) a branch on the right side which immediately divides into two—a branch for each of the branchiæ. The branches run up and down the body close to the parietes and enter the branchiæ: the efferent branch passes down the opposite side of the body-wall in a corresponding position, but I did not succeed in seeing its actual opening into the dorsal vessel— presuming always that such an opening exists; but at the points where these vessels should open it always happened that the dorsal vessel was much contracted, while the end of the efferent branchial vessel was much dilated. This looks as if the flow of blood into the dorsal vessel was hindered at the moment of death by the contraction of the latter obliterating its lumen, and thus rendering the actual communication so slender as to escape attention.

Another important point with regard to the circulatory organs

of this Oligochæte is the presence of an extensive integumental network of capillaries. These consist of a large number of longitudinal trunks united by transverse vessels so as to constitute a network. A portion of this network is illustrated in fig. 7, which figure also shows the branchial loops: the branchial loops are not connected with the network shown in the figure.

The aquatic Oligochæta are, as a rule, not provided with an integumental blood-plexus; indeed, until recently the absence of such would be considered to distinguish the earthworms from the limicolous Oligochæta. Nevertheless Ilyodrilus appears, from the figures given by Stolc,[1] to possess an integumental vascular plexus which seems to be as fully developed as in Branchiura. In the small tract of body-wall illustrated in fig. 7 I observed at least three longitudinal trunks.

In the anterior part of the body, at any rate, the vascular network arises from paired trunks, which take origin from the supra-intestinal blood-vessel (see fig. 9).

The existence of an integumental network is, as has been already mentioned, a rare occurrence among the aquatic Oligochæta; the interest attaching to its existence in Branchiura is increased by the fact that the worm possesses special branchial organs. In about seven or eight of the anterior segments two of the longitudinal trunks of the integumental network are particularly enlarged, and in one specimen, at any rate, were very conspicuous; they run, as shown in the figure (fig. 9, *L. V.*), on either side of the nerve-cord, but below the ventral blood-vessel. I have not attempted to represent their branching, which is, I believe, connected with the system of vessels derived from the supra-intestinal blood-trunk.

I regard these vessels as the homologues of the "intestino-tegumentary" trunks of earthworms; their presence in Branchiura removes another structural barrier between the "Terricolæ" and "Limicolæ" of Claparède.

Finally, with regard to the circulatory system, I have to mention the existence of a pair of intestinal hearts; they lie in

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[1] "Monografie Českých Tubificidů," 'Abh. Böhm. Ges.,' 1888, pl. ii, fig. 3.

the 8th segment (fig. 9, *H'.*), and connect the supra-œsophageal with the ventral blood-vessel. They appear not to be contractile, and coexist in the 8th segment with the usual pair of contractile hearts. In each segment of the body in this region there is, as has been mentioned, a pair of vessels given off from the supra-intestinal trunk at about the middle of the segment, which join the integumental network. In the 8th segment a corresponding pair of vessels are present, which arise from the intestinal hearts.

The existence not only of a supra-intestinal vessel, but of intestinal hearts connecting it with the ventral vessel, was unknown among the Limicolæ until the publication of Stolc's important work upon the Bohemian Tubificidæ. Such hearts exist in the genera Bothrioneuron and Lophochæta. I have also described an "intestinal" heart in the remarkable fresh-water Oligochæte Phreodrilus,[1] which appears to be the representative of a distinct family, though showing some points of resemblance to the Tubificidæ.

§ Nephridia, Alimentary Tract, &c.

The perivisceral cavity appears to contain no free corpuscles. The anterior septa are thicker than the following ones.

The nephridia are constructed on the same plan as in Tubifex. The first pair were in the 12th segment. The nephridium communicates with the exterior by a pear-shaped vesicle (see fig. 14), dilated where it receives the excretory tubule and gradually narrowing towards the external orifice, which is very small and placed in front of the ventral seta bundle, at a point corresponding to about the middle of each bundle.

The alimentary tract is like that of Tubifex. The buccal cavity alone is not ciliated; the rest of the tube lying behind the buccal cavity is ciliated.

The œsophagus only differs from the intestine, which commences in the 11th segment, by its smaller calibre; it is much

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[1] "On Two New Genera of Aquatic Oligochæta," 'Trans. Roy. Soc. Edin.,' vol. xxxvi.

longer than in Tubifex, therefore : the brown peritoneal cells commence in the 6th segment. There are septal glands present, which lie in Segments 4, 5, and 6. The intestine is attached to the parietes by muscular bands.

§ Generative Organs.

In worms not sexually mature, two pairs of gonads in an immature condition were to be seen (in longitudinal sections) in Segments 10 and 11. I presume that these are the testes and ovaries, though there was no means of telling their nature from their structure. In another specimen which had advanced a little, but not much, further towards maturity the gonads were bigger, and stretched right across the segment, and their nature (testis and ovary) could be established from their minute structure, but there was no trace of ducts or of spermathecæ.

After examining five or six specimens of Branchiura which were sexually immature, I was so fortunate as to discover a single specimen with fully developed sexual organs. This individual was very much longer than the others, the relative size being shown in the figure (fig. 1, A, B). It was an inch and a half to two inches in length. The presence of the sexual organs gave a milky-white appearance to some of the anterior segments. This was chiefly owing to the sperm-sacs.

With regard to the external differences produced by the development of the sexual organs I may direct attention to fig. 8. The clitellum occupies three segments (10 to 12). The epidermis covering this region of the body is deeper than elsewhere, but still, as in aquatic Oligochæta, consists of one layer of cells only.

On to the 10th segment open the spermathecæ. The paired apertures are situated behind the ventral seta bundles.

The atrial pores are upon Segment 11. They correspond in position to the spermathecal pores, but lie where the ventral setæ would be were these present. The ventral setæ of Seg-

ment 11 are totally absent, nor are they replaced by penial setæ.

Between Segments 11/12 are the oviducal pores. On the 12th segment the first pair of nephridiopores are to be seen, as already mentioned.

With regard to the internal parts of the generative system, the position of the testes and ovaries has been already mentioned.

The sperm-sacs are very greatly developed; they extend from the 9th to the 17th segment; they lie dorsally as well as on both sides of the intestine.

The sperm-duct opens by a large funnel on each side into the interior of the 10th segment; the sperm-duct is comparatively short, and after a few windings opens, as shown in fig. 12, into a straightish tube which leads to the exterior. In that fignre the relations of the different parts of the whole efferent apparatus are shown; the whole apparatus differs in many points from the corresponding organs of other Tubificidæ. There is a large sac (fig. 1, *At.*) on each side of the body in the 11th segment; this has a somewhat oval contour, and presents the histological structure indicated in the figure. The lumen is narrow, and lined with a single layer of epithelial cells, which has been hardly at all stained by the borax carmine; they are clear in appearance, and their nuclei are pushed back to the base of the cell : this seems to indicate that the cells are in full secretory activity. Outside of this epithelial lining is a layer of muscular fibres about equal in thickness to the epithelium, the fibres of which are mostly arranged in a circular direction. This muscular layer is covered by a very thick layer of cells, five or six deep, which seem to represent a very much thickened peritoneal coating.

This sac has a structnre which evidently corresponds to that of the atrium of other Oligochæta, particularly of R h y n c h e l-m i s[1] and M o n i l i g a s t e r,[2] and more particularly of the latter;

[1] VEJDOVSKY, "Anatomische Studien an R y n c h e l m i s L i m o s e l l a, &c.," ' Zeit. wiss. Zool.,' Bd. xxvii, Taf. xxiv, fig. 1.

[2] BEDDARD, " On the Structure of Three New Species of Earthworms, &c.," ' Quart. Journ. Micr. Sci.,' vol. xxix, pl. xii, fig. 11.

but in Branchiura, instead of receiving the vas deferens, as it does in the two genera mentioned, this organ forms a diverticulum of the vas deferens.   The difference is in fact precisely analogous to that which exists between Eudrilus and Pontodrilus.   A tubular gland exists in both: in Eudrilus the vasa deferentia open into it; in Pontodrilus they open at its base into a duct leading from it.   In the latter case there is no valid reason against regarding the two structures as homologous, and I am of opinion that the glandular sacs of Branchiura which have just been described represent a portion of the atrium.   This glandular section of the atrium passes abruptly into a narrow tube (a in fig. 1), which receives the sperm-duct just at its commencement: the sperm-duct becomes exceedingly narrow just before it opens into this tube, which, like the sperm-duct, is ciliated.   The distal part of the atrium, at first ciliated, loses the cilia further down, and the character of the lining epithelium abruptly changes: the cells become taller, and the cellular membrane as a whole is thrown into folds.   Both regions of the tube are enveloped by a thick muscular coat, but the most distal portion is probably protrusible, since it is surrounded by a space, which is, I think, as in Tubifex, &c., lined by epithelium.   The male efferent apparatus of Branchiura differs, therefore, in a number of points from the corresponding structures of other Tubificidæ.   The division of the atrium into two parts is commonly seen in the Tubificidæ, and is particularly well marked in Bothrioneuron Vejdovskyanum,[1] but in no Tubifex is the glandular part so greatly developed, nor is there any other genus in which the vas deferens joins the muscular part of the atrium, thus making the glandular part into a cæcum.   This particular relation of the two parts of the atrium to the vas deferens is common among earthworms.

The spermathecæ lie in Segment 10.   They are more or less pear-shaped, narrowing towards the external opening. They have no cæca or glandular appendages of any kind. They contained spermatozoa not aggregated into bundles.

[1] Stolc, loc. cit., Taf. iv, fig. 7, *zchv* and *at*.

The ovaries, as already mentioned, lie in the 11th segment. In the sexually mature worm I found masses of developing ova in Segments 12 and 13 ; in the latter segment they were in close contact with the sperm-sacs, and appeared to be enveloped in a common sheath. In Segments 18 and 19 were two or three fully mature ova, which, as in most aquatic worms, are of large size, and are filled with spherical yolk bodies.

For a long time the existence of oviducts in the Tubificidæ was unknown, and the ova were believed to leave the body in some way through the penis sheath. These structures were first found by Stolc in Ilyodrilus and Psammoryctes, and subsequently by myself in Clitellio and Hemitubifex.[1] I have found them in Branchiura, where they are of precisely the same structure as in other Tubificidæ.

Affinities.

It will be apparent from the above account that Branchiura must be referred to the Tubificidæ, of which family, however, it forms a very distinct new genus. It is difficult to say to which of the remaining genera of the family it comes nearest; it is, indeed, nearly equally remote from all. The absence of prostates ("Cementdrüsen"), as well as the presence of the integumental network of blood-vessels, perhaps brings Branchiura into nearer proximity to Ilyodrilus than to any other Tubificidæ. Stolc has shown how Ilyodrilus connects the Tubificidæ with the Naidomorpha ; but the relationship of the Tubificidæ with the higher forms is not yet clear. I may point out, therefore, that the glandular part of the atrium in Branchiura shows in its structure a certain amount of resemblance to that of the Lumbriculidæ (Rhynchelmis), and to that of the simply organised earthworm Moniligaster. Its relations to the vas deferens are unlike what occurs in any of the lower Oligochæta, but are paralleled in many earthworms (e. g. Pontodrilus, Cryptodrilus).

It is impossible at present to distinguish generic from

[1] "On Certain Points in the Structure of Clitellio" (Claparède), 'Proc. Zool. Soc.,' 1888, p. 485.

specific characters, and so the following attempt at generic and specific definition must be regarded as only tentative.

Branchiura, nov. gen.

Hinder 50—80 segments furnished with a series of branchial processes, a pair to each segment, arising one from the dorsal and one from the ventral median lines. Setæ capilliform and uncinate, the former kind only found in the dorsal bundles of the anterior segments. An integumental capillary plexus present. Atria consisting of a large oval sac enveloped, as in Lumbriculidæ, by a thick layer of peritoneal cells, and of a narrow tube leading to exterior; vas deferens opens at junction of glandular and non-glandular part of atrium.

Branchiura Sowerbyi, n. sp.

Two pairs of specially dilated hearts in Segments 9, 10. Intestine begins in 11; pigmented peritoneal cells appear first in Segment 6.

EXPLANATION OF PLATE XIX,

Illustrating Mr. Frank E. Beddard's paper on "A New Branchiate Oligochæte (Branchiura Sowerbyi)."

Fig. 1.—Branchiura Sowerbyi, nat. size. *A.* Larger (sexual) individual. *B.* Smaller individual, representing the average size.

Fig. 2.—Branchiura Sowerbyi, an individual slightly magnified.

Fig. 3.—Setæ of dorsal bundle. *a.* Capilliform seta. *b.* Uncinate seta. *c.* Sigmoid setæ.

Fig. 4.—Prostomium and three anterior segments.

Fig. 5.—Branchiæ, showing transverse wrinkles due to contraction. *Br.* Dorsal series. *Br'.* Ventral series.

Fig. 6.—A single branchia, showing details of structure. *c.* Cuticle. *Ep.* Epidermis, beneath which lies blood-capillary. *M.* Muscular fibrils stretching across lumen. *D.* "Diaphragm."

Fig. 7.—A part of integumental network of posterior segments. *Br.* Branchia. *Int.* Intestine.

Fig. 8.—Ventral external view of Segments 9—14. *cl.* Clitellum. *s.* Ventral seta bundles. *Sp.* Spermathecal pores. ♂. Atrial pores. ♀. Oviducal pores. *np.* Nephridiopores.

Fig. 9.—Diagram to illustrate principal blood-vessels of Segment 8. *al.* Alimentary canal. *N.* Nerve-cord. *D. V.* Dorsal vessel. *V. V.* Ventral vessel. *H.* Contractile heart. *S. i. V.* Supra-intestinal vessel. *H'.* Intestinal heart, giving off on each side a vessel which joins the integumental network. *L. V.* Lateral vessels connected with integumental network.

Fig. 10.—Principal trunks of vascular system in anterior region of body. Lettering as in Fig. 9.

Fig. 11.—Corpuscles from axial cavity of branchia.

Fig. 12.—Male efferent apparatus. *F.* Funnel of vas deferens. *Vd.* Vas deferens. *X.* Junction of vas deferens with atrium (*At.*). *Cav.* Cavity of atrium. *Vd'.* Coils of vas deferens lying behind atrium. *a.* Ciliated thin-walled tube leading from atrium to *b*, protrusible penis, separated by a space from its muscular sheath.

Fig. 13.—Transverse section through body in branchial region. *Br. d.* Dorsal branchia. *Br. v.* Ventral branchia. *Int.* Intestine. *N.* Nerve-cord. *sp.* Septum separating dorsal from ventral part of cœlom. *D. V.* Dorsal vessel. *V. V.* Ventral vessel; the latter gives off a branch, which immediately divides to supply dorsal and ventral branchiæ.

Fig. 14.—Extenal opening of nephridium through a dilated vesicle (*N.*). *s.* Ventral seta.

Fig. 15.—Longitudinal section through head, to show position of brain, which lies in prostomium and first segment of the body.

The Formation of the Germ-layers in Crangon vulgaris.

By

W. F. R. Weldon, M.A., F.R.S.,

Fellow of St. John's College, Cambridge; Jodrell Professor of Zoology in University College, London.

With Plates XX, XXI, and XXII.

THE development of the common shrimp has recently been studied by Kingsley,[1] whose papers contain full references to the work of other authors on Crustacean embryology. To these, and to the memoir of Nusbaum [2] on the development of Mysis, the reader is referred for an account of the literature of the subject.

Professor Kingsley's account of the later development of many important organs, especially of those of mesoblastic origin, is somewhat meagre; I hoped, during a recent visit to the Laboratory of the Marine Biological Association at Plymouth, to supplement his work by observations on the later history of the mesoderm. A preliminary study of the mode of formation of the germinal layers has, however, led me to form a conception of the early development of Crangon which differs widely from that arrived at by Kingsley. My observations on the early stages are, therefore, described in what follows.

Embryos of various stages were obtained by the examination of newly-caught females; any attempt to rear the eggs in

[1] 'Bulletin of the Essex Institute,' xviii, p. 99, and xxi, p. 1, Salem, Mass., 1886 and 1889.

[2] 'Archives de Zoologie Expérimentale,' v, 1887.

captivity being rendered useless by the great abundance of
material procured for me by the fishermen of the Association.
The gravid females were killed by immersion in a solution of
corrosive sublimate, warmed to about 50° C., and were after-
wards treated with increasingly strong alcohol in the usual
way. The shells were easily removed from the eggs with
needles; and the eggs themselves were stained, either with
picrocarmine in the case of those intended for sections, or
when intended for surface views with Grenacher's alum-
carmine.

The youngest eggs observed were already divided super-
ficially into four equal segments. The egg at this stage is
somewhat elliptical, having a long diameter of 0·5 mm., a short
diameter of 0·4 mm. It is surrounded by a single, delicate,
transparent shell-membrane, which is, as pointed out by
Kingsley, completely filled by the living egg. The appearance
of the hardened egg, after removal of the shell, is seen in
fig. 1, where the whole surface of each blastomere is seen to
be crowded with small spherules of yolk. This spherical con-
dition of the yolk-particles was well retained in all my speci-
mens, though Kingsley appears to have found that the yolk
spherules broke up and ran together into masses during the
process of hardening. The yolk spheres are so densely
crowded on the surface that the nuclei cannot at this stage be
rendered visible in the uninjured egg. In sections a single
nucleus, surrounded by a small mass of protoplasm, is found
in the centre of each blastomere. The protoplasmic mass
sends processes into the yolk, which can easily be followed for
a certain distance; and there can be little doubt that a proto-
plasmic reticulum extends between the yolk spherules through
the whole substance of each blastomere. Whether a proto-
plasmic connection between the adjacent blastomeres is present
or absent I have been unable to determine.

It is to be noticed that during the stage with four nuclei
the segmentation furrows are simple superficial grooves,
which do not extend to any considerable distance below the
surface of the egg, so that the great mass of the yolk is as yet

undivided.   Kingsley, who has described the unsegmented egg and the first two nuclear divisions, states that no furrows appear until four nuclei are present.   The rudimentary condition of the furrows in my specimens with four nuclei affords a strong confirmation of this statement.

In the next stage observed eight cells were present, arranged in the manner shown in fig. 2.   Round the equator of the egg were four cells arranged in a ring, while at each pole were two cells.   The upper and lower cells of fig. 2 are unfortunately so placed that each conceals a second cell.   Sections through the eight-celled embryo show that the segmentation furrows are now much deeper than during the four-celled stage.   The drawing given in Pl. XX, fig. 10, represents, as accurately as may be, the appearance of a median section.   Two of the blastomeres appear to be completely separated, by well-marked fissures, from the rest; but the three remaining cells, through which the section passes, are connected by their central ends with a small, irregular, unsegmented mass of yolk.   In other sections of the series a similar communication with the central mass could be demonstrated in the case of every cell.

I have taken some little trouble in order to determine whether this central mass of yolk, with which all the cells appear to communicate, is a mere result of manipulation, or whether it is really present in the living egg.   In transparent preparations of the hardened egg, or in living specimens, it seems easy to trace the cell outlines almost to the centre; and examination of specimens in this manner led E. van Beneden to the conclusion that the segmentation was total.   But it is difficult to be quite sure of the distinction of the central extremities of cells which are seen through a maze of spherical outlines due to the superficial yolk; and, on the whole, from the great uniformity of the appearances seen in section, it seems probable that the segmentation is really centrolecithal and incomplete, though the central mass of unsegmented yolk is smaller than in many Decapods.

The stage with sixteen cells appears to follow immediately upon that just described, by division of each of the eight blas-

tomeres into two. In this way an equatorial band of eight cells is produced, with a cap of four cells at each pole. An egg at this stage, seen from one pole, is shown in fig. 3. The central yolk-mass is more evident in section than before; and there seems in some cases to be a well-marked difference between the character of the unsegmented mass and that of the yolk contained in the blastomeres themselves. An extreme case of such difference is shown in fig. 11, where the central mass consists of small, highly-refracting spherules, packed closely together, and contrasting strongly with the larger, less refringent masses of the peripheral yolk. This contrast between the two kinds of yolk varies greatly in extent, and may, perhaps, be due to variations in the action of reagents. In the later stages it entirely disappears. As will be noticed in fig. 11, the development of the appearances referred to is frequently accompanied by a very distinct separation between the central yolk-mass and the surrounding blastomeres.

In spite of Kingsley's definite statement to the contrary, I feel sure that the central mass consists, both now and during the whole period of segmentation, of yolk containing only a minimal quantity of protoplasmic reticulum, and entirely devoid of nuclei. Kingsley's statements, which will be discussed below, led me to pay especial attention to the constitution of the central mass; and I have absolutely failed to demonstrate the presence of central nuclei, or of central masses of protoplasm, at any period of segmentation. In sections stained with picrocarmine the contrast is so striking between the brilliant pink of the nuclei, the rosy colour of the protoplasm, and the bright yellow of the yolk, that it would be difficult to overlook a nucleus in the most cursory examination of a series of sections.

The nuclei, as will be seen from fig. 3, do not appear on the surface during the stage with sixteen cells. The stage with thirty-two cells I have not seen; but at sixty-four cells the nuclei are distinctly visible on the surface of the blastomeres (fig. 4).

The regular character of the segmentation is preserved until

128 cells are present, the cell outlines still extending nearly to the centre of the egg. The median section drawn in fig. 12 shows that the central yolk-mass is at this stage composed of elements similar to those of the rest of the yolk. After this period the regularity appears to be lost, for in the next stage I have only been able to count 175 nuclei.

During the later stages of segmentation the nuclei, with their surrounding protoplasm, approach more and more closely to the surface of the egg, while the segmentation furrows become less and less distinct. In the stage with 175 nuclei the furrows can only be traced through about half the distance from the periphery of the egg to the centre (see fig. 13) ; and at the same time the central yolk exhibits a marked tendency to run together into large, irregular masses under the influence of reagents.

The later stages of the segmentation have not been followed in detail. At the close of this period the egg is surrounded by a layer of cells which are, except in one small region, uniform in character; while the yolk is devoid of nuclei. At one point, which marks the posterior end of the future embryo, is a shallow depression (fig. 5, *Blp.*) lined by cells which are richer in protoplasm than their neighbours, and which are about to divide and to pass again into the yolk.

Professor Kingsley's account of the segmentation differs in many important points from that which is here put forward. According to this observer, the segmentation furrows are throughout confined to the surface of the yolk, so that the greater part of the substance of the egg remains permanently undivided. At the stage with "about sixteen cells," he describes and figures (first paper, p. 106, and fig. 4) a central nucleated mass of protoplasm in the position of the mass described above as unsegmented yolk. He says:

" As will be seen from fig. 4, most of the protoplasm has reached the surface of the egg, but there still remains some near the centre of the yolk. Whether this is the same as the protoplasm described by several authors . . . . I cannot say ; but I am certain not only that it is derived from the first seg-

mentation nucleus, but that it plays a part in the formation of the blastoderm."

The later history of this central mass is described as follows:

" While the cells which have reached the surface and which have thus formed a blastoderm are undergoing division, this central protoplasm also divides and migrates, though much more slowly, to the surface," giving rise to the blastoporic patch of cells described above.

This account of the segmentation is so definite, and at the same time so inconsistent with anything which I have myself been able to observe, that I cannot offer any plausible suggestion as to the cause of the differences between Professor Kingsley and myself, unless it be that the mode of segmentation in the same species really differs on the opposite shores of the Atlantic.

The process of formation of the endoderm and mesoderm, which commences at the close of the period of segmentation, proceeds so slowly that these layers are not completely established before the assumption of the Nauplius condition. The stages between the blastosphere already described and the perfect Nauplius will, therefore, be considered together.

The changes in external appearance may be gathered from figs. 5—9. At the close of segmentation the egg is covered, as already stated, by a single layer of cells, which are uniform in character except over a small, depressed area, representing the blastopore. This blastoporic area marks the posterior extremity of the future embryo, and that surface of the egg on which it lies is often slightly flattened, giving an indication of the ventral surface. The relation of the blastoderm to the egg is, however, subject to change, owing to the readiness with which each egg is deformed by pressure.

After the establishment of the blastoporic area, the cells of the ventral surface thicken, and those on each side of the middle line divide, so that an irregular band extends forwards from the blastopore on either side, in which the nuclei are more

crowded than over the remainder of the egg.   The distribution of nuclei at this stage is shown in fig. 5a.

Shortly after the establishment of two ventral bands of crowded nuclei, each band becomes divided into three distinct regions: an anterior optic lobe, corresponding to the cephalic lobe described by Reichenbach in Astacus ;[1] a median region, in which the nuclei are less densely crowded; and a posterior thickened and densely nucleated region, corresponding to the thoracico-abdo-minal plate of Reichenbach, or to the ventral (neuro-muscular) plates described by Kleinenberg in the larva of Lopadorhynchus.[2] The optic lobes (fig. 6, o. p.) are characterised by the more or less definitely concentric arrangement of their closely-packed nuclei.   The region between these and the ventral plates will ultimately give rise to the two pairs of antennæ and to the mandibles; while the post-mandibular ectoderm, together with the whole mesoderm of the body, arises from the ventral plates. These ventral neuro-muscular plates (fig. 6, n. m. p.) are more conspicuous than the remaining portions of the ventral bands, because their surface is externally concave (compare the section, fig. 16).   The nuclei in these plates are arranged in irregularly concentric rings, like those of the optic lobes.   The irregularity of these nuclei is perhaps exaggerated in the figure.   The blastopore is not closed, as Kingsley erroneously supposes, but may be distinctly recognised as a very small pit, surrounded by a ring of nuclei, between the posterior margins of the ventral plates (see fig. 6, Blp.).   This embryo, therefore, corresponds fairly well with Reichenbach's stage, as shown in his fig. 3, pl. ii; the only important difference between the two arising from the very small size of the blastoporic patch in Crangon as compared with that of Astacus.

There is a considerable gap between the stage just described and that shown in fig. 7; the changes which occur in the interval will, however, be readily understood from the figures themselves, and from the sections of intermediate stages to be presently described.

[1] 'Studien z. Entw. d. Flusskrebsse,' Frankfurt a/M.
[2] 'Zeitschr. f. w. Zoologie,' Bd. xliv, 1886.

The embryo drawn in fig. 7 corresponds to Reichenbach's stage EF, figured on pl. iii, fig. 8, of his work. The optic lobes have much the same appearance as that already seen in fig. 6, while the bands connecting these with the ventral plates have made considerable progress. The first antennæ are already visible as well-marked hemispherical projections, while a ganglionic rudiment appears as an aggregation of nuclei at the base of each. The second antennæ are indicated, behind the first, by a slight increase in the crowding of the nuclei, which in this place exhibit to a marked degree the curious arrangement in intersecting curves which is so well seen in Reichenbach's figures of Astacus. Behind the second antennæ, and just in front of the ventral plates, the mandibles are already present as a pair of slight projections. The concavity of the ventral plates is well marked at this stage, and causes these structures to appear separated from one another in the middle line by a raised ventral crest. A quite similar horizontal ridge seems to bound the ventral plates anteriorly; and this ridge has apparently been mistaken by Kingsley for the commencement of a pit, which will ultimately, according to him, produce the ventral flexure of the body characteristic of the later stages. Kingsley appears to have examined a section passing through the blastoporic pit (which he calls " proctodeum ") and through the concavity of one ventral plate, which he has regarded as representing the commencement of an abdominal flexure; but his figures are very difficult to reconcile with the appearances seen in sections by myself.

Between the first antennæ is a median aggregation of nuclei; and behind this, on a level with the posterior border of the antennules, is a small depression, which is distinctly visible in sections, but which could not be satisfactorily shown in fig. 7. This depression is the mouth; and from its position in this and in the next stage (compare fig. 8) the first antennæ are evidently præoral from the very earliest period at which the mouth is visible. This view of the relations between the mouth and the first antennæ is, I think, unquestionably justified by the figures referred to, together with those of the

sections represented in figs. 23 and 24; but it is in flat contra-
diction to Kingsley's statements, and to his remarkable fig.
32 (second paper, pl. i), in which a black dot placed between
the optic lobes is called the mouth.

It is to be observed that during the stages of figs. 6 and 7,
the ventral bands which form the blastodermic area occupy
nearly half the surface of the egg. During the following
stages a remarkable shrinking occurs; so that the Nauplius
occupies a portion of the surface of the egg smaller than that
occupied by the newly-established germinal bands. Kingsley
has called attention to this shrinking of the embryonic area in
Crangon; and a similar shrinking was observed in Pagurus
by Paul Mayer.

At the stage represented in fig. 8, all the Nauplius appen-
dages have already become distinct. The optic plate has the
form of a rounded lobe, distinctly marked off from the rest of
the blastoderm behind, but passing gradually into it in front.
The first antennæ are larger than the other appendages, and
are dilated at their extremities. Their præoral position is at
this time unmistakable, the mouth appearing as a narrow
transversely elongated slit, bounded by closely-set nuclei, at
the level of the interval between the first and second pairs of
appendages. The second antennæ and the mandibles are
simple, rounded papillæ. The ventral plates have ceased to
be prominent externally, though they are, as will be seen,
easily recognised in section. The thoracico-abdominal rudi-
ment has the form of a rounded papilla, projecting slightly
from the posterior surface of the blastoderm, but connected
with the cephalic region by an even slope. There is at this
stage no sharp flexure of the posterior part of the embryo
upon the head, such as is seen in the next stage. It need
hardly be pointed out, that the existence of such a stage as
that here described is quite incompatible with the formation
of an abdominal flexure at an earlier period by the appear-
ance of the epiblastic pit described by Kingsley. The nuclei
of the whole post-oral region of the body exhibit a marked
tendency towards an arrangement in parallel rows, lying

transversely to the long axis of the embryo. In the thoracico-abdominal region this transverse arrangement is particularly well seen ; the nuclei in this region forming a series of rings, concentrically arranged round a point which occupies the apex of the papilla, and which indicates the position of the now closed blastopore.

The last stage figured is a fully-formed Nauplius (see fig. 9) which has already undergone an ecdysis. The external appearance of this embryo at this stage is greatly modified by the growth of the thoracico-abdominal papilla, which has greatly increased in size, and has at the same time become folded forwards over the cephalic blastoderm. The optic lobes are larger than before, and each lobe is divided into an outer retinal portion, in which the nuclei are larger, and exhibit a more definitely concentric arrangement; and an inner, ganglionic region, with smaller and more densely crowded nuclei. The optic ganglion is continuous with that at the base of the first antennæ. The first antennæ themselves are much larger than before ; the free portion of each projects transversely outwards for nearly half its extent, and then becomes bent at right angles, so as to project directly backwards. The bases of the first two antennæ are connected by a prominent ridge, which is crowded with nuclei, and which overhangs the mouth ; so that the mouth itself is not visible in the uninjured Nauplius. The second antennæ arise behind the first, and below (dorsal to) the transverse swelling just described ; they are already distinctly biramous, the outer branch being the larger. The mandibles are partly concealed by the antennæ, and are still relatively small ; they are seen in section to be distinctly biramous. The thoracico-abdominal rudiment is now much swollen at its base, and is prolonged at its extremity into a flattened papilla of considerable size, with a rounded and very slightly emarginate apex, which is folded over the cephalic portion of the embryo. This mode of origin of the ventral flexure, by the growth of a papilla which bends forwards as it becomes larger, is quite similar to that described by Reichenbach in Astacus, and by Nusbaum in

Mysis. It is, however, quite incompatible with the account given by Kingsley, and already referred to, of the process in Crangon itself. The transverse arrangement of the nuclei is somewhat obscured in surface views by the peculiar curvature of the embryo; but it is, as will be seen, more evident in sections during this stage than in younger embryos.

From the stage represented in fig. 7 to the Nauplius stage a small patch of thickened ectoderm-cells—the " dorsal organ " —is present on the posterior dorsal surface of the embryo. I have nothing to add to the account of this structure which has been given by Kingsley. Its later history, together with that of other organs, I hope to describe in a future paper.

The internal changes which accompany the development may now be described.

Immediately after the formation of the blastoporic area shown in fig. 5, an invagination commences. The cells of the blastoporic area divide, and apparently become amœboid, some of them travelling from the surface of the blastoderm into the substance of the yolk. Certain of these cells, which evidently correspond to the "lower-layer cells" (vitellophags) of Nusbaum, send out processes in all directions among the spherules of the yolk, and become irregularly distributed through its substance. Other cells remain, for a considerable time after their invagination, in the immediate neighbourhood of the blastopore. As will appear directly, I am inclined to regard the whole of the invaginated cells as forming endoderm; and I have failed to find any evidence of an ultimate formation of blood-corpuscles from the cells which first split off from the blastoderm. I have also failed to recognise the mesoderm cells spoken of by Kingsley, who distinguishes " some cells with large nuclei and amœboid outlines, which are plainly budding out from cells at the mouth of the blastopore, and sinking into the yolk." In Kingsley's figure (first paper, fig. 9) there is no apparent evidence of any difference between the various cells which are undergoing invagination; and no explanation is attempted of the manner in which these so-called mesoderm-cells separate from the rest of the un-

doubted endoderm, and migrate towards the ventral surface of
the yolk to form the mesodermic bands.

The appearance of a section passing through the blastopore
during this process of invagination is shown in fig. 14. The
cells of the ectoderm, which cover the greater part of the sur-
face of the embryo, are seen to be even more superficial than
at the close of segmentation; and the divisions between them
extend only for a very short distance into the yolk. The
great mass of the yolk is therefore undivided; and into this
undivided mass the ectoderm-cells wander. The figure is
drawn with a considerable amount of care, and represents with
fair accuracy the appearance of a typical section. It will, I
think, be admitted that there is no difference in character
between the invaginated cells so great as to enable any
observer to say that some are endodermic and others meso-
dermic in nature.

Immediately after the invagination of the amœboid cells
shown in fig. 14, the ventral surface of the blastoderm
thickens, as is indicated by the crowding of the nuclei in
fig. 5a.

In embryos of the age of fig. 6, the endoderm is separated
into two distinct portions : one of these is formed by the cells
which were first invaginated (vitellophags of Nusbaum), and
which have by this time become scattered irregularly through
the substance of the yolk (figs. 15 and 16, En'); the other
forms a more or less compact mass of cells, confined to the
posterior portion of the embryo, and continuous with the cells
of the persistent blastoporic area (fig. 15, En''). The posterior
mass of endoderm frequently contains a small lumen, such as
that shown in fig. 15 ; but this lumen is not always demon-
strable, and I have never been able to show that it communi-
cates with the exterior.

The relations of the ectoderm at this stage will be under-
stood from figs. 15 and 16, one of which represents a median,
the other a lateral longitudinal section. The ectoderm is
everywhere one cell deep ; in the middle ventral line (fig. 15)
the cells are only slightly thicker than those outside the em-

bryonic area; but in the region of the ventral bands the thickening is greater, especially in the optic lobes and in the ventral plates. The optic lobes (fig. 16, *o. p.*) consist of long, closely-packed cells, with elongated nuclei, the cells of the ventral plate being broader in proportion to their length, with more nearly spherical nuclei. The concavity of the ventral plate is fairly well seen in fig. 16.

The condition of an embryo intermediate between figs. 6 and 7 is represented in figs. 17—22. The endoderm is seen to differ from that of younger embryos chiefly in the greater size of the posterior portion, which is still continuous with the blastoporic area. In the specimen from which the transverse sections, figs. 20 and 21, were prepared I could find no trace of a lumen in the posterior endoderm. An egg, taken from the same mother, was cut longitudinally, and the median section (fig. 22) shows a distinct cavity, which does not communicate with the exterior. The lumen is present in about half my series of sections through embryos of this age. It is very difficult to determine whether the increase in the amount of posterior endoderm which has taken place since the last stage is due to a continuation of the process of invagination, or to simple division of the previously invaginated cells. The appearances indicated by figs. 20—22 seem equally consistent with either view. An interesting feature of the posterior endoderm is its apparent tendency to become continuous with the ectoderm in the middle ventral line. The section drawn in fig. 21 is near the anterior limit of the original blastoporic area, and that shown in fig. 20 is the sixth of a fairly thick series of sections in front of it. In the anterior section the large endoderm-cell seemed to be distinctly continuous with the small cells between the ventral plates. This kind of appearance will be noticed in sections through the later stages. It is, of course, possible that these appearances of a fusion between endoderm and ectoderm are merely accidental; but it is equally possible that they are indications of a ventral elongation of the blastopore, in which case they will be regarded by many morphologists as of great importance.

The anterior scattered endoderm-cells have increased in number since the last stage, partly, no doubt, by division of previously scattered cells, but partly by the migration of cells from the posterior endoderm. Such a migration of cells is distinctly indicated in fig. 22. A fair idea of the number and distribution of these scattered cells in the anterior part of the body may be gathered from figs. 18 and 19.

The ectoderm has undergone few changes of importance. The optic lobes (fig. 17) have the same structure as before; behind these, in the region of the first antennæ, the ventral ectoderm is slightly thickened (fig. 18), while still more posteriorly the stomodæum is already visible as a slight pit, hollowed out in a thickened mass of ectoderm. It will be understood that the apparent symmetry of the mouth in fig. 19 is due to the obliquity of the section.

In the region of the trunk the ventral plates are well seen (figs. 20 and 21) as concave thickened plates. Anteriorly these plates are still one cell thick, but behind the cells are in places arranged in two layers. In some sections (one of which I have purposely drawn in fig. 21) the appearances are consistent with the possibility that the cells of the inner layer (the future mesoderm) are added to the ventral plate by migration of cells from the primitive endoderm; but I feel convinced, from the examination of many series of sections, that this is not the case. The elongated cell on the right-hand side of fig. 20, the spindle in an undoubted ectoderm-cell of fig. 27, and the general appearance of the ventral plates in fig. 29 give evidence, of a kind which might have been indefinitely multiplied, that the doubling of the layers in the ventral plates is really due to a division of the ectoderm-cells. The doubtful case shown in fig. 21 has been given principally in order to do the fullest possible justice to Professor Kingsley's contention that the primitive mesoderm is entirely invaginated from the blastopore.

When the stage represented in fig. 7 is fully attained, both ectoderm and endoderm have made important progress. The endoderm, in the region of the ventral plates, is in much the

same condition as before, but the lumen, which was at least occasionally present during the earlier stages, has now entirely disappeared ; and the whole posterior portion of the endoderm is now a branched vacuolated mass. The blastoporic area is no longer conspicuous in surface views of the embryo, though its position is indicated by the angle between the posterior portions of the ventral plates (fig. 7, *Blp.*) ; but the region of continuity between ectoderm and endoderm in the middle ventral line is even more conspicuous than before (fig. 27). The separation between the lateral endoderm and the ventral plates is even more conspicuous than before. The cells of the anterior endoderm have become more abundant ; and while certain of their number remain scattered through the yolk, others are beginning to arrange themselves in a layer on the ventral surface of the yolk, so that the alimentary canal begins to acquire a definite epithelial floor. This layer of endoderm is already fairly complete in the region of the mandibles and of the second antennæ, while further forwards it is still imperfect (compare figs. 23—26, *En'*). Posteriorly, the epithelial layer of anterior endoderm becomes continuous with the posterior mass already described. This anterior layer of endodermal epithelium appears to correspond, on the one hand, with the similar layer of anterior endoderm described by Nusbaum in Mysis, and on the other with the layer of flattened cells, fusiform in section, which are stated by Kingsley to represent the cephalic mesoderm.

The ectoderm has become specialised, in the region of each optic lobe, into a retinal plate, which is one cell thick, and a ganglionic region, consisting of several layers of cells. The appearance of the optic lobes in section is nearly the same as that shown in fig. 28, from an older embryo. The first antennæ appear immediately behind the optic lobes (fig. 23, *Ant. i*), and at the inner side of the base of each is an aggregation of ectodermal nuclei representing a commencing ganglion (fig. 23, *N. S.*). Five sections behind the level of fig. 23 appears the stomodæum (fig. 24, *Stom.*), on each side of which is an aggregation of nervous nuclei. The antennules are almost

entirely free from the body in the section figured. The second antennæ are represented simply by densely nucleated portions of ectoderm (fig. 25) which do not as yet project beyond the surface, while the mandibles are already visible as slight projections (fig. 26, *Md.*).

The ventral plates (fig. 27, *n. m. p.*) are thicker than in previous stages, both because the individual cells are larger, and because they are arranged more completely than before in a double layer. The section figured is especially fortunate, because the spindle which appears on the right-hand side shows distinct indication of a transverse division of the ectoderm-cell to which it belongs. The very remarkable appearance of the nuclei of the left ventral plate may, perhaps, be an indication that these also are about to divide.

In the next stage (fig. 8) the principal changes affect the external form. The endoderm is practically unaltered, except for the further addition of wandering anterior cells to the ventral cephalic layer. The ectoderm is pulled out, as already stated, into well-marked processes which form the three pairs of appendages, and the structure of the optic lobes is slightly more complex than before. The division into a retinal plate (fig. 28, *r. p.*) and a ganglionic region (*o. g.*) is sufficiently obvious. The ganglia are confluent in the middle line, and partly overlap the retinal plate. The section figured being somewhat oblique, the retinal plate appears free on one side, while on the other it is partly covered by nervous cells.

The ventral plates (fig. 29, *n. m. p.*) have much the same appearance as that seen in older embryos of the last stage ; they are, however, still more distinctly two-layered. Between these plates, in the middle line, the appearance of a fusion between ectoderm and endoderm is still retained.

With the growth of the thoracico-abdominal papilla the endoderm and the ventral plates undergo important changes. A horizontal longitudinal section through the papilla of an embryo intermediate between figs. 7 and 8 is shown in fig. 30. With the growth of the thoracico-abdominal papilla, the posterior endoderm is drawn upwards into the cavity of that

structure; and at the same time the endodermal cells arrange themselves in the form of a tube, closed posteriorly and dorsally, where it is in contact with the former blastoporic area, and widely open anteriorly, where its funnel-shaped mouth embraces the yolk, its ventral wall being continuous with the ventral sheet of cephalic endoderm already described. The ventral plates commence at this time to increase in size; and at the posterior extremity of each a pair of large cells is found (fig. 30, *m. b.* and *e. b.*), one cell being especially related to each of the two layers which form the plate.   These cells are evidently homologous with the "Knospungszellen" of Reichenbach, and may be spoken of as ectoblasts and mesoblasts respectively.

I regret that in spite of numerous attempts I have been unable to find an embryo which showed the exact mode of origin of these large cells; but there can, I think, be little doubt that they arise as specialisations of cells which already existed in the ventral plates during previous stages, and not by the addition to these plates of new cells derived from the endoderm.

When the embryo has reached the stage represented in fig. 19, the budding cells have increased in number, and each has a fairly definite relation to a band of cells on the ventral side of the embryo.   The flexure of the thoracico-abdominal papilla prevents these cells from appearing in surface views; but their relations will be apparent from the horizontal longitudinal sections of the papilla which are represented in figs. 31—33, and which cut the embryo in planes parallel to the line *x y* in fig. 34, and perpendicular to the plane of the paper. Of these sections, fig. 31 represents the most dorsal, fig. 33 the most ventral.   In fig. 31 the tubular portion of the posterior endoderm is well seen; and its perfect continuity with the cells of the first-formed endoderm, which by this time form a fairly regular layer on the ventral surface of the yolk, is also evident.

The section passes through a single mesoblast on each side, which lies at the extremity of a considerable plate of well-

differentiated mesoderm. On one side there is a single ecto-
blast outside the mesoblast, on the other there are two such
cells. In fig. 32, which is ventral to fig. 31 and separated
from it by the thickness of two sections, two more mesoblasts
are present, and the mesoderm extends forwards from these in
the form of a pair of broad bands, meeting in the middle line
below the alimentary canal, but not as yet extending into the
cephalic region. In several series of sections, cutting embryos
of this stage in various planes, I have been able to recognise
four large mesoblasts, and four only. I am, therefore, inclined
to believe that the number is constant. Outside the meso-
blasts in fig. 32 are seen two more ectoblasts; but the definite
relation of the ectoblasts to the cells of the ventral and lateral
ectoderm can only be fully realised by the examination of
superficial sections, parallel to the surface of the ectoderm.
Such a section is represented in fig. 33, where four ectoblasts
are seen, each of which is at the posterior extremity of a row
of ectodermal cells. The nuclei of these ectoderm cells are so
arranged that they fall with equal facility into transverse or
longitudinal series. The transverse arrangement is most strik-
ing in views of the whole embryo, and has already been alluded
to; but the reality of the relation to the ectoblasts, indicated
in fig. 33, can hardly be doubted. This arrangement of the
ectoderm-cells can be traced for a considerable distance along
the ventral face of the cephalic region; but those sections in
which it is best seen must necessarily cut the embryo in various
oblique planes, and explanation of them would be long and
tedious; figures of such preparations have, therefore, been
omitted.

The relations of mesoderm and endoderm can, perhaps, be
more clearly gathered from the lateral longitudinal section
shown in fig. 34, in which the connection between the tubular
posterior endoderm and the ventral layer produced by the
rearrangement of the first-formed anterior endoderm-cells is
particularly well seen.

The structure of the remaining organs of the Nauplius may
be most conveniently considered in connection with the history

of the later development, and a fuller description of the embryo at this stage is therefore deferred.

The agreement between the account here given of the formation of the layers in Crangon, and that given by Nusbaum of the corresponding process in Mysis, is on the whole fairly complete; but there are, nevertheless, certain points of difference which are not unimportant. The cells which are here spoken of as anterior endoderm correspond closely in their mode of origin with Nusbaum's "Vitellophags," being cells which separate from the rest of the blastoderm before the main body of the endoderm, and which immediately wander through the yolk. The corresponding cells of Mysis differ from those seen by me in the fact that they are separated from the superficial portions of the blastoderm at a slightly earlier stage; but they resemble the cells of Crangon in arising from the blastoporic area, which serves in both cases as a centre of dispersion, from which the cells scatter through the yolk. The statement repeatedly made by Nusbaum that these cells take no part in the formation of the alimentary epithelium does not seem to me to be proved either by his descriptions or by his figures. The extreme rarity of the "Vitellophags" in all those stages in which an endodermic epithelium occurs makes an interpretation of Nusbaum's figures, similar to that which is here offered in the case of Crangon, seem perfectly possible. The formation of a tubular endodermal tract, open in front towards the yolk, and early connected with a ventral anterior sheet of endoderm is a point in which the the two genera closely agree.

The formation of the mesoderm from two ventral ectodermal plates is also described by Nusbaum. In Mysis, however, these plates appear to extend further forwards than in the shrimp, and a formation of a zone of large budding cells does not occur.

The many points of difference between Professor Kingsley and myself have been already alluded to, and I can only express regret for their existence, without being able in any

way to suggest a possible reason for the extremely different results at which we have arrived.

The account here given of the early development suggests many interesting comparisons with corresponding stages of other forms, and especially with Lopadorhynchus, but I prefer to postpone a discussion of these points until I am in a position to describe the later history of Crangon.

EXPLANATION OF PLATES XX, XXI, and XXII,

Illustrating Professor Weldon's paper on " The Formation of the Germ-layers in Crangon vulgaris."

List of Reference Letters.

Ant. i. First antenna. *Ant. ii.* Second antenna. *Blp.* Blastopore. *c. o. p.* circumoral portion of ventral plate. *Eb.* Ectoblast. *En'.* Endoderm which is just invaginated as scattered cells. *En''.* Continuous endoderm of later invagination. *Mb.* Mesoblast. *Md.* Mandible. *me.* Mesoderm. *n. m. p.* Neuro-muscular plate. *N. S.* Nerve-cord. *o. g.* Optic ganglion. *o. p.* optic plate. *r. p.* retinal plate. *Stom.* Stomodæum. *Th. abd.* Forecast of the thoracico-abdominal segments.

Figs. 1—4.—External views of segmentation.

Fig. 5.—Embryo with newly-formed blastopore.

Fig. 5a.—Outline of embryo slightly older than Fig. 5, showing the crowding of nuclei in the region of the future ventral bands.

Fig. 6.—Embryo with fully-formed ventral bands and conspicuous blastopore.

Fig. 7.—Embryo with optic plate, first antennæ, and mandibles.

Fig. 8.—Embryo with Nauplius appendages, before the formation of an abdominal flexure.

Fig. 9.—Fully-formed Nauplius, after ecdysis, with well-marked abdominal flexure.

Fig. 10.—Section through an eight-celled egg.

Fig. 11.—Section through an embryo with sixteen cells.

Fig. 12.—Section through an embryo with 128 cells.

Fɪɢ. 13.—Portion of a section through the centre of the egg at the close of segmentation.

Fɪɢ. 14.—Section passing through the blastopore at the stage shown in Fig. 5.

Fɪɢ. 15.—Median longitudinal section through an embryo of the age of Fig. 6.

Fɪɢ. 16.—Lateral section from the same series.

Fɪɢs. 17—21.—Transverse sections through an embryo slightly older than Fig. 6.

Fɪɢ. 22.—Posterior portion of an obliquely longitudinal section through an embryo of the age of Fig. 6, showing cavity in the endoderm.

Fɪɢs. 23—27.—Successive transverse sections through an embryo of the age of Fig. 7.

Fɪɢ. 28.—Transverse section through the optic ganglion at the age of Fig. 8.

Fɪɢ. 29.—Transverse section through the thoracico-abdomen at the age of Fig. 8.

Fɪɢ. 30.—Horizontal section through the abdominal papilla, at stage between Fig. 8 and Fig. 9.

Fɪɢs. 31—33.—Successive horizontal sections through the abdominal papilla at the age of Fig. 9. The sections are parallel to the line $x\,y$ in Fig. 34, and perpendicular to the plane of the paper.

Fɪɢ. 34.—Slightly lateral longitudinal section through Fig. 9.

The Pigment Cells of the Retina.

By

J. S. Boden and F. C. Sprawson,
Students of Medicine, King's College, London.

In preparing a specimen of the retinal pigment cells from a sheep's eye in the practical histology class at King's College one of us (S.) noticed that these were not all hexagonal in shape, as is usually described, but that many had seven sides ; and on searching through the specimen, cells with varying numbers of sides were found. On showing this to Professor Halliburton he suggested that we should examine a large number of specimens from different animals, in order to ascertain if the same irregularities exist there. This we have done, and the present paper contains the results of the investigation. The descriptions and figures in the various text-books are taken from Max Schultze's work on the subject.[1] In all, the cells are represented as being perfect hexagons, and the existence of cells of other shapes is not mentioned.

The method of preparation we employed was as follows :— The eye was removed from the body, and an incision having heen made through the sclerotic, it was placed in Müller's fluid for a few days. It was then transferred to very dilute Müller's fluid for a day or two in order to macerate it a little, and the eyeball freely opened ; the black pigment layer can be stripped off fairly easily. Portions of this were mounted in glycerine or Farrant's solution, and examined unstained. In some cases, especially when we examined that portion of the

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[1] 'Archiv f. mikr. Anat.,' vol. ii, and Stricker's 'Handbook of Histology.'

epithelium which in the region of the tapetum lucidum con-
tains no pigment, the specimens were first stained with dilute
magenta before being mounted.

The specimens were taken from the eyes of the sheep, ox,
rabbit, kitten, pig, frog, and hen.   In all cases the results
were the same.   Hexagonal cells are certainly the most
numerous ; heptagonal cells come next, and scattered at inter-
vals throughout a preparation, cells with four, five, eight, nine,
ten, and eleven sides are also found.   In all cases there is
never anything of the nature of a gap ; the mosaic appears to
be always perfect.   This is true for all parts of the epithelium
—that is to say, in the cells richly laden with pigment in the
tapetum nigrum, and also in the unpigmented cells over the
tapetum lucidum when that is present.

We included the hen in the list of animals the eyes of which
we examined, as it was from the retinal epithelium of this
animal that Max Schultze's figures were drawn.   Here the
shape of the cells is certainly much more uniform than in
mammalian eyes, but we saw amidst the hexagonal cells several
with five, seven, eight, and nine sides.

There can be no doubt that the polygonal shape of the cells
is due to mutual pressure, and if all the cells were of the same
size they would be necessarily hexagonal.   In the process of
growth, however, some cells, receiving presumably more nutri-
ment, grow to a greater size than their fellows.   The result of
this is that the bigger cells come into relation with a larger
number of ordinary sized cells, and so become more than six-
sided ; and in a similar manner the smaller cells are less than
six-sided.   This view is fully borne out by a number of
measurements we have made.   The number of sides in a cell
varies directly as its size.

We give below the measurements we have made in the case
of the sheep and ox.   The measurements were made with the
aid of a camera lucida, and the numbers below give the longest
and shortest diameter of the cells.

Number of sides.		Pigment layer from eye of Sheep.		Pigment layer from eye of Ox.
4	.	$16\mu \times 13\mu$	.	$14\mu \times 12\mu$.
4	.	$15\mu \times 15\mu$	.	$11\mu \times 8·5\mu$.
5	.	$19\mu \times 16\mu$	.	$13\mu \times 12\mu$.
6	.	$28\mu \times 15\mu$	.	$17\mu \times 16\mu$.
6	.	$20\mu \times 19·5\mu$		
7	.	$20\mu \times 16·5\mu$	.	$32\mu \times 17\mu$.
8	.	$28·5\mu \times 19\mu$	.	$32\mu \times 22\mu$.
8	.	$34·5\mu \times 22\mu$		
9	.	$24\mu \times 21\mu$	.	$34·5\mu \times 21\mu$.
9	.	$32\mu \times 24·5\mu$		
10	.	$38\mu \times 27\mu$	.	$42\mu \times 25\mu$.

Among the mammals examined we noticed that the pigment cells from the rabbit's eye were exceptionally large, the hexagonal cells measuring as much as the eight- to ten-sided cells from the retina of the ox and sheep.

In our examination of the retinal epithelium our chief result has thus been that the term hexagonal as applied to it is not correct; polygonal would be better. Among minor points we have also observed that many of the larger cells— namely, those with eight, nine, and ten sides—have two nuclei. With regard to the shape of the pigment particles in the cells, most of them appear to be fusiform or rod-shaped, as described by Max Schultze. Many, however, are spherical or approximately so, and certain others are spherical with a rod-like projection from one side of the sphere, as though the spherical granule were elongating to form a rod.

NOTE.—Since the above was written we have had an opportunity of examining the retinal pigment cells from a human fœtus (aged about seven months). The cells there presented the same general characters as described above, but the proportion of non-hexagonal cells was smaller than in the eyes of the other mammals we have examined.

We have also found, in Jabez Hogg's book on the 'Microscope,' a figure of the retinal pigment cells which indicates their polygonal rather than hexagonal shape, but in the descriptive letterpress no allusion is made to this fact.

Observations upon the Development of the Segmentation Cavity, the Archenteron, the Germinal Layers, and the Amnion in Mammals.

By

Arthur Robinson, M.D.,

Senior Demonstrator of Anatomy at the Owens College, Manchester.

With Plates XXIII, XXIV, XXV, XXVI, and XXVII.

SUMMARY.

A.—General description of the development of the ova of the rat and mouse up to the period of completion of the blastodermic vesicle, and comparison with the results obtained by Professors Fraser, Duval, and Selenka.

Results.—There is a segmentation cavity, which is not the blastodermic cavity. The segmentation cavity disappears simultaneously with the appearance of the archenteron. The archenteron appears amidst the hypoblast. The young ovum consists principally of hypoblast, which becomes vacuolated to form the cavity of the yolk-sac. The cavity of the yolk-sac is never bounded by epiblast alone. The epiblast extends over the outer surface of the hypoblast, not the hypoblast over the inner surface of the epiblast. The hypoblast never becomes entirely surrounded by epiblast.

B.—Description of the formation of the mesoblast.

Results.—The mesoblast is formed partly from the peristomal cells in the region of the primitive streak, partly from the embryonic hypoblast, and partly from the extra-embryonic hypoblast. Mesoblastic formation commences at the posterior end of the embryonic area, not anteriorly, as in the hedgehog. In the embryonic area the pericardial mesoblast is the last formed.

C.—Description of the formation of the chorda dorsalis.

Results.—The chorda is formed entirely from the primitive hypoblast, to which it last remains adherent at the dorsal end of the bucco-pharyngeal membrane and the anterior end of the primitive streak. There is no " Kopffortsatz " of the primitive streak.

D.—Comparison of the ova of the rat and mouse with the ova of other mammals and the lower vertebrates.

    Conclusions.—The ova of mammals do not differ essentially from the ova of other vertebrates. They do not consist in the early stages of an epiblastic vesicle containing an inner mass of epiblast and hypoblast, but of a large hypoblastic mass which supports a small epiblastic disc. The ova of mammals present all the characteristic features of comparatively large-yolked ova.

E.—Description of the formation of the amnion and discussion of the relation of amnion formation to "inversion."

    Conclusions.—There is a pro-amnion in the rat and mouse. Pro-amnion formation and "inversion" are distinct processes, and "inversion" is not precocious pro-amnion formation. The whole of the amnion is formed from the tail-fold.

Comparison of amnion formation in the rat and mouse with amnion formation in man and other mammals.

F.—Description of the formation of the cœlom.

    Results.—The cœlom commences bilaterally and in the embryonic area. The pericardial cœlom is an extension of the embryonic cœlom from behind forwards. It does not communicate with the anterior portion of the extra-embryonic cœlom.

THE formation and the extension of the germinal layers, their relation to the segmentation cavity and archenteron, and the derivation of the chorda dorsalis in the mammalia are developmental problems which have initiated many investigations. From the results which have hitherto been obtained only very general conclusions can be drawn, and upon many important points the recorded observations are contradictory. This is all the more noteworthy inasmuch as many of the observations have been made upon the same kind of animal; others, however, upon animals belonging to widely divergent species.

    The blastodermic cavity of the mammalian ovum lies, at first, between the epiblast and hypoblast and corresponds in position with the segmentation cavity of the lower Vertebrata. In the rabbit (2), rat (9, 13, 45), mouse (9, 13, 44), guinea-pig (45), shrew (23), mole (16), bat (4), and opossum (46) it is said to become surrounded by the hypoblast. If this actually occurs, then the mammalia are separated from all the other Vertebrata by a peculiarity which as yet has received no

satisfactory explanation, for in none of the other Vertebrata
does the segmentation cavity become surrounded by hypoblast.

The relationship of the blastodermic cavity to the archen-
teron is disputed. Van Beneden believes the former cavity to
be a yolk-space which has no genetic association with the
primitive alimentary cavity, which is represented in the mam-
malia by the notochordal canal, from the dorsal wall of which
the notochord is separated. In opposition to this opinion
Bonnet (5, 6) and Hubrecht (23) have shown that a portion of
the blastodermic cavity becomes converted into the enteric
canal, and that at least a part of the chorda dorsalis is
developed from the hypoblast which forms the wall of the
primitive cavity. It must be noted, however, that Kölliker
(26) and Keibel (25) deny the hypoblastic origin of the chorda,
and that they look upon this organ as a purely mesoblastic
structure which is evolved from the "Kopffortsatz" of the
primitive streak.

Quite recently an attempt to reconcile these opposed con-
clusions has been made (23), and for this purpose the hypo-
thesis has been advanced that the hypoblast in the Mammalia
is formed in two separate portions. One of these, which ulti-
mately surrounds the blastodermic cavity, becomes precociously
segregated and separated, and is therefore termed the cœno-
genetic hypoblast. The second portion, the phylogenetic
hypoblast, undergoes a modified embolic invagination at a
later period, part of it appearing as the "Kopffortsatz" of the
primitive streak. The chorda dorsalis is formed partly from
the cœnogenetic hypoblast and partly from the "Kopffortsatz,"
and thus entirely from the inner germinal layer, as in the lower
Vertebrata.

The mode of formation of the middle germinal layer is also
a subject of dispute. It is said to arise from the epiblast alone
(12, 26, 27, 44), or partly from the epiblast and partly from
the hypoblast (1, 5). There is also difference of opinion with
regard to the position in which the middle layer is first formed.
It is said by some to arise only in the posterior part of the
germinal area in the region of the primitive streak (12, 27).

Bonnet has described it as originating partly in the region of the primitive streak and partly in the marginal zone of the germinal area from the hypoblast (5). More recently three chief seats of mesoblastic formation have been described,—the anterior part of the germinal area, the marginal zone of the germinal area, and the primitive streak (23).

Under these circumstances no apology is needed for the publication of the results of a series of observations, dealing with the questions in dispute, which have been carried on during a period of several years, upon the developing ova of Mus musculus and Mus decumanus, which were selected on account of the comparative ease with which they can be obtained in large numbers. Although, at first sight, the peculiar "inversion of the layers" which occurs in the early stages might be held as an objection to their use for the study of ordinary developmental phenomena, experience proves that this is not the case. On the contrary, the "inversion" is an aid to the investigator, for it facilitates observations on the extension of the middle germinal layer and upon the comparative rate of growth of the primitive streak.

Some of the observations to be referred to in the subsequent pages have already been recorded in a thesis presented to the University of Edinburgh for the degree of Doctor of Medicine, in 1890 (41). Since that period, however, amongst a number of fresh specimens which have served to confirm the most important conclusions drawn from my previous work, I have found some intermediate stages which have thrown further light upon some of the phenomena, and which have enabled me to form certain definite conclusions concerning the processes of cœlom formation and extension, and the relation of amnion formation to the "inversion of the layers."

As the results of my observations are not in harmony with those obtained by previous observers (9, 13, 44, 45), it is advisable to precede the record of results by a short account of the methods used in the preparation of the specimens, so that it may be shown at the outset that the differences to be noted are not due either to the manipulation of the specimens

or to the action of the reagents used in the preservation and staining processes.

After the pregnant female had been killed with chloroform the uterus was removed by cutting through the broad ligaments and the upper part of the vagina. The whole uterus was then immersed in either picro-sulphuric, picro-nitric, or picro-hydro-chloric solution, in which it was left for an amount of time which varied with the size of the object, from a few hours in the case of small specimens up to two days in the case of large embryos. The acid was removed from the specimens by methylated spirit; the uterus was then placed in borax carmine solution, from which it was transferred to acidulated alcohol.

After the completion of the staining the uterus was cut into segments corresponding to the contained ova, and the segments were passed through absolute alcohol and turpentine into paraffin, in which they were embedded. The sections of the segments, cut by the rocking microtome, were cemented to the slide by collodion and oil of cloves; they were cleared in turpentine and mounted in Canada balsam.

The sections were made in three planes:

1. Transversely to the long axis of the uterus.

2. Parallel to the long axis of the uterus, but at right angles to the plane of the broad ligament.

3. Parallel to the long axis of the uterus and the broad ligament.

In their relation to the uterus as it lies in the body these sections may be termed respectively—

(1) Vertical-transverse.

(2) Horizontal-longitudinal or coronal.

(3) Vertical-longitudinal or sagittal.

The first and second series were the most useful, especially in the very early stages.

General Description of the Various Stages, and Comparison with the Results obtained by Previous Observers.

After fecundation the ovum of the rat, whilst it still retains its spherical form, becomes, according to Tafani's observations (51), divided into a number of blastomeres of equal size and texture, and then, as the segmentation continues, the spherical form is lost and an oval form is assumed. My youngest specimen is an ovum in the oval stage. The uterus in which it was found was cut into vertical-transverse sections, and the ovum, which lies free in the antimesometrial end of one of the radii of a triradiate cavity, is divided into eight sections, the fourth of which is represented in fig. 1. The longest diameter of the ovum measures 64·6 μ, and the shortest 41·8 μ. The long axis of the ovum is at right angles to the long axis of the uterine cavity.

The ovum consists of a number of large cells. There is no trace of the polar bodies, which are said by Tafani to be present up to this period. There is no vitelline membrane, but the cells of the ovum are closely packed together, and their forms are irregular from mutual compression. The protoplasm of the cells is very slightly granular, and it stains but faintly with carmine.

Each cell contains a large more or less spherical nucleus, which is bounded by a distinct nuclear membrane, and which contains a reticulate and nodulated chromoplasm, but no nucleoli distinct from the nodes of the reticulum. The ovum at this period, about the fourth day, is a solid morula, and its cells present no appearance of arrangement into the two groups mentioned by Tafani.

The next stage in my possession is that represented in fig. 3. It is a vesicle with an excentric cavity: one wall of the cavity, which for convenience may be termed upper, is formed by a single layer of flattened cells (ED), whose nuclei are comparatively large in proportion to the cell body. The

other wall of the cavity, the lower, is formed by a mass of cells (*HY*) of larger size and more irregular shape.

This ovum measures in its greatest diameter 57 μ, and in its least diameter 46 μ. Its long axis is at right angles to the long axis of the uterine canal.

It is apparently a little younger than the mouse ovum figured by Selenka (**44**, in Taf. i, fig. 1), and about the same age (five days) as that represented by Duval (**9**, pl. i, fig. 73).

Fig. 2, Pl. XXIII, represents a section of another ovum from the same uterus as the ovum depicted in fig. 3. It is vesicular, and measures 79 μ in its longest diameter, which lies at right angles to the long axis of the uterus, and 41 μ in its shortest diameter. The ovum is divided into eight sections, and the fifth of these is represented by fig. 2. The cells forming the upper wall of the cavity are flatter than the cells in the same position in the ovum represented in fig. 3, Pl. XXIII, and the floor of the cavity is formed over part of its extent by only a single layer of large cells. The blastodermic cavity is considerably larger than in the other ovum from the same uterus.

Fig. 4, Pl. XXIII, represents the sixth section of a mouse ovum, which has been divided into eleven sections. The long diameter of this ovum, which lies in the long axis of the uterine canal, measures 49 μ, and its short diameter is 26 μ. It is at about the end of the fifth or the commencement of the sixth day of development, and corresponds with the ovum represented in Taf. i, fig. 1, by Selenka (**44**), and in pl. i, fig. 76, by Duval (**9**). It is a nucleated protoplasmic vesicle in which no cell outlines are distinguishable. The roof of the vesicle (*ED*) is formed by a layer of protoplasm, which contains a single row of nuclei, the long axes of which are parallel with the surface of the vesicle. The floor of the vesicle is a mass of granular protoplasm (*HY*), containing many nuclei which are larger and more rounded than those in the roof. The protoplasm of the roof stains with carmine more deeply than that of the floor.

A section of another mouse ovum at the sixth day is repre-

sented in fig. 5, Pl. XXIII. This ovum also lies free in the uterine canal. Its long axis measures 125 μ and lies parallel with the long axis of the uterine canal. Its short axis is 26 μ long. The ovum is cut in its long axis, but somewhat obliquely, so that the total length is not fully represented in any one section, but the sixth out of a series of nine sections has the greatest length, and fig. 5 is a representation of that section.

In this ovum both the floor and the roof of the cavity are more extensive than in the mouse ovum previously described (compare figs. 4 and 5, Pl. XXIII). In the ovum represented in fig. 5, however, the nuclei of the floor are comparatively very large, and the protoplasm surrounding them is more or less distinctly marked out into cell areas; but, as in the younger ovum (fig. 4), the protoplasm of the floor is less granular, and it stains less deeply with carmine than the protoplasm of the roof (see fig. 5).

The youngest mouse ovum described by Duval (9, pl. i, figs. 73 and 74) is a blastodermic vesicle of the fifth day, which consists, according to his description, of two parts, an outer formed by a single layer of epiblast-cells, on the inner surface of which, at the proximal pole of the ovum (that is, the pole lying next the mesometrial side of the uterus), is a small mass of hypoblast. During the fifth day the epiblast of the proximal pole of the ovum is said to proliferate, and as the spherical outline of the vesicle is maintained the thickened epiblast projects into the interior of the vesicle, pushing the hypoblast before it. The youngest mouse ovum described by Selenka has attained this stage (44, Taf. i, fig. 1); and he distinguishes its component cells into two groups,—an outer layer of flattened cells, which he calls Reichert's membrane; and an inner mass, which he subdivides into a proximal portion or epiblast, and a distal portion or hypoblast.

Fraser's description of an ovum at six days twelve hours (13) corresponds very closely with that given by Selenka, except that he calls all the external layer of epiblast Rauber's cell layer, whilst Selenka restricts the term " Rauber's cells"

to that portion of Reichert's membrane which covers the epiblast of the inner cell mass.

According to both Selenka and Duval, the proliferation of the epiblast at the proximal pole of the ovum now proceeds rapidly, and the ovum assumes a more distinctly oval form (**44**, Taf. i, figs. 6, 7, 9, and 10; and **9**, pl. i, figs. 83 and 84).

During this period (about the seventh day) Selenka figures a distinct line of demarcation between Rauber's cells and the epiblast of the inner mass, but Duval draws no such distinction; on the contrary, he figures and describes the epiblast of the inner mass as continuous with the outer layer of cells.

My specimens of these early stages of development of the ova of the mouse and the rat (fourth, fifth, sixth, and seventh days) do not confirm the interpretation which has been given by Selenka, Fraser, and Duval of the phenomena of these periods.

Towards the close of the period of segmentation, and before the blastodermic cavity appears, the ovum acquires an oval form (fig. 1, Pl. XXIII), which it retains until about the twelfth day of gestation. During the fifth day the long axis of the ovum changes its direction. On the fourth day (fig. 1, Pl. XXIII) and the commencement of the fifth day (figs. 2 and 3, Pl. XXIII) the long axis of the ovum runs from side to side; by the middle of the fifth day (fig. 4, Pl. I) it extends from roof to floor, or, according to Duval's nomenclature, from proximal to distal end. I have been entirely unable to find a spherical blastodermic vesicle of the fifth day similar to those figured by Selenka and Duval.

When the blastodermic cavity appears, its wall, over somewhat less than half the extent of the ovum, is formed by a single layer of cells, and the remainder of the wall is constituted by a mass of cells which is not separable into an inner mass of hypoblast and an outer layer of epiblast. At this period (figs. 2 and 3, Pl. XXIII) the blastodermic vesicle lies free in the uterine cavity. Its thin and thick walls are directed towards the sides of the uterine cavity, and the extremities of its long axis towards the uterine margins.

During the fifth day (figs. 4 and 5, Pl. XXIII) the thin portion of the wall of the blastodermic vesicle increases very considerably in extent, its apical portion is projected away from the thickened portion of the wall, and thus the long axis of the ovum comes to lie at right angles to its former position. In the meantime, however, the ovum has altered its position in relation to the wall of the uterine cavity. The most prominent points of its thick and thin walls are no longer opposed to the sides of the uterine canal ; they are directed towards the ends of that channel, or, in other words, the long axis of the ovum lies parallel with the long axis of the uterine canal, and there is still no separation of the thick portion of the wall of the vesicle into an inner and outer layer.

During the sixth day important changes take place in the relations of the ovum to the uterus, and of the various parts of the ovum to each other. The inversion of the layers commences, and the first traces of the archenteron appear (figs. 6 and 7, Pl. XXIII).

The ovum lies in a cylindrical crypt in the thickened mucous membrane of the distal (antimesometrial) side of the uterine canal. The long axis of the crypt is at right angles to the long axis of the uterus, and therefore the ends of the ovum occupying the crypt are now distinctly proximal or mesometrial (P, fig. 6), and distal or antimesometrial (D).

The crypt is lined by uterine epithelium, which is reduced to a layer of flat cells where it is opposed to the sides of the ovum, with which, however, it is not united, but over the remaining parts of the walls of the crypt the epithelium has a cubical or low columnar form.

Fig. 6 is a camera drawing of the seventh section of an ovum, which is divided longitudinally into fourteen sections. The greatest length of this ovum is 95 μ, and its greatest breadth 49 μ.

It is a vesicle. Its distal wall is formed by a multinucleated mass of protoplasm indistinctly divided into cell areas. The protoplasm of the distal pole stains but faintly with carmine, and it corresponds in all essential respects with the

thick protoplasmic floor of the blastodermic vesicle of the earlier stages (compare figs. 4 and 5 with fig. 6, Pl. XXIII). The lateral walls of the vesicle consist of a thin layer of nucleated protoplasm, which is more granular and stains more deeply with carmine than the protoplasm of the distal pole. It clearly resembles the protoplasmic roof of younger vesicles (compare figs. 4 and 6, Pl. XXIII).

The proximal wall (P) of the vesicle is thicker than the lateral walls, to which it is otherwise similar, and it is continuous with a mass of nucleated protoplasm which occupies the interior of the vesicle. This mass of protoplasm is very granular, it stains comparatively deeply with carmine, it contains many large nuclei, and it is indistinctly divided into cell areas. The optical characters of the protoplasm of the inner mass are similar to those of the lateral and proximal walls, but the nuclei of the latter are much smaller than those of the inner mass.

In the interior of the inner mass is a small cavity, from which a dark line extends to the surface of the proximal pole of the ovum.

It seems probable that, during the sixth day, the thin roof of the blastodermic vesicle is invaginated into the blastodermic cavity; that its nuclei enlarge and proliferate; and that the sides of the invagination cavity are gradually approximated (figs. 3, 4, 5, and 6, Pl. XXIII). The thick floor of the blastodermic vesicle of the earlier stages (figs. 3, 4, 5, and 6, Pl. XXIII) becomes the distal pole of the vesicle at a later stage. It does not become divided into an outer layer and an inner mass, for the inner mass is undoubtedly formed by the invagination of the thin roof of the vesicle.

Fig. 7, Pl. XXIII, is a representation of the tenth section of an ovum from the same uterus as the ovum shown in fig. 6, but it is more advanced than the latter. It is divided longitudinally, and also slightly obliquely, so that none of the fourteen sections represent the full length of the ovum.

The section depicted in fig. 7 is 64 μ long (from proximal to distal end), and 76 μ broad.

The depression at the proximal pole (*P*) probably represents the remains of the invagination cavity, and from the bottom of it a dark line is traceable for a short distance into the inner cell mass, which does not contain a cavity.

The diminution in height of this ovum as compared with the specimen represented in fig. 6, Pl. XXIII, is due to the projection of the inner cell mass into the protoplasm of the distal pole of the ovum, and by the same event the segmentation cavity (*S C*) has been transformed into a circular canal which encircles the proximal end of the inner cell mass. A second circular canal (*V C*) is seen, distal to the modified blastodermic cavity. It is situated in the protoplasmic mass which forms the floor of the blastodermic vesicle. It is the rudiment of the yolk-sac.

The protoplasm of the distal pole of the ovum is apparently dividing into two layers, for a line of demarcation is already visible on the section, extending between the two segments of the yolk-sac through the distal protoplasmic mass.

It is during the sixth day, therefore, that the various component parts of the blastodermic vesicle present, for the first time, clear indications of their nature and ultimate fate.

The thick faintly staining floor of the early blastodermic vesicle is the distal, not the proximal wall. It does not divide into an outer layer of epiblast and an inner of hypoblast, but in it a cavity develops, and it becomes the hypoblastic lining of the alimentary canal and yolk-sac of the embryo.

The thin roof of the young blastodermic vesicle is the proximal, not the distal wall. A portion of it is invaginated into the cavity of the vesicle, thus producing the inner cell mass. A portion of it ultimately becomes the embryonic epiblast, and the remainder takes part in the formation of the placenta.

The comparison which Duval makes between the early stages of the ovum of the mouse and that of the rabbit cannot be sustained, for before the sixth day there is no inner cell

mass in the blastodermic cavity of the mouse, not even the hypoblastic mass which Duval describes ; and when the inner cell mass does appear it is purely epiblastic, not partly epiblastic and partly hypoblastic, as is the case in the rabbit, according to the description of Kölliker (27) and Rauber (38).

During the latter part of the sixth day the remains of the blastodermic cavity are entirely obliterated, yet the ovum retains a vesicular form; but the cavity in its interior at the end of the sixth day is the cavity of the yolk-sac, which has now become more distinctly developed.

Fig. 8, Pl. XXIII, represents a section of an ovum found in a series of vertical transverse sections of the uterus. The ovum is at about the commencement of the seventh day of development, and corresponds to the ova figured by Selenka (44, Taf. i, fig. 7) and by Duval (9, pl. i, fig. 83).

It has been cut longitudinally. It is 121 μ long, and 64 μ broad. It consists principally of a large hypoblastic sac, upon the proximal end of which rests a conical cap of ectoderm ($E\ D$).

Comparison of figs. 6, 7, and 8, Pl. XXIII, shows that the large yolk-sac in fig. 8 has been produced by the vacuolation of the multinucleated mass of protoplasm which formed the floor of the blastodermic vesicle, and the rudiment of the hypoblast. The vacuolation commences during the sixth day in the periphery of the proximal portion of the hypoblast, and from thence it extends throughout the whole mass, converting it into a sac. The cavity of the sac is traversed by nucleated protoplasmic strands, which are the remains of the central portion of the original mass.

The proximal and distal walls of the yolk-sac are comparatively thick, especially the proximal, which is most intimately associated with protoplasmic strands of the interior. The lateral walls are thin, and they lie in contact with the walls of the crypt in which the ovum is contained. The epithelial lining of the uterus has disappeared from certain portions of the area with which the walls of the yolk-sac are in contact,

and consequently in these situations the fœtal hypoblast is in direct contact with the maternal submucosa (42).

At the commencement of the seventh day the original roof of the blastodermic cavity is separable into two parts : (1) an internal (*E*), oval in form, which is projected against and partially invaginates the central portion of the proximal wall of the hypoblast sac; and (2) an external (*T*), which covers and extends beyond the internal, resting by its circular margin on the peripheral part of the proximal end of the hypoblast.

The figures of ova at this stage given by Selenka and Duval differ somewhat from each other. Selenka figures and describes the inner mass of epiblast (Taf. i, fig. 44), but Duval sees no line of demarcation between the two, and he figures the inner mass and outer layer as directly continuous with each other (**9**, pl. i, fig. 83). Both Selenka and Duval describe the lateral and distal walls of the yolk-sac as epiblast, the only hypoblast at this stage, according to their accounts, being that which constitutes the proximal wall of the yolk-sac and the irregular protoplasmic projections which extend from this mass towards the lateral and distal walls of the sac. The sac itself is, in their opinion, the remains of the cavity of the blastodermic vesicle, which only becomes converted into the yolk-sac by the extension of the hypoblast from the proximal end round the inner surface of the epiblastic wall, in a manner somewhat similar to that described in the rabbit by Van Beneden (**2**), in the mole by Heape (**16**), and in the shrew by Hubrecht (**23**).

It has already been shown that the history of the ovum during the sixth day precludes such an interpretation of the appearances found at the commencement of the seventh day, and the following summary of important events of the former period will serve as a basis for the description of the changes which take place during the seventh day.

I. Changes in the Longitudinal and Transverse Diameters of the Ovum.

Towards the end of the fifth day, or at the commencement of the sixth day, the longitudinal axis of the blastodermic vesicle is 125 μ long (fig. 5). During the sixth day that axis of the ovum is diminished, first to 95 μ (fig. 6), and then to 64 μ, after which it again increases, and at the commencement of the seventh day it is 121 μ. During the same period the transverse diameter increases from 26 μ at the end of the fifth day to 64 μ at the end of the sixth day.

The first diminution in the length of the longitudinal axis is due to the invagination of the roof of the blastodermic vesicle into the cavity of the vesicle (fig. 6, Pl. XXIII); and were it not for this invagination the longitudinal axis of the ovum at the commencement of the sixth day would measure 139 μ (length of vesicle, fig. 6, Pl. XXIII, 95 μ; length of mass of invagination cells, 44 μ).

The second diminution of the longitudinal axis results from the sinking of the invaginated mass of cells into the floor of the blastodermic cavity (fig. 7, Pl. XXIII).

The increase in the longitudinal diameter towards the end of the sixth day is produced by the formation and distension of the cavity of the yolk-sac in the midst of the floor of the blastodermic vesicle ($Y\,S$, fig. 8, Pl. XXIII).

The gradual increase of breadth is due partly to gradual multiplication of the cells of the ovum, and partly to the appearance and distension of the yolk-sac cavity.

II. The Disappearance of the Primitive Cavity of the Blastoderm.

The occlusion of this cavity is brought about by the invagination of the roof into its interior (fig. 7, Pl. XXIII), and by the growth of the yolk-sac (figs. 7 and 8, Pl. XXIII).

III.　The Separation of the Invaginated from the Non-invaginated Portion of the Roof.

When the invagination is completed (fig. 6, Pl. XXIII) the inner mass is attached to the external layer by a constricted pedicle which apparently becomes broader (fig. 7, Pl. XXIII), occupying about half of the superficial area of the roof of the blastodermic vesicle. Subsequently the superficial layer of the central area is separated by delamination from the inner mass, but remains continuous with the peripheral portion of the roof, and thus at the commencement of the seventh day the original roof of the blastodermic vesicle is separated into an outer layer (T) and an inner oval mass (E, fig. 8, Pl. XXIII).

Therefore at the commencement of the seventh day the ovum consists of three distinct portions :

1. A comparatively large yolk-sac, with walls of irregular thickness and a cavity which is traversed and divided by nucleated protoplasmic strands. Both the walls and the internal reticulum of the yolk-sac are formed by hypoblast ($H\ Y$).

2. An oval mass of nucleated protoplasm which rests upon and slightly invaginates the middle ova of the proximal wall of the yolk-sac. This is the rudiment of the epiblast of the embryo and the amnion. It will be termed in the further description the epiblast (E).

3. A nucleated layer of protoplasm which covers the epiblast and, extending beyond it, rests upon the peripheral area of the proximal end of the yolk-sac. This layer is the trophoblast (T); it takes no part in the formation of the embryo or amnion, but is developed into a portion of the placenta.

During the seventh day the uterine epithelium entirely disappears from the wall of the crypt in which the ovum lies, over an area corresponding to the lateral surface of the ovum, and both the trophoblast and the hypoblast of the ovum become intimately attached to the maternal submucosa. The ovum rapidly increases in size, and the inversion of the layers is

complete; but a full description of the events of the seventh day will be more rapidly given and more easily understood after a consideration of the sections represented in figs. 9, 10, 11, and 12, Pl. XXIII.

Fig. 9, Pl. XXIII, is the eighth of a series of sixteen longitudinal sections of a mouse ovum, cut in vertical transverse sections of the uterus in the early part of the seventh day. The sections are slightly oblique, and the distal end of the yolk-sac is somewhat curved, so that the whole length of the ovum, 232 μ, does not appear in any one section.

As compared with the ovum represented in fig. 8 the yolk-sac is longer, but not so wide; indeed, in the distal portion of the sac the walls are in contact (fig. 9).

The walls of the yolk-sac consist of nucleated protoplasm, which stains but faintly with carmine. The protoplasm is most abundant and the nuclei are most numerous in the proximal wall of the sac, which is partially invaginated by the epiblast (E), and from the proximal wall a few protoplasmic strands extend to the lateral walls of the cavity. The lateral walls of the yolk-sac are thin, except in the distal portion (fig. 9), where the thickness of the walls is more regular and the nuclei are placed at more uniform distances.

The epiblast (E) is an oval mass of protoplasm, 61 μ long and 28 μ broad. It contains many nuclei, which are very irregular in size. It is entirely surrounded in its distal half by hypoblast ($H\ Y$), and in its proximal half by trophoblast (T), and it is not separable into distinct cell territories.

The trophoblast (T) forms the proximal end of the ovum; it is 22 μ thick. It covers the proximal end of the epiblast, and, extending round it slightly, overlaps the proximal part of the hypoblast (see the left side of fig. 9). Like the epiblast, the trophoblast is not divided into cells. Its nuclei are not so large or so irregular in size as those of the epiblast.

Both the epiblast and the trophoblast stain more deeply with carmine than the hypoblast.

Fig. 10, Pl. XXIII, is a representation of an oblique longitudinal section of a mouse ovum at about the middle of the

seventh day.   The ovum is $159\,\mu$ long and $61\,\mu$ broad, and
it is divided into fourteen sections.   Fig. 10 represents the
eighth section.

The yolk-sac is invaginated upon itself from the proximal
towards the distal end.   In the cavity of invagination the epi-
blast and part of the trophoblast are situated.   The nuclei of
the hypoblast (HY) form a single row.   They are placed at
fairly regular intervals over the trophoblast and epiblast,
but more irregularly in the remaining portions of the wall of
the yolk-sac.   The hypoblastic protoplasm stains less deeply
with carmine than the protoplasm of the epiblast and tropho-
blast.

The mass of epiblast (E) is $33\,\mu$ long and $41\,\mu$ broad.   It
contains no cavity.   It is not distinctly divided into cell areas,
and it contains many very large nuclei.

The trophoblast (T) is $106\,\mu$ long and $45\,\mu$ broad.   It also
is a multinucleated protoplasmic mass.   The nuclei are placed
at varying intervals, and they are of irregular but generally
large size.

The distal end of the trophoblast rests against the epiblast,
from which, however, it is quite distinct.

Laterally the trophoblast is partially surrounded by the
invaginated portion of the yolk-sac, and the part of the tropho-
blast outside the yolk-sac lies in contact with the uterine sub-
mucosa.   Proximally it is free in the cavity of the uterine
crypt.

There is a distinct cavity projecting into the interior of the
trophoblast from its distal end.

Fig. 11, Pl. XXIII, represents the eighth of a series of seven-
teen sections of an ovum of a mouse at the end of the seventh or
the commencement of the eighth day.   The ovum is $171\,\mu$ long
and $87\,\mu$ broad.   It is divided longitudinally, and evidently
consists of the same three portions recognised in the three pre-
ceding ova.   The yolk-sac is more distinctly invaginated than
in the preceding stage.   It encloses the epiblast (E) and the
greater part of the trophoblast (T).   The hypoblast (HY)
which forms the yolk-sac wall is thickest over the epiblast and

the trophoblast, thinnest in the remainder of its extent, where it lies in contact with the uterine tissues.

The hypoblastic protoplasm still stains more faintly than that of the epiblast and trophoblast.   On the left side of the figure, at the point marked with an asterisk, there is an indication of a constriction between the epiblast and trophoblast, and at this point the hypoblastic protoplasm is aggregated, and contains two large nuclei.

The epiblast (E) is 53 μ long and 49 μ broad.   It contains a small oval cavity which is situated nearer the proximal than the distal end.   The nuclei of the epiblast are large, and the majority of them are arranged parallel with and near to the periphery of the mass.

The greater part of the trophoblast (T) is contained within the cavity formed by the invagination of the yolk-sac, and the margins of the invagination cavity appear to be contracted round the trophoblast, which is constricted as it passes from the interior to the exterior of the cavity.   The distal end of the trophoblast lies in close contact with the epiblast.   That portion of the trophoblast which lies outside the yolk-sac is attached by its surface to the uterine tissues, but its proximal end lies free in the uterine crypt.

Fig. 12, Pl. XXIII, represents the seventh section of a rat's ovum, which is divided longitudinally into thirteen sections. The ovum is about the same age as that of the mouse last described—that is, it is about at the end of the seventh or the commencement of the eighth day.   The greatest length of the ovum is 209 μ, and its greatest breadth 91 μ.

A detailed description of this ovum is unnecessary because its general features correspond with those of the mouse ovum last described, but there are some special points which require attention.

The invaginated portion of the yolk-sac is distinctly constricted round the region where the epiblast and trophoblast lie in contact with each other, and in this region the hypoblast is thicker than elsewhere; indeed, on the right-hand side of the figure it is seen to contain a double row of nuclei.

The hypoblast of the rat's ovum at this stage is more distinctly divided into cells than that of the mouse ovum (compare figs. 11 and 12), and this is more especially the case round the proximal part of the epiblast.

The epiblast is 76 μ long and 53 μ broad. It contains an oval cavity which is situated in the centre of the mass.

The trophoblast, which is 102 μ long and 26 μ broad, is not constricted where it enters the invagination cavity, but it contains in this situation a small cavity. The trophoblastic nuclei are arranged with some degree of order round the cavity, but distally and proximally they are scattered irregularly in the protoplasm.

The account given by Duval (9) of the events of the seventh day varies somewhat from Selenka's (44, 45) description of the changes which take place in ova of apparently the same period, and as the results of my own observations of this period differ materially upon many points from those of the observers named, it is necessary that I should give a short account of their results and a comparison with my own before entering upon a summary of the latter.

1. The Uterine Epithelium.

It is during the seventh day that the uterine epithelium in the neighbourhood of the ovum disappears. This disappearance is due to a simple atrophy, according to Selenka's figures ; whilst according to Duval's account, which is confirmed by my specimens, the disappearance is preceded by a degeneration. The outlines of the uterine epithelium cells are lost, and the nuclei become smaller and more numerous ; subsequently both the protoplasm and the nuclei are absorbed. Possibly the cells become pabulum for the growing ovum, as Duval suggests. There is no proof that this is the case beyond the fact that the disappearance of the uterine epithelium and the increase of the ovum are simultaneous events, and it is quite as probable that the uterine epithelium is absorbed by the wandering cells from the uterine capillaries, which have in the meantime become much dilated, for such cells are known to possess the

power of consuming degenerated tissues, and it has yet to be shown that the cells of the developing ovum are possessed of similar gastronomic powers.

2. The Ectoderm at the Seventh Day.

Both Selenka and Duval figure and describe the ectoderm at this stage as being formed by a number of distinctly outlined cells. My specimens, on the contrary, show clearly that the ectoderm is simply a nucleated protoplasmic mass. According to Duval the ectoderm surrounds the whole vesicle into which it projects at the proximal end as a plug-like mass, which is due to the rapid proliferation taking place in that situation.

Selenka also describes an outer ectodermal wall on the sides and distal end of the vesicle which I have been entirely unable to find, and the cells figured by Duval as the representatives of this layer in the later stages are, in my opinion, modified cells of the uterine mucosa. I base this opinion upon the fact that in my specimens the cells in question are not present in the early stages, for in the situations where they are figured by Selenka and Duval the hypoblast of the ovum is in contact with the uterine wall, and that they appear at a later period between the hypoblast and the uterine tissues simultaneously at irregular points, not as a layer extending gradually from the trophoblast.

The ectoderm in ova of apparently the seventh day is described and figured by Selenka as consisting of two parts, the "formative epiblast" and the "Träger," or, in other words, the epiblast and trophoblast. Selenka also draws attention to a cavity which appears in the trophoblast.

Duval neither refers to nor figures the separation of the ectoderm into two layers, yet the separation is so evident (figs. 9, 10, 11, and 12, Pl. XXIII) that it is scarcely possible that he has not observed it ; and apparently he has not seen the cavity in the trophoblast (fig. 10, Pl. XXIII), possibly because it is present for a very short period, and he has missed the exact stage in which it occurs. This primary cavity in the trophoblast is a special feature which must be due to some peculiarity of growth, but Selenka's explanation that it is formed

by the increase of the trophoblast in the form of a blind sac towards the centre of the blastodermic vesicle is scarcely satisfactory, for it is evident that between the stages represented in figs. 8 and 10, Pl. XXIII, the increase of the epiblast and of the trophoblast on the distal side of the cavity ($T\,C$) represented in fig. 10 is sufficient alone to account for the diminution of the distance between the proximal and distal walls of the yolk-sac during the same period. It appears more probable, therefore, that the primary cavity of trophoblast does not result from invagination of the trophoblast, but from the rapid growth of the peripheral portion of that part of it which would in the case of an unattached ovum gradually extend over the surface of the yolk-sac. Such extension, however, is prevented in the mouse by the close apposition of the wall of the yolk-sac to the uterine mucosa, and, consequently, the proliferating trophoblast takes the only other possible course, and spreads itself along the wall of the crypt in which the ovum lies, leaving the central portion of the cavity of the crypt unobliterated for a time. If this explanation is correct the invagination of the yolk-sac is not in any way due to invagination of the trophoblast, for there is no true invagination of that portion of the ectoderm.

The primary cavity of the trophoblast entirely disappears, and has nothing whatever to do with the secondary cavity, which appears at a later period.

The secondary cavity, according to Selenka's figures in his first communication (44, Taf. xi, figs. 16 and 17), is formed by the extension into the trophoblast of the cavity which appears in the epiblast at the seventh day, but in the third portion of his studies he figures the secondary cavity of the trophoblast and the cavity of the epiblast as present simultaneously and separately, both in the mouse and the rat (45, Taf. xvi, figs. 57 and 63). In the rat the two cavities which are afterwards found undoubtedly arise independently of each other. Duval has so represented them (9, pl. ii, fig. 92, and pl. iii, fig. 100), and figures representing the rat's ovum on Pl. XXIII are further confirmative of Selenka's account. In the mouse,

however, I have not been able to find two similar cavities, neither apparently has Duval; and as the appearance of some of my sections corresponds very closely as regards this point with the figures given by Selenka in the first part of his studies (**44**), I am inclined to the belief that the cavity of the trophoblast in the mouse does not arise independently, but is the result of the extension of the cavity of the epiblast into the trophoblastic mass.

Summary of the Events of the Seventh Day.

The Epiblast.—The epiblast is projected by the increase of the trophoblast against the proximal wall of the yolk-sac which is gradually invaginated, and thus at the commencement of the eighth day the epiblast lies at the distal end of a long cavity, which is bounded by hypoblast (fig. 11, Pl. XXIII). Whilst this alteration of position is taking place the epiblast increases in amount, changes from an oval (fig. 9, Pl. XXIII) to a more circular form (fig. 10, Pl. XXIII), and a cavity develops in its interior (figs. 11 and 12, Pl. XXIII). This cavity is afterwards extended and becomes the cavity of the amniotic sac, from which the central canal of the nervous system is separated by the closure of the medullary folds.

The Trophoblast.—This portion of the ectoderm proliferates more rapidly than the epiblast, and the rapid growth of its central portion is the main cause of the invagination of the yolk-sac, its length increasing from $22\,\mu$ (fig. 9) to $106\,\mu$ (fig. 10). The increase of the margin of the trophoblast and its extension along the sides of the uterine crypt result in the appearance of a cavity apparently within the trophoblast ($T\,C.'$, fig. 10), the primary cavity of the trophoblast. This cavity is entirely obliterated by the approximation and fusion of its lateral boundaries, after which the trophoblast has, for a time, the appearance of a solid rod, which lies partially within and partially outside the cavity formed by the invagination of the yolk-sac (fig. 11), and for the convenience of description in the later stages these two portions will be respectively the distal and the proximal. Afterwards, towards the end of the

seventh day, a new cavity appears in the distal part of the trophoblast, either by extension from the cavity of the epiblast, as in the mouse, or independently, as in the rat ($T C$, fig. 12, Pl. XXIII).

The Hypoblast.—The hypoblast forms the wall of the yolk-sac, which is gradually invaginated upon itself, chiefly by the growth of the trophoblast, but apparently not altogether so, for at the end of the seventh day (fig. 11, Pl. XXIII) the yolk-sac surrounds a greater portion of the trophoblast than it did at the middle of the same day (fig. 10, Pl. XXIII) ; and as the distal end (D) of the yolk-sac remains at a definite distance from the distal end of the uterine crypt, and as the invaginated proximal end (P) of the sac is no nearer the distal at the end than at the middle of the seventh day, it is evident that during the latter portion of the seventh day there is no further extension distally of the trophoblast, consequently the yolk-sac must have extended towards the proximal end of the uterine crypt over the outer surface of the trophoblast, or there has been simultaneous growth of the distal trophoblast and the hypoblast covering it.

It is during the seventh day that the invagination of the yolk-sac is completed; and though the invaginated portion afterwards increases in length, it does so simultaneously with the growth of the epiblast and trophoblast which it encloses.

After the completion of the invagination two areas of the hypoblast are clearly defined ; (1) the invaginated or internal portion ($H Y I$), and (2) the non-invaginated or external portion ($H Y E$). The former lies in contact partly with the epiblast and partly with the trophoblast; the latter is closely attached to the uterine mucosa. The only portion of the hypoblast which takes part in the formation of the embryo is the greater part of that which lies in contact with the epiblast, and it therefore is the embryonic hypoblast as distinguished from the remainder, which is extra-embryonic hypoblast.

The Eighth and Ninth Days.

In the early part of the eighth day the cavity in the distal

trophoblast of the mouse becomes more extensive, and in the rat the cavities of the epiblast and trophoblast meet and fuse ; thus in both animals the epiblast and distal trophoblast assume the form of hollow cylinders closed at one end (fig. 13).

The epiblastic cylinder is closed at its distal end, the trophoblastic at its proximal, and the open ends of the two cylinders are in close apposition, but not indistinguishably fused, for the characters of each portion of the ectoderm, after treatment with carmine, are still quite distinctive, the protoplasm of the trophoblast being tinged much more faintly than that of the epiblast.

For a time the united cavities of the epiblast and trophoblast increase in size, together with the general growth of the ovum, and this increase continues until in the latter part of the eighth day the mesoblast appears round the margin of the epiblast where it is in apposition with the trophoblast (fig. 14, Pl. XXIII).

The growth of the mesoblast causes a constriction of the opposed margins of the epiblast and trophoblast; in other words, it gives rise to the amnion folds (*Am.*), which are small on account of the contracted margins of the embryonic field.

It is at this period, the latter part of the eighth day, that it is possible to recognise for the first time the exact position of the embryonic area and its relations to the uterine walls.

During the preceding stages the epiblast has grown in a narrow cylindrical uterine crypt, and has assumed a corresponding cylindrical form.   The long axis cylinder lies at right angles to the long axis of the uterine canal, and it may for convenience be considered to possess four surfaces—two directed towards the sides of the uterine cavity, and two towards its extremities.   At the proximal end of one of the former surfaces a nodular mass of mesoblast appears (fig. 14, Pl. XXIV), indicating the posterior extremity of the primitive streak, and therefore the posterior end of the embryonic area. From the posterior nodular mass of mesoblast two wing-like plates rapidly extend round the sides of the cylinder and meet on the opposite surface, just where the epiblast and tropho-

blast are in contact (fig. 15, Pl. XXIV); and at the first glance
it appears probable that the point immediately behind the
fusion of the mesoblastic plates is the anterior end of the em-
bryonic area. Fraser and Duval have looked upon it in this
light. If this supposition were correct it is evident that the
anterior and posterior extremities of the embryonic area would
be directly opposite to each other, and that the central point
of the distal end of the cylinder would be the central point of
the embryonic area. This is, however, not the case, for it
soon becomes evident that a portion of the cylinder imme-
diately distal to the fusion of the mesoblastic plates forms for
a time a small pro-amniotic fold (AMP, fig. 15); therefore,
in the dorsal curvature of the embryonic area, which is one
of the peculiar features of the inverted layers, the bend does
not occur across, but some distance in front of the centre of
the area, and the neurenteric canal, which is situated a short
distance posterior to the centre of the young embryonic area,
is well behind the bend (NC, fig. 15). Further, it will
eventually be shown that portions of the lateral walls of the
cylinder become split up and take part in the formation of the
amnion; consequently these portions also are extra-embryonic.
In other words, within the area of the epiblast the whole of
the embryonic and part of the extra-embryonic area are
included.

The only portion of the extra-embryonic area included in
the epiblastic area is that which takes part in the formation
of the amnion, and the constriction which separates the amnion
from the false amnion does not pass through the epiblast as
Selenka figures it (45, Taf. xvi, figs. 64 and 67), but between
the epiblast and the trophoblast (see fig. 15).

The bent long axis of the embryonic area lies in a plane
which is at right angles with the long axis of the uterine canal.
The bend of the embryonic axis is opposite the inferior or ven-
tral border of the uterus; therefore the axial portion of the
embryonic area in front of the bend lies parallel with one sur-
face of the uterus, and the portion behind the bend is parallel
with the opposite surface of the uterus. One of these two

parts of the axial portion of the embryonic area corresponds in length with the whole of one side of the epiblastic cylinder. It is at the proximal end of this portion that the mesoblast first appears, marking the posterior or caudal end of the embryonic area; therefore, for convenience of reference, I shall call this side the caudal side (*CAS*, fig. 15), although it includes a little more than the caudal half of the embryonic area. The opposite side is the cephalic side (*CES*) of the embryonic cylinder.

As the long axis of the embryonic area lies in a plane at right angles to the long axis of the uterus until the eleventh day, when the twisting of the embryo commences, to obtain longitudinal sections of the embryonic area between the eighth and eleventh days it is necessary to cut the uterus into vertical transverse sections.

Coronal sections of the uterus between the eighth and eleventh days contain transverse sections of the embryonic cylinder; the cephalic caudal sides of the cylinder appear in each section. The section of the cephalic side represents part of the anterior portion of the embryonic area; the section through the caudal side part of the posterior portion of the embryonic area, except in the case of those sections which have passed through the distal end of the cylinder beyond the neurenteric canal (fig. 14 *A*), and have, therefore, cut the anterior part of the embryonic area twice—once on the cephalic and once on the caudal side of the cylinder. It must also be remembered that transverse sections which pass through the embryonic cylinder on the proximal side of the neurenteric canal cut the anterior and posterior halves of the embryonic area at unequal distances from the central point of the area.

Returning now to the consideration of the main changes of form which take place during the eighth and ninth days, it has been shown that the appearance of the mesoblast at the posterior end of the embryonic area (figs. 14 and 15, Pl. XXIV) gives rise to the tail amnion fold (*AFT*, figs. 14 and 15). The appearance of the mesoblast at the cephalic end of the em-

bryonic area produces the head amnion fold (*AFH,* fig. 15).
The continued growth of the mesoblast and the appearance
and extension of the body-cavity (figs. 15 *A* and 15 *B*) are
associated with the completion of the amnion folds and the
separation of the cavity of the trophoblast from that of the
epiblast, the latter cavity now becoming the amniotic sac;
at the same time the distal margins of the trophoblast are
fused, and thus the trophoblastic cylinder acquires a distal
wall (*DWT,* fig. 15 *B*), which is gradually invaginated into
the cavity of the trophoblast coincidently with the extension
of the cœlom, and finally the cavity of the trophoblast is
obliterated (fig. 19, Pl. XXVII).

Whilst the cœlom is extending a solid mass of mesoblast is
projected into it from the posterior end of the embryonic area.
This is the rudiment of the allantois (fig. 19, Pl. XXVII).

According to Fraser's account (13) the mesoblast is pro-
duced by the budding off of cells from the epiblast at the
hinder end of the embryonic area, over which portion of the
ovum the middle layer spreads rapidly in the form of two
lateral plates, which are not continuous across the middle line.
In addition to this embryonic portion the mesoblast also
spreads in another direction, splitting at the anterior and pos-
terior ends of the embryonic area; one part of it passes over
the amniotic portion of the neuramniotic cavity; the other
passes internal to the hypoblast over the free surface of the
epiblastic wall of the false amnion cavity.

Selenka also ascribes to the mesoderm a purely epiblastic
origin, for he describes it as formed by "eine zellenwucher-
ung des Ektoderms in Gestalt einer Platt auf, welche sich in
den Spaltraum zwischen Ektoderm und Entoderm hinein-
drängt. Gegen den Träger hin wuchert gleichzeitig aus dem
äusseren hinteren Umschlagsrande der Primitivrinne die
Allantois als Knopse hervor" (44, p. 17).

If these opinions stood alone, they would go far to support
Kölliker's (26) description of the mesoderm as a secondary
formation from the epiblast; but Duval is convinced "que
l'étude du blastoderme de la souris, malgré ses dispositions si

aberrantes, vient confirmer la loi générale que nous sommes efforcé d'établir dans d'autres études, à savoir que le méso-derme provient de l'entoderme primitif" (**9**).

Figs. 13 to 13 G represent sections of ova from the same uterus.   The ova are those of a rat, and they are in a state of development corresponding to about the early part of the second half of the eighth day in the mouse.   Figs. 13 to 13 F represent sections of an ovum in which the embryonic area was 266 μ long.   Fig. 13 G is a section of an ovum with an embryonic area 270 μ long, and therefore probably a little further developed than the ovum represented in figs. 13 to 13 F, although it was contained in the same uterine cavity.

Mesial longitudinal sections, figs. 13 to 13 B, at this period reveal clearly the several constituent portions of the ovum. The proximal part of the trophoblast (T. P.), which projects into the uterine crypt, to the wall of which it is attached, rests by its flange-like margin on the edge of the yolk-sac.   The distal portion of the trophoblast (T. D.) is enclosed with the inva-ginated hypoblast, and rests by its distal free margin upon the epiblast.

The epiblast on the caudal side of the cylinder (C. A. S, figs. 13 A and 13 B, Pl. XXIII) is slightly thicker than that on the cephalic side (C E S).   In the distal portion of the caudal half of the epiblast there is a very narrow neurenteric canal (N. C., fig. 13 A, Pl. XXIII), which passes obliquely through the epiblast into the cavity of the yolk-sac.   The epiblast is entirely sepa-rate from the hypoblast, except at the margins of the neuren-teric canal, where the two layers are fused.   In the middle line immediately distal to (in front of) the neurenteric canal the caudal part of the epiblast is thickened, and immediately proximal (posterior) to the neurenteric canal the caudal portion of the epiblast is thinner than elsewhere (fig. 13 A, Pl. XXIII).   At the proximal end of its caudal half (posterior end of embryonic area) the epiblast is thicker than in any other part of its extent, and in this situation it forms a distinct projecting knob-like process (fig. 13 B, Pl. XXIII).

The invaginated hypoblast (HY I., figs. 13 and 13 B) consists

of large, ill-defined, columnar cells, where it covers the distal portion of the trophoblast. As a rule, each cell of this portion of the hypoblast contains a large round nucleus, but on the caudal side of the distal trophoblast, and immediately proximal to the epiblast, nuclear proliferation is proceeding in the hypoblast (fig. 13 *B*, Pl. XXIII).

The invaginated hypoblast (*HY I.*, figs. 13, 13 *A*, and 13 *B*, Pl. XXIII) which surrounds the epiblast is much thinner than that which surrounds the distal trophoblast, and it is not divided into cell areas, except round the proximal end of the epiblastic cylinder (fig. 13 *B*), where the hypoblast which lies in contact with the epiblast gradually merges into and assumes the same characters as the hypoblast which surrounds the trophoblast. At the margins of the neurenteric canal this layer of the hypoblast becomes continuous with the epiblast (fig. 13 *A*).

The uninvaginated hypoblast (*HY E.*, fig. 13) consists of a single layer of flattened cells, which are spindle-shaped in section. Proximally it is slightly overlapped by the flange-like margin of the proximal portion of the trophoblast, but in the remainder of its extent it lies in direct contact with the maternal tissues, and in many places is bathed by maternal blood (42).

Transverse sections of the epiblast cylinder and the surrounding hypoblast render still more clear the peculiarities observable at this period.

Fig. 13 *C* is the twelfth of a series of forty-seven sections of an epiblastic cylinder 266 μ long. It passes through the cylinder immediately proximal to the neurenteric canal, and it confirms the appearances seen in fig. 13 *A* in the same position. On the caudal side of the cylinder the epiblast is thinnest, apparently on account of a depression of its internal surface. This depression is the anterior extremity of the primitive groove, which extends backwards (towards the proximal end of the caudal surface) for a distance of 17 μ; it is present, therefore, on sections 13 and 14, on the latter of which it is very shallow, but it is not found on section 15.

Behind the posterior extremity of the primitive groove the

epiblast is thickened in the middle line on the caudal side; this thickening is specially marked from the fifteenth to the eighteenth sections—that is, for a distance of 23 μ. The characters of the thickening are shown in fig. 13 D, Pl. XXIV, which represents the sixteenth section. Beyond the sixteenth section the epiblast on the caudal side is still thicker than on the cephalic side, but only to a very slight extent (see figs. 13 E and 13 F, Pl. XXIV, the twenty-fourth and thirtieth sections respectively), until the proximal end of the cylinder is reached, and here (fig. 13 G, Pl. XXIV) the epiblast on the caudal side in the middle line is much thicker than the epiblast on the cephalic side.

The relations of the hypoblast to the epiblast are rendered still more evident by transverse than they were by the longitudinal sections. On the cephalic and caudal sides of the distal portion of the epiblast, the central portion of the germinal area, the hypoblast is a thin layer of nucleated protoplasm, which lies in close contact with the epiblast (fig. 13 C). As the proximal portion of the tube is approached (figs. 13 D to 13 F) the hypoblast on both the cephalic and caudal sides becomes thicker. In other words, in the central portion of the embryonic area the hypoblast is a thin layer, and it is continuous with the epiblast round the margins of a narrow neurenteric canal (fig. 13 A, Pl. XXIII), which lies in the mesial plane of the embryonic area behind its centre.

In the peripheral part of the embryonic area the hypoblast is thicker than in the central portion; but it is, up to this time, entirely distinct from the epiblast. Within a very short period, however, when the long axis of the embryonic area has increased to 270 μ, the thickened epiblast of its posterior extremity fuses with the underlying hypoblast (fig. 13 G). This fusion appears to take place without any active participation of the hypoblast, for at the forty-third section from the distal end of the caudal epiblast, where the commencement of the fusion is first noticeable, there is no sign of proliferation of the hypoblastic nuclei, and the fusion appears to be brought about by the gradual flowing together and admixture of the

darkly staining epiblastic and faintly staining hypoblastic protoplasm. But though there is no active proliferation of the hypoblast in the first stages of its fusion with the epiblast in the posterior portion of the embryonic area, such prolifera-tion does not long remain absent, for at the posterior end of the area of fusion, which has an extent of 67·5 μ, there are distinct signs of activity in the nuclei of the hypoblast.

The position of the thickened area of epiblast, between the neurenteric canal and the proximal end of the epiblast (fig. 13), that is, between the neurenteric canal and the posterior end of the embryonic area in the mid-axial line, indicates that it is the rudiment of the primitive streak.

The groove which appears upon the surface of the streak is, therefore, the primitive groove. In an embryonic area about 532 μ long, the groove is only about 17 μ long. It is deepest in front at the neurenteric canal. In an embryonic area 540 μ long the primitive groove is 190 μ long; it is present from the neurenteric canal to within 5 μ of the posterior end of the embryonic area, therefore it extends backwards, and as the groove extends, the thickened epiblast of the primitive streak becomes thinned out beneath it for a time (compare fig. 13 C, with fig. 13 D, Pl. XXIV).

When the epiblast tube has attained a length of 288 μ in the rat, and the germinal area is therefore about 576 μ long (fig. 14), the general relations are similar to those described when the germinal area was 532 μ long, except that from the posterior end of the primitive streak a nodular mass of mesoblast projects between the trophoblast and hypoblast. Selenka (44) has termed this mass the " Allantoisknospe." It will, however, be shown, in connection with the description of formation of the extra-embryonic cœlom, that the true allantoic projection does not appear until a considerably later period—that is, after the formation of the caudal and cephalic portions of the extra-embryonic cœlom.

The neurenteric canal is still present, but is just as indis-tinctly marked as in the preceding stage (N C, fig. 14 A, Pl. XXIV), except at its lower part where a distinct space is visible.

In longitudinal sections of the central portion of the embry-
onic area (fig. 14 *A*) it is impossible to discover any separation
between the epiblast and hypoblast at the anterior end of the
primitive streak.   In front of the neurenteric canal both the
epiblast and hypoblast are thickened, and in the latter layer
the nuclei are no longer arranged in a definite single row, but
are more or less irregularly scattered throughout the proto-
plasm.   Further forward, towards the cephalic end of the
embryonic area, the hypoblast becomes thinner and its nuclei
less numerous.

Transverse sections of an ovum from the same uterus
confirm all the most important points observable on longitu-
dinal sections, and they give much more information concern-
ing the formation of the mesoderm.

Figs. 14 *B* to 14 *I* are representations of transverse sections
which have passed in an oblique plane through an ovum with
an epiblast cylinder about 288 *μ* long.   The cylinder is
divided into sixty-one sections, and the tenth section (fig.
14 *B*) passes just in front of the fusion of epiblast and hypo-
blast in the anterior boundary of the neurenteric canal along
the line (*b*) (fig. 14 *A*, Pl. XXIV).   The epiblastic cylinder is
surrounded on all sides by a ring of hypoblast.   The epiblast is
thickened on the caudal side (*CA S.*), where it projects
towards but does not touch the hypoblast, which is thickened
in the same region.   The hypoblast, however, is not only
thickened on the caudal side in the middle line, but also
laterally over an area equal to about a third of the circumfer-
ence of the section, and in the same regions its nuclei are more
numerous than elsewhere, and they are irregularly arranged in
two rows.

The next section, the eleventh (fig. 14 *C*), passes through
the anterior boundary of the neurenteric canal along the line
c (fig. 14 *A*).   On the caudal side of the section the epiblast
and hypoblast are fused, and laterally for about half the cir-
cumference of the section the hypoblast is thickened, and its
nuclei are arranged in a double row.

As the sections proceed towards the proximal end of the

epiblastic cylinder—that is, forwards towards the anterior end
of the cephalic side (*CE S.*) and backwards towards the
posterior end of the embryonic area on the caudal side
(*CA S.*), it becomes evident that the thickening of the hypo-
blast gradually extends further and further round the circum-
ference of the cylinder (see figs. 14 *D*, the fifteenth section,
and 14 *E*, the thirty-third section) ; and it is further noticeable
that at the thirty-third section spaces begin to appear in the
thickened hypoblastic layer. These spaces gradually assume
a more definite arrangement (fig. 14 *F*) at the fortieth section,
and at the fifty-second section (fig. 14 *G*) they have fused,
separating a third layer, the mesoblast, from the thickened
hypoblast, except to the right of the section on the cephalic
side, to which the thickening of the hypoblast has now ex-
tended. At the fifty-eighth section (fig. 14 *H*), which passes
in the direction of the line *H* in fig. 14, the massive meso-
blastic plates are entirely separated from the hypoblast, but
they remain connected on the caudal side with the primitive
streak. In this region the greater part of the hypoblast
appears to be formed of large columnar cells, which contain
large rounded nuclei at their bases ; but in the middle line on
the cephalic side the hypoblast is still a homogeneous proto-
plasmic layer which contains a double row of nuclei.

At the sixtieth section, 9 μ further forward on the cephalic
side, the apices of the mesoblastic plates are again fused with
the hypoblast, from which they do not again separate. Beyond
the proximal end of the epiblastic cylinder the mesoblast
forms a continuous sheet, which intervenes between the tro-
phoblast and hypoblast in the middle line on the caudal side;
and as the sections pass further forward on the cephalic, and
further backward on the caudal side, the margins of the meso-
blastic plate recede gradually from the cephalic towards the
caudal side, and thirty-six micromillimetres beyond the
posterior end of the primitive streak the mesoblast is only
found on the caudal side (fig. 14 *I*, Pl. XXIV), where it forms
a thin layer not distinctly separated from the hypoblast, in
which the nuclei are in an active state of proliferation, on the

caudal (*CA S.*) and lateral aspects of the cylinder ; whilst on the cephalic side (*CE S.*), at this period, they show no distinct evidence of activity.

In mice embryos at the commencement of the ninth day of gestation many of the succeeding stages of mesoblastic formation may be readily followed. They are shown in the series of figs. 15 to 15 *H*, which represent sections of different mice ova from one uterus. The differences between them are therefore small, but some of the ova are evidently a little more advanced than others.

Longitudinal sections of the least developed of the series are shown in figs. 15, 15 *C*, and 15 *D*, Pl. XXIV. The neurenteric canal is still present (figs. 15 and 15 *D*) at the centre of the embryonic area, which is about 544 μ long. The upper aperture of the canal is not distinct, but its lower opening is moderately well marked, though the canal is only an exceedingly narrow channel. In front of its lower opening the epiblast and hypoblast are fused, and for a short distance towards the cephalic extremity of the embryonic area the hypoblast is thickened, and contains a number of irregularly placed nuclei.

Behind the neurenteric canal, in the anterior region of the primitive streak, the epiblast and hypoblast are not separable, though a series of flattened cells on the inferior surface of the streak suggest a thin and expanded hypoblastic layer. This suggestion is, however, not confirmed by transverse sections of a slightly more advanced embryo (figs. 15 *F* and 15 *G*, Pl. XXV), which show that in the region of the anterior part of the primitive streak the epiblast and hypoblast are fused. In longitudinal sections (fig. 15) the hypoblast beneath the posterior third of the primitive streak appears as a distinct layer, and transverse sections of the same region show that it is separated from the mesoblast by a cleft-like space (fig. 15 *H*).

The extension of the mesoblast round the sides of the cylinder is much more advanced than in the preceding stage, for at the ninth section from the distal end of the cylinder

(fig. 15 F) the third layer has already arrived at the margins
of the cephalic surface where it is not yet separated from the
hypoblast.   It ceases as a distinct layer at the same distance
from the middle line through twenty-five sections which pass
through a gradually expanding cylinder, but after the circum-
ference of the cylinder becomes uniform the mesoblast advances
nearer to the middle line on the cephalic side, and imme-
diately beyond the extra-embryonic area the mesoblastic plates
of opposite sides meet and fuse with each other.   When the
mesoblastic plates first meet and fuse in the middle line on the
cephalic side, they are united with the hypoblast, which pro-
jects as a thickened ridge (figs. 15 and 15 C, Pl. XXIV) against
the epiblast, and thus produces a portion of the cephalic amnion
fold.   Immediately to the distal side of the ridge a small
portion of both the epiblast and hypoblast is carried inwards
towards the interior of the cylinder, and in this very short
double layer is the rudiment of the true pro-amniotic fold.
Almost immediately afterwards the apex of the ridge becomes
separated from the hypoblast, and thus in the middle line on
the cephalic side a continuous layer of mesoblast is produced
which intervenes between the hypoblast and epiblast imme-
diately in front of the embryonic area (see fig. 15 H), which
represents an oblique section along the line H (fig. 15 B).

In front of the advancing margins of the mesoblastic plates
the hypoblast is thickened, and contains the nuclei irregularly
arranged in more than one row (figs. 15 F and 15 G).   That
the same conditions occur wherever the mesoblast is about to
separate from the hypoblast is shown not only by transverse,
but also by lateral longitudinal sections (fig. 15 E, Pl. XXIV).

The next succeeding stages of mesoblast formation are
represented in the transverse sections of a rat ovum depicted
in figs. 16 to 16 F, Pl. XXV, and Pl. XXVI, fig. 16 H.   From
a mere glance at the series it appears that the diameter of the
cylindrical ovum is very much increased, and that the increase
in size of the cylinder is not due to distension accompanied
by thinning of all the wall, for the hypoblast is not thinner
than in preceding stages, and the mesoblast is evidently thicker.

The inner and middle layers have, therefore, grown simultaneously with the increase in size of the ovum.   The changes in the epiblast, however, do not indicate any very great activity in that layer.   In sections of the distal end of the cylinder, where the extension of the cavity has not been great, the epiblast is a uniformly thick ring (fig. 16 *B*) continuous with the mesoblast in the primitive streak on the caudal side (*CA S.*) of the section, and abutting directly against the thickened hypoblast in the middle line on the cephalic side (*CE S.*); but towards the proximal end, where the cylinder is most dilated, the epiblast gradually becomes thinner, first on the lateral aspects (figs. 16 *C* and 16 *D*), and then on the cephalic side of the cylinder (fig. 16 *E*), where it becomes reduced first to a thin layer of nucleated protoplasm, and in ova at a little later period to a thin layer of flattened squames.

The mesoblast is continuous with the epiblast and hypoblast in the primitive streak from its anterior extremity (fig. 16 *A*) backwards for a distance of 23·9 μ, and for the same distance it is still adherent to the hypoblast at the sides of the streak (fig. 16 *B*).   Further backward in the embryonic area to the end of the primitive streak, a distance of 311 μ, the mesoblast is separated from the hypoblast, but is continuous with the epiblast (figs. 16 *C* to 16 *E*, Pl. XXV, and figs. 16 *G* and *H*, Pl. XXVI).

In front of the primitive streak the mesoblastic plates end at the side of a rod-like thickening of the hypoblast (figs. 16 *A* to 16 *D*).

In the distal portion of the cylinder the mesoblast extends as a thick continuous layer from the primitive streak on the caudal side to the margins of the hypoblastic ridge on the cephalic side (fig. 16 *B*); but 120 μ from the end of the embryonic area, where the cylinder is expanded and the lateral epiblast much thinned, the mesoblast presents indications of separation into two portions on each side (fig. 16 *C*, right side), and at 55 μ from the end of the embryonic area the separation is completed on the right side (fig. 16 *D*).   One portion on each side belongs to the cephalic end of the

embryonic area; it lies free between epiblast and hypoblast, its thickened internal extremity abuts against the hypoblastic ridge, and its external extremity contains a small cavity, a portion of the cœlom. The other portion is more extensive; it also lies free between epiblast and hypoblast, except in the middle line on the caudal side, where it is continuous with the epiblast in the primitive streak. It belongs, therefore, to the caudal section of the embryonic area. In its peripheral margin a small section of the cœlomic cavity is visible.

In the anterior portion of the embryonic area, in front of the hypoblastic ridge, the mesoblast extends across the middle line, and it presents peculiarities which are worthy of notice.

In mesial longitudinal sections of a mouse embryo at this period the hypoblastic ridge, recognisable by the regularity of arrangement and the oval shape of its nuclei (fig. 17 A, Pl. XXVI), terminates abruptly just at the point where the superjacent epiblast begins to thin. In front of it, beneath the thin epiblast of the anterior portion of the embryonic area, the hypoblast is irregularly thickened, and it contains many nuclei rounded in form, but varying in size.

At this period transverse sections of the most anterior part of the embryonic area show that the mesoblast on the cephalic side of the cylinder becomes gradually reduced in amount from behind forwards (compare fig. 16 D, 60 μ behind the anterior extremity of the area, with fig. 16 E, 15 μ behind the same point). Still further forward the reduction continues, until this portion of the mesoblast entirely disappears (fig. 16 G, Pl. XXVI) at the edge of the embryonic area, whilst at the same time the caudal mesoblast encroaches towards the cephalic side; but the two extremities of the caudal mesoblast do not reach the middle line on the cephalic side simultaneously with the disappearance of the cephalic mesoblast. In front of the point where the cephalic mesoblast ends for a distance of 20 μ the epiblast of the extra-embryonic area is in contact with the hypoblast (fig. 16 G, Pl. XXVI). Immediately in front of this bilaminar area the extra-embryonic mesoblast appears between the epiblast and hypoblast as a series of islets, each of which

contains a cavity (fig. 16 *H*). These islets and their cavities merely indicate an irregular indented margin of the extra-embryonic cœlom, for as the sections are traced forwards the islets fuse one by one with the extra-embryonic portion of the caudal mesoblast.

In the extra-embryonic area the somatic mesoblast (*S M.*) is represented by a single layer of flattened cells which covers the outer surface of the amnion (figs. 16 *H* and 16 *F*) and the inner surface of the trophoblast (fig. 19, Pl. XXVII). It is continuous laterally with the undivided paraxial mesoblast, which lies at the sides of the chorda in the anterior portion of the embryonic area, and with the primitive streak in the posterior portion of the area. At the posterior end of the embryonic area the somatic mesoblast is continued into the rudiment of the solid allantois (fig. 19, Pl. XXVII, which is a semi-diagrammatic figure of an older embryo), and at the anterior end it becomes continuous with the splanchnic mesoblast of the extra-embryonic region, but is entirely distinct from the axial mesoblast of the anterior portion of the embryonic region, being separated from it by the bilaminar area before mentioned.

The spaces in the mesoblast of the embryonic area in front of the hypoblastic ridge lie laterally (fig. 16 *E*), and are directly continuous behind with the cœlomic spaces in the paraxial mesoblast, but anteriorly they disappear.

The splanchnic mesoblast differs entirely from the somatic, for instead of forming a single layer of flattened cells it has gained new connections with the hypoblast at many irregularly distant points, and in these situations proliferation of the hypoblastic nuclei occurs, and the splanchnic mesoblast is thickened (figs. 16 *F* and 16 *H*).

In the axial line of the embryonic area the distinction between the primitive streak and the region in front of it is still well marked. A short distance behind the centre of the embryonic area a distinct line of demarcation, the remains of the neurenteric canal (*N C.*, fig. 17, Pl. XXVI), bounds the anterior end of the primitive streak. Immediately behind this

line no layers are distinguishable (fig. 17, Pl. XXVI), and the large rounded nuclei have no definite arrangement.

In front of the remains of the neurenteric canal the epiblast and hypoblast are fused for a distance of 21 μ (fig. 17, Pl. XXVI). In this region of fusion, as in the primitive streak, the nuclei are large, rounded in shape, and not definitely arranged ; but further forward, where the two layers are separate, the nuclei of the hypoblastic ridge, to which attention has been drawn in the description of transverse sections, are generally large, of oval shape, and assume a somewhat palisade-like arrangement (fig. 17). They retain this form and arrangement for only a short distance, and then, when the hypoblastic ridge is narrowed (fig. 16 B), the nuclei are smaller and rounder; still further forward they are flattened, but in the thickened anterior portion of the ridge (figs. 16 C and 16 D) the nuclei are again large and oval.

The nuclei of the epiblast which is immediately superjacent to the hypoblastic ridge are also, for the most part, oval, and their long axes lie at right angles to the surfaces of the layer (figs. 16 C, 16 D, and 17).

During the latter part of the ninth day in the mouse, and at a corresponding period in the rat, the most important changes which take place are those in the anterior portion of the embryonic area.

In a mesial longitudinal section (fig. 18 A, Pl. XXVI) an axial mass of mesoblast is seen between the epiblast and hypoblast at the anterior end of the embryonic region. It is quite separate from the epiblast above it, and from the splanchnic mesoblast of the extra-embryonic region in front of it, though the latter now lies in close relation with the axial mesoblast, for the didermic area which previously intervened between them has disappeared in this embryo, but in another embryo from the same uterus a small portion not more than 8 μ in antero-posterior length remains (fig. 18 B).

At one point the inferior surface of the axial mesoblast is fused with the hypoblast, from which it is apparently receiving a further addition of nuclei. The hypoblast beneath the

anterior axial mesoblast is thickened.   It is continuous behind
with the hypoblastic ridge, which it joins at a slight angle, and
in front with the extra-embryonic hypoblast.

In mesial longitudinal sections the hypoblastic ridge extends
backwards to the anterior end of the primitive streak, imme-
diately in front of which it is considerably thickened, and
becomes continuous with the epiblast (fig. 18).   There is at
this stage no trace of the neurenteric canal, but the more
regular arrangement of the nuclei in both hypoblast and epi-
blast in front of the primitive streak, and their less regular
arrangement in the streak itself, clearly indicate the former
position of the canal.

The peculiar relations of the mesoblast of the anterior por-
tion of the embryonic area, in front of the hypoblastic ridge,
are most clearly revealed by transverse sections.   Beneath the
epiblast the hypoblast is bent towards the yolk-sac (fig. 18 C).
The mesoblast extends across the mouth of the bay-shaped de-
pression, and is attached laterally to the hypoblast.   Beyond
the marginal attachments of the anterior portion of the em-
bryonic mesoblast small mesoblastic islets are found in the
extra-embryonic area ($M I.$).   These are the first rudiments of
the blood islets, and they are not connected with the embryonic
mesoblast.

Further back, at the anterior end of the hypoblastic ridge
(fig. 18 A), the anterior axial mesoblast terminates in the
middle line, but it becomes continuous by its lateral margins
with the paraxial mesoblast of the anterior portion of the em-
bryonic region (fig. 18 D).   The paraxial mesoblastic plates
are widely separated anteriorly, for a distance of 40 μ, by the
broad anterior extremity of the hypoblastic ridge, and late-
rally they are continuous with the hypoblast, from which they
seem to be receiving many new nuclei.   In the interior of the
plates, but nearer their outer than their inner extremities,
cavities are developed (fig. 18 D).

More posteriorly the inner ends of the paraxial mesoblastic
plates approach nearer to each other, simultaneously with the
narrowing of the hypoblastic ridge, and they assume a trian-

gular form as on the left side of fig. 18 E. Their peripheral extremities remain continuous with the hypoblast, and the cavities which they contain become larger.

Still more posteriorly (fig. 18 F, Pl. XXVII) the inner portions of the paraxial mesoblastic plates become divided up into a number of stellate cells, and their outer parts assume a more epithelial arrangement. One hundred and thirty micromillimetres behind the anterior extremity of the hypoblastic ridge the lateral portions of the paraxial mesoblastic plates are separated from the hypoblast, but they have become continuous with the somatic and splanchnic mesoblast of the extra-embryonic area (fig. 18 F).

The changes in the form of the hypoblastic ridge are also most readily observable in transverse sections. Anteriorly it is widely expanded, thickened at its lateral margins, and slightly curved (fig. 18 D); more posteriorly, apparently by folding, it is converted into a wedge-shaped ridge (fig. 18 E); then it becomes reduced to a single row of rounded nuclei embedded in a small amount of protoplasm, and this thin layer is excluded from the vitelline cavity by a single layer of flat cells (fig. 18 F); still more posteriorly, whilst it retains the same characters, it resumes its place in the dorsal wall of the vitelline cavity, and in the last 40 μ of its extent it is a broad curved plate similar to that represented in fig. 20, Pl. XXVII.

At the end of the ninth day a longitudinal section of the embryonic area may be divided into four parts.

1. From the anterior end of the embryonic area to the anterior extremity of the hypoblastic ridge (from A to B, fig. 18 A).

2. From the anterior to the posterior end of the expanded anterior portion of the hypoblastic ridge (from B to C, fig. 18 A).

3. From the posterior end of 2 to the anterior end of the primitive streak.

4. From the anterior end of the primitive streak to the posterior end of the embryonic area.

During the tenth day, simultaneously with the appearance

of the cephalic projection, the posterior end of the second portion is carried upwards, that is, away from the yolk-sac, and forward. Thus the second portion is first horizontal (fig. 18 *A*), but as its posterior extremity rises it becomes oblique, then vertical, and at last again oblique, but with the extremity which was posterior now anterior (fig. 19 *A*) ; and the whole of the mesial portion of this area forms the anterior wall of the fore-gut—that is, it becomes the bucco-pharyngeal membrane.

Whilst this movement of the second portion is taking place the first portion retains its original position (fig. 19 *A*, Pl. XXVII), and the cavity which, in the latter part of the ninth day, was only found laterally in the mesoblast of this area, now extends across the middle line.

During the course of the tenth day the anterior end of this portion of the embryonic area is folded backwards until it forms the anterior boundary of the umbilical orifice, and its two surfaces are reversed in position. In this manner the ventral wall of the fore-gut is completed. The cavity in the mesoblast of the ventral wall of the fore-gut is evidently the mesial portion of the pericardial cavity ; it extends from the bucco-pharyngeal membrane to the anterior boundary of the umbilical orifice.

The hypoblast of the bucco-pharyngeal membrane is not distinguishable, in mesial longitudinal sections, from the remainder of the hypoblastic ridge, which is seen to become gradually thinner as it is traced backwards along the dorsal wall of the fore-gut.

In transverse sections of embryos in the early part of the tenth day the characters of the hypoblastic ridge behind the bucco-pharyngeal membrane are very similar to those described at the end of the ninth day, but it is, in relation to the embryo, proportionally longer; and it is separated from the vitelline cavity for a somewhat greater distance by the ingrowth of a layer of flat cells beneath it, and the characteristic features of its posterior extremity are better marked.

Its posterior part, for a distance of 70 μ, has the form of a

broad curved plate (fig. 20, Pl. XXVII,), which lies between the paraxial plates of mesoblast, and is directly continuous with the remainder of the hypoblast; this is succeeded by a thickened rod of hypoblast (fig. 20 A), which bears the same relation to the mesoblast and the remainder of the hypoblast as the curved plate in front of it. The rod-like mass is 40 μ long. It terminates posteriorly in the anterior end of the primitive streak, where it is fused dorsally with the epiblast, and laterally with the mesoblast (fig. 20 B).

At the eleventh day, when the neural groove is closed from the lumbar region to the mid-brain, and the folding of the posterior end of the embryonic area has resulted in the enclosure of the posterior part of the gut, the hypoblastic ridge is entirely separated from the remainder of the hypo-blast, except at the dorsal end of the bucco-pharyngeal mem-brane in front, and at the anterior extremity of the primitive streak behind (figs. 21 and 21 A). In the latter situation the posterior end of the ridge is not only continuous with the dorsal wall of the gut (figs. 21 and 21 A), but also with the mesoblast of the primitive streak, and through it with the epiblast; the connection is more evident in transverse than in longitudinal sections (fig. 21 B).

The folding of the posterior end of the embryonic area mainly affects the region of the primitive streak, which is bent upon itself twice; first at the junction of its anterior and middle thirds, and secondly at the junction of its middle and posterior thirds. The upper surface of the anterior third enters into the formation of the neural canal, and after the fusion of the medullary folds it is entirely shut off from the surface of the body.

The superficial surface of the middle third of the primitive streak remains on the surface of the body between the base of the tail and the anus.

The anus is formed through the upper part of the posterior third of the streak, where at the eleventh day the anal membrane is very clearly defined (fig. 21).

I have not yet been able to trace the early stages of the

formation of the hind gut ; but when the caudal portion of the gut is well developed the relations of the floor of the neural tube to the mesoblast, and of the chorda to the termination of the gut and the mesoblast, are the same as those found at the anterior end of the primitive streak at the eleventh day. It seems probable, therefore, that the hind gut is formed from the hypoblast of the anterior third of the primitive streak, and that the anterior portion of the streak forms the growing point of the tail, in which case the fate of the primitive streak of mammals is exactly similar to the fate of the primitive streak of Rana temporaria (40).

The Segmentation Cavity and the Didermic Blastocyst.

It is during the period which precedes the completion of the didermic condition that peculiarities appear in the mammalian ovum which render its comparison with the ova of other Vertebrates a matter of some difficulty.

A complete didermic vesicle—that is, a vesicle with a double wall entirely surrounding the cavity, and with no intervening stratum anywhere present between the two layers—exists both amongst the lower and the higher Vertebrata : it is found, e. g., in the Amphioxus, Cyclostomes, Ganoids, Amphibians, and in Mammals. In a large number of Vertebrates, however, a third layer appears before the second layer is completed, and it is both customary and convenient to speak of a developing vesicle as didermic, even when only part of its wall is formed by two of the primitive germinal layers ; a didermic stage may, therefore, be considered to be universal in the development of the Vertebrate ovum. The mode of formation of the didermic vesicle and the relations which its two layers bear to each other vary considerably.

In Amphioxus (15, 28) the segmentation of the ovum is followed by the formation of a monodermic vesicle with a central cavity, the segmentation cavity ; and the didermic condition is produced by the invagination of a portion of the wall of the vesicle ; at the same time the segmentation cavity is

obliterated, and a new cavity, that of the archenteron, appears. The latter is from the first in communication with the exterior by a widely open orifice, the blastopore.   In the majority of Vertebrates, however, the inner boundary of the didermic vesicle, the hypoblast, is not produced as a distinct layer of cells, but either as a solid mass of food-laden cells or food-laden protoplasm, which is not divided into cells.   The former condition is met with in Amphibia (1), Cyclostomes (1, 14, 30, 47), Ganoids (1); the latter in Teleosteans (1, 7), Elasmobranchs (1) ; Lacertilians (50), Chelonians (1, 35), and Aves (1).

The large hypoblastic mass in all these cases is produced by an increase of the protoplasm destined to form the ventral wall of the archenteron ; consequently the latter cavity, when it first appears, is situated excentrically.   It lies, however, within the hypoblast, and is therefore entirely separated from the segmentation cavity, which is represented either by a large cavity, which gradually disappears simultaneously with the increase of the archenteron, as in Elasmobranchs (1), Cyclostomes (1, 14, 30, 47), Ganoids (1), and Amphibians (1); or by a small and also transient cavity, as in birds (10) ; or from the first by a narrow space, as in the Lacertilians (22).

The increase of the ventral hypoblast is associated with modifications, both in the formation of the archenteron and also in the completion of the didermic vesicle.

In Amphibians the archenteron is produced by cleavage, which takes place excentrically in the hypoblastic mass (22 a, 36, 40).   It commences peripherally at the posterior part of the ovum, and passes forward into its interior.   The cavity is apparently formed in a very similar but somewhat modified way in Teleosteans.   In Elasmobranchs (1), Cyclostomes (1, 14, 30, 47), and Ganoids (1) the dorsal wall of the archenteron is said to be formed by embolic invagination, and its ventral wall by the hypertrophied ventral hypoblast, which appears during the segmentation.   In all these cases, however, the archenteron commences before the inclusion of the hypoblast is completed, and there can be little doubt that the retardation

of epiblast formation over the surface of the ventral hypoblast is in some way associated with the increased amount of food material deposited in the ovum.

The archenteron in the Cephalochorda, Elasmobranchs, Cyclostomes, Teleosteans, Ganoids, and Amphibians is from the first in direct connection with the exterior by an opening, at the margins of which the epiblast and hypoblast are continuous; this is the true blastopore, which becomes occluded by the fusion of its margins, either from before backwards or from behind forwards, giving rise to a primitive streak in all the orders mentioned except the Cephalochords (40). Cunningham (8) and Rabl (37) have already drawn attention to the fact that increase in the posterior part of the ventral hypoblast splits the ventral lip of the blastopore, and carries the two halves outwards and forwards, the dorsal lip remaining as a fixed point; as the proliferating epiblast extends the opening is re-formed from the dorsal to the ventral side, the ventral portion of the blastoporic rim being the last part completed. If the increase of the ventral hypoblast takes place more anteriorly a "yolk hernia" results; that is, the formation of the epiblast is interfered with a short distance in front of the ventral lip of the blastopore, and it is not difficult to understand how, by the subsequent increase of the more posteriorly situated ventral hypoblast, such a yolk hernia might be prolonged backwards until it became continuous with the blastopore. In a case of this kind the process of completion of the epiblastic layer would follow in the reverse way, and the posterior lip of the blastopore would be completed before the "yolk hernia" was covered in. Apparently the Elasmobranchii present us with examples of a process of this kind in its simplest form. The supposititious opening in the epiblast, through which the yolk hernia projects, is the yolk blastopore, and at its margins the epiblast and yolk are in close contact, if not absolutely fused.

In lizards and birds there is also a yolk blastopore which at its origin is apparently situated further forward than in the Elasmobranchs, but as in them, by the great increase of the

yolk, it has been extended back into the true blastopore. We may imagine that the true blastopore in lizards and birds has been split, as compared with the opening of the yolk blastopore, at a comparatively late period; therefore its margins are not so far apart as the margins of the yolk blasto- pore, and as the epiblast extends they are swung back into position long before the yolk blastopore is closed.

In all the Vertebrata below the Sauropsida and mammals the fusion of the hypoblast and epiblast in the margins of the true blastopore remains during the closure of that orifice and for some time after.

In lizards and birds there is an early separation of the two primitive layers in the region of the true blastopore; the separation is, however, only temporary, and before the yolk blastopore is closed the two layers have reunited in the primi- tive streak, which becomes perforated by the neurenteric canal. It is difficult to suggest any satisfactory explanation of this phenomenon; possibly the proliferation of the posterior part of the ventral hypoblast in the lower Vertebrates has been in some way associated with a tendency to increased rapidity of epiblast production in order that the early enclosure of the hypoblast might be ensured. In the passage from the lower Vertebrates to the Protamniota the tendency to rapid epiblast formation was retained, but the situation of the ventral hypo- blastic proliferation was transferred to a more anterior posi- tion; and consequently, as the area to be covered in the region of the true blastopore was reduced, the rapidly growing epi- blast encroached upon the orifice and brought about its early occlusion, after which the two layers, previously united in its margins, became separated; and when this separation occurred, the cells forming the boundary of the orifice, which may be termed from this position peristomal cells, remained attached to the epiblast, and continued quiescent until the period of mesoblast formation commenced, when they proliferated and produced a ridge-like thickening on the under surface of the epiblast, which was the earliest rudiment of the primitive streak. These phenomena are repeated in lizards and birds.

In the former, however, the earlier stages have probably been shortened, for no one appears to have observed the closure of the blastoporic lips; but in birds Duval (**10**) has described the process of concrescence which precedes the formation of the primitive streak.

The tendency to early separation of epiblast and hypoblast in the region of the blastopore has been transmitted from the Protamniota, not only to the Lacertilia, but also to Mammalia; but in the latter order, due probably to the altered condition in which the ovum is placed during the early stages of its development, many other modifications of the ordinary developmental processes also appear. Dealing, however, with the rat, mouse, and hedgehog only, it is not difficult to trace a fairly close correspondence between mammalian and lacertilian development. If an ovum with a transmitted tendency to comparatively great hypoblastic proliferation becomes embedded, at an early period, in a decidual tissue from which nutriment can be easily absorbed, it is quite possible that the hypoblast may acquire an attachment to the uterine tissues, in which case the spread of the epiblast will be prevented and the yolk blastopore will remain permanently open, as in the rat and the mouse. Under these circumstances solid yolk formation will no longer be necessary, and it will probably cease; but, as Hubrecht has already pointed out (**23**, p. 529), the exposure of a large ovular surface to the maternal tissues would still be a distinct advantage; and thus, instead of the yolk-sac disappearing in the passage from the Protamniota to the Mammalia, it has been retained, and has acquired the form of a large hollow sac, which becomes filled and distended by maternal fluids passed into it either by secretion or osmosis. Nevertheless even in the rat and the mouse the yolk-sac is relatively small when compared with the yolk-sac of the Lacertilia, and there can be but little doubt that during the passage from the meroblastic Protamniota to the holoblastic mammal a considerable reduction in the rapidity of hypoblastic proliferation has occurred; and this reduction has been so great in the hedgehog that at an early period the yolk blastopore becomes closed by the

spreading epiblast: its situation, however, is marked, at least for a time, by the adherence of the hypoblast and epiblast (24). In such cases the maternal fluids must pass through both layers before they can distend the cavity of the yolk-sac and bring about the extension of the nutritive area; and apparently to facilitate the passage the epiblast soon becomes permeated by maternal blood, which circulates through spaces in the epiblastic layer.

In another respect also there is a close resemblance between the ova of rats and mice and those of Lacertilians, for at an early period all these ova consist of a small area of epiblast resting upon the upper pole of a large yolk-sac; the mesial portion of the epiblastic area is separated from the underlying hypoblast by a distinct space, which may be looked upon as a remnant of the segmentation cavity; the middle portion of the epiblast is thick, but its margins are thin, and, as in reptiles, they lie in direct contact with the hypoblast. The fact that the hypoblast in the rat and the mouse does not become entirely surrounded by epiblast as in the Lacertilia seems to be entirely due to close apposition of the hypoblast and the decidua in the former animals. The hedgehog's ovum differs from the lacertilian ovum in the opposite direction, for in it the yolk blastopore becomes closed at a comparatively early period, and the two primitive layers are for a time almost entirely separated from each other by a distinct space. Still, in the rat, the mouse, and the hedgehog, in spite of the modifications brought about by the reduction of the hypoblast and the inclusion in maternal decidua, the general resemblance to the lacertilian ovum is retained, and it is probably due to inherited tendencies transmitted both to the Lacertilia and Mammalia from a common ancestor, one of the protamniotic Vertebrates.

Further, in the rat, mouse, and hedgehog, as in lizards, tortoises, birds, and all the lower Vertebrates, a space appears within the hypoblastic mass. Van Beneden asserts that this is merely a space in the yolk substance, a "lecithopore," not comparable with the archenteron of other Vertebrates. The

phenomena observable in the rat and mouse do not substantiate this inference; on the contrary, they very definitely indicate the reverse conclusion, for a portion of the cavity becomes the true enteric canal, and from its walls both notochord and meso-blast are formed, as in lower Vertebrates. But although, in the main features of the early stages of development, the ova of the rat, mouse, and hedgehog correspond closely with the ova of the Lacertilia and the lower Vertebrata, in the ova of the majority of the Mammalia which have been investigated, peculiarities in the formation of the didermic vesicle are de-scribed which have hitherto received no satisfactory explana-tion, and there is room for difference of opinion concerning the nature of the cavity contained within the two primitive layers.

In the cat there seems reason to believe, from Professor Schafer's account of an early ovum (43), that the processes by which the didermic vesicle is formed correspond with those which obtain in the hedgehog, but we have no definite proof that this is the case. Bonnet's account of the sheep does not extend to the stages which precede the completion of the didermic vesicle. Precise accounts have been given of the de-velopmental features which precede the completion of the didermic stage in the opossum (46), rabbit (2, 27), mole (17), shrew (23), bat (4), and the guinea-pig (45). In the ova of all these mammals, according to the descriptions given, the cavity which afterwards becomes the cavity of the yolk-sac lies at first between the epiblast and the hypoblast, and it is afterwards enclosed by the hypoblast, which extends round the inner sur-face of the epiblastic wall of the blastodermic vesicle; in other words, a cavity which lies outside the hypoblast, between it and the epiblast, and which is therefore in the position of the segmentation cavity of the rat, mouse, hedgehog, and the lower Vertebrates, becomes converted into the cavity of the yolk-sac. This is a distinguishing feature, and, so far as I am aware, no connecting link has been found which can be looked upon as uniting the ova in which the epiblast grows round the hypoblast to the ova in which the hypoblast is said to grow

round the inner surface of the epiblast. The figures which are given as representations of the phenomenon do not afford conclusive proof of its occurrence; in point of fact, they are as adaptable to an opposed hypothesis as to that which they are commonly supposed to support.

Since the publication, in 1880, of van Beneden's monograph on the early development of the rabbit's ovum (2), the epiblastic nature of the greater part of the wall of the primitive blastocyst has been almost universally accepted; most of the accounts of mammalian development which have been published since its appearance have been correspondingly modified.

According to van Beneden's description the first division separates the ovum into two segments of unequal size and of different appearance. The descendants of one of these segments at the seventy-second hour form a mass of cells which are completely surrounded by the descendants of the other segment. Van Beneden termed the outer layer the epiblast and the inner mass the primitive hypoblast, and his figures (figs. 1 and 4, Pl. IV) represent these two constituent portions very clearly. At the period of the publication of his memoir van Beneden believed that the inner mass eventually gave rise to the definite hypoblast and the mesoblast, and that the outer layer formed the epiblast. It has, however, been conclusively proved, by the observations of Kölliker, that from the inner mass all three layers of the germ arise, and it is doubtful whether the outer layer takes part in the formation of the germinal epiblast (17) or whether it disappears entirely in the germinal area (27, p. 33). However this may be, the evident difficulty which exists in the determination of the question is an indication that differences of appearance cannot alone be taken as sufficient evidence of the special nature of the cells under observation; yet it is mainly upon such differences, seen in optical sections only, that van Beneden bases his description of an outer epiblastic layer and an inner hypoblastic mass in the very early stages. Further, these differences do not seem to have been noticed by Rauber,

who, in describing the appearance of the blastodermic cavity, attributes it not, as van Beneden does, to the separation of the inner mass from an outer layer, but to the appearance of a cavity amidst a number of similar elements. He says, " Die Furchungshöhle bildet sich durch einen langsam wach-senden Serum-Erguss im Innern des vollständig in gleich-grosse Furchungskugeln zerlegten Dotters, an excentrisch gelegnen Stelle der ganzen Erkugel " (**38**, p. 104).

After the appearance of the blastodermic cavity the blasto-cyst is rapidly expanded ; between the seventy-fifth and ninety-fourth hours it becomes doubled in size, and at the latter period the wall of the vesicle, which measures 0·28 mm. in diameter, consists in the greater part of its extent of a single layer of flattened cells ; the remainder of the wall is formed by a mass of cells, and of these, those situated most externally are apparently, according to van Beneden, similar in appear-ance to the flat cells which form the greater part of the wall of the vesicle, with which they are said to be directly con-tinuous ; and thus has arisen the description of a vesicle with a wall formed by a layer of flattened cells, and containing an inner mass which is adherent to a portion of the vesicle wall. It is admitted, however, that the inner mass is intimately attached to the outer layer, and upon this point Rauber speaks very clearly. " Nur an einer Stelle ist die so gebildete Keim-blase nicht einschichtig und sind die Furchungskugeln nicht platz geworden, sondern eine dunkle Gruppe von Furchungs-kugeln ragt daselbt mit convexer Oberfläche in das Innere der Keimblase vor, eine biconvex Scheibe dastellend."

" Diese Scheibe liegt nicht etwas lose und verschiebar der wand der Keimblase an, sie ändert nicht ihren Platz an der wand der letzteren, wenn das El bewegt wird, sondern sie bildet einen integrirenden Theil der Keimblasenwand selbst und geht mit ihrer Urmundrandung in den einschichtigen Theil der Keimblase über " (**38**, p. 104).

It is admitted that the inner layer of the inner mass is the germ of the primitive hypoblast, and that the cells of this layer assume a somewhat more flattened form at the peri-

phery of the mass where they are in contact, if not in direct continuity, with the flat cells which form the greater part of the wall of the vesicle.

It is stated that the flat cells on the outer surface of the inner mass either disappear entirely, or they fuse with the cells immediately beneath them to form the epiblast of the germ; and in either case, after the flat cells over the outer surface of the inner mass have disappeared, it is just as possible that the remainder of the vesicle wall hangs in continuity with the peripheral flattened cells derived from the inner layer of the inner mass—that is, with the hypoblast, as with those cells which constitute the epiblast; but in the case of the rabbit there is no evidence which will completely substantiate a statement that either the one or the other of these possibilities occurs. It is certain that the portion of the vesicle wall, which is at first formed by a single layer of flattened cells, eventually becomes didermic, and that the change from the single to the double-layered condition commences in the vicinity of the inner mass, whence it gradually extends to the opposite pole of the ovum. It is stated that the didermic condition is produced by the extension of the hypoblast round the inner surface of the primitive wall, but no satisfactory proof has been brought forward in support of this statement. The cells of the extending layer are from the first flattened, like those over which they are extending, and nuclear division has not been clearly demonstrated either in the inner or the outer layer.

It appears to me, therefore, that the evidence which has been obtained from the study of the rabbit's ovum does not conclusively substantiate the statement that the outer wall of the primitive blastocyst is epiblastic in nature, and that the hypoblast extends round its inner surface.

If the evidence in support of the latter view is incomplete in the case of the rabbit, it is still more so in the case of the bat; for although (fig. 6, Pl. XXIII) van Beneden and Julin (4) figure the greater part of the blastocyst wall as composed of a single layer of flattened cells, there is no definite proof forth-

coming that those cells are of epiblastic nature.   It is true
that similar cells overlap the inner mass, but the fate of the
overlapping cells is unknown, and it is quite possible that, as
in the rabbit, they disappear and leave the remainder of the
vesicle wall in continuity with the peripheral hypoblastic cells
of the inner mass.   Concerning the attainment of the dider-
mic condition in the bat, van Beneden and Julin make a most
significant statement.   They say that " l'hypoblast, que s'étale
progressivement en partant de la tache embryonnaire à la face
interne de l'épiblast, ne gagne jamais le pole inférieur de la
vésicale blastodermique ; une zone monodermique persiste
jusqu'à une phase très avancée du développement" (4, p.
569).   Now, if there is any close relationship between the
mammalian ovum and the ova of other Vertebrates, it is much
more probable that the epiblast extends over the surface of
the hypoblast, leaving for a time a portion of the latter layer
uncovered, than that the cavity of the yolk-sac is for a long
time bounded partially by epiblast ; and if this should prove to
be the case, then the ovum of the bat is intermediate in
position, as regards the extension of the outer layer over the
inner, between the ova of the rat and the mouse on the one
hand, and the ovum of the hedgehog on the other.

In the mole, Heape describes appearances in the early
stages very similar to those already noted as occurring in the
rabbit.   Towards the end of the segmentation period the
ovum consists of a solid mass of cells which, in optical section,
appear to be separable into an outer layer and an inner mass ;
at a later stage a cavity appears, as in the rabbit, and the
inner mass still remains adherent to a portion of the outer
layer.   Heape assumes that the outer layer is epiblastic, and
certainly his figures (17, pl. xxix, figs. 17, 18, and 19) give the
idea that the inner mass and the outer layer are quite dis-
tinct ; but, as Heape has shown, the outer layer disappears, as
a layer, from over the outer surface of the inner mass by
fusing with the latter to form the embryonic epiblast.   In the
stages which precede this fusion (pl. xxix, figs. 21, 22, and
23) the outer layer is intimately connected with the margin

of the epiblastic portion of the inner mass, and the cells of the outer layer over the surface of the inner mass assume a cubical or low columnar form, whilst the remainder of the wall of the blastocyst is formed by flattened cells. It is at this period that the hypoblast is differentiated from the inner part of the inner mass, and in these early stages (figs. 21 and 22) the connection between it and the flat-celled portion of the blastocyst wall appears to be just as intimate as the connection of the latter with the cells of the outer layer which lie over the inner mass and take part in the formation of the epiblast, and it is not possible to determine from the figures mentioned where the hypoblast ends.

In the shrew, also, Hubrecht has shown (23) that the germinal epiblast and the hypoblast are formed from the inner mass of the blastocyst, which is very similar to the blastocyst of the rabbit and of the mole, but the fate of the outer layer over the inner mass is uncertain. Hubrecht says that the hypoblast-cells spread over the inner surface of the epiblastic wall of the blastocyst, and that they are distinguishable by their appearance, but his illustrations (pl. xxxv) are far from being convincing ; they are, indeed, open to the same objections which have already been urged against the figures representing the development of the rabbit.

Selenka's representations of the appearances observable in the early stages of development of the guinea-pig's ovum lend themselves very readily to the support of the idea that the greater portion of the blastocyst wall is formed by the hypoblast, for in Taf. xi, figs. 3, 5, 6, 7, and 9, in the fourth part of 'Studien über Entwickelungsgeschichte der Thiere' (45), the connection between the hypoblastic layer which is invaginated into the cavity of the blastocyst by the germinal epiblast, and by the formation and the extension of the "Interamnionhöhle," and the layer of flattened cells which forms the greater portion of the blastocyst wall is, at all events, quite as intimate as the connection of the latter layer with the epiblastic "Träger."

In the opossum Selenka has demonstrated the appearance

of a segmentation cavity which remains more or less open to the exterior, and which may contain one or more large cells (**46**, Taf. xvii, fig. 8).   Apparently this cavity remains and becomes the cavity of the blastocyst; the cell or cells in the interior and those at the margin of the opening in the wall of the cavity proliferate, and thus the vesicle becomes very similar in appearance to the blastocyst of the bat (**46**, Taf. xxiii, fig. 6; Taf. xvi, fig. 11), except that the greater portion of the wall is formed of columnar instead of flattened cells; but the vesicle in both cases consists of an outer wall to which the inner mass is adherent, and the outer wall is incomplete over the centre of the inner mass, where a depression, called the blastopore, is noticeable.   In this case also there is no absolute proof that the columnar cells which form the greater part of the blastocyst wall are any more closely related to the cells which cover the inner mass than they are to the cells which form the margin of the latter part of the ovum—that is, there is no more proof that they are epiblastic than that they are hypoblastic, and at a more advanced stage after the cells of the monodermic portion of the blastocyst wall have become flattened (**46**, Taf. xviii, fig. 3), and the extension of a second layer over the primitively monodermic wall has commenced, it is absolutely impossible to assert with any degree of certainty which of the two layers of the didermic area is the one undergoing extension, for in the marginal portion of the didermic region the cells of both layers are flattened, and on the right-hand side of the figure the appearance seems to be, if anything, more in favour of the extension of the outer layer over the inner than the inner over the outer.

As in all the Vertebrata except the Mammalia, the archenteric space appears within the hypoblast; and as in the rat, the mouse, and the hedgehog amongst the mammals, the blastocyst cavity, which in all probability is merely a modification and extension of the archenteric cavity, appears in a situation similar to that occupied by the archenteron of the lower Vertebrates, it seems improbable that in other mammals a cavity which bears similar relations to the embryo should be bounded,

in the greater part of its extent and for a considerable period, by epiblast alone. I have endeavoured to show that the observations which have been brought forward in support of such an improbable occurrence are not conclusive, and that the facts are capable of a very different interpretation, which may be summarised as follows :

The phenomena observed in the early stages of development of the ova of the rabbit, the guinea-pig, the mole, the shrew, the bat, and the opossum do not differ essentially from the phenomena observable in the ova of all other Vertebrata.

The blastodermic cavity in the ova of the mammals in question is in reality a modified archenteric space which has been extended coincidently with the disappearance of the solid yolk, for the purpose of maintaining an extensive fœtal area in close relation with the maternal tissues.

The whole of the immediate boundary wall of the space in question is from the first constituted by the hypoblast, in the midst of which it has appeared.

The only epiblast present in the early stages in the ova of the above animals is a small mass which is situated upon one pole of hypoblastic mass, which constitutes the greater part of each ovum.

This view appears to me not only more probable than that usually held, but also more capable of direct support; and although most of the facts and opinions upon which it is based have been already referred to, it is advisable, even at the expense of a certain amount of repetition, to conclude this section with a short summary of the most important.

1. There seems to be every probability that the Mammalia and Sauropsida have been produced from a common ancestral stock, whose descendants have diverged along two different lines.

2. It is generally admitted that the ova of this common ancestral stock, the "Protamniota," were provided with a large amount of food-yolk, and there can be but little doubt that all true yolk is to be considered as modified hypoblast.

3. In the descent from the "Protamniota" to the Saurop-

sida an increasingly early and complete separation of the ovum from all sources whence maternal nutriment might be derived has possibly occurred, and consequently there has been increased yolk formation and general increase in the size of the ovum; but in the descent from the "Protamniota" to the Mammalia the ova have been placed in conditions progressively more favorable to the direct nutrition of the developing germ by the maternal tissues, and the ovum has therefore diminished in size.

4. One of the peculiar features of all ova with a large or comparatively large amount of yolk, e. g. the ova of the Elasmobranchs, Teleosteans, Lacertilians, and Aves, is the great preponderance of the hypoblast over the epiblast. The latter portion of the ovum in the very early stages is merely a small disc, which rests upon one pole of a large hypoblastic mass. It is only as development proceeds that the margins of the epiblastic disc extend until the mass of hypoblast is completely enclosed, as in the Elasmobranchii, the Teleostei, and the Lacertilia, or almost completely enclosed as in Aves; and we have no reason for supposing that in the large-yolked protamniotic ova any great variation from this arrangement prevailed.

5. In the developing ova of all the Vertebrata below the Protamniota two cavities appear at different periods: the first, the segmentation cavity, lies between the epiblast and hypoblast; it is transitory in character, and disappears coincidently with the formation and extension of the second cavity—the archenteron—which appears within the hypoblast.

The segmentation cavity is in some cases very small, and it may be that this peculiarity was at first associated with rapid yolk formation occurring during the segmentation period, part of the yolk being deposited in the situation that would otherwise have been occupied by the segmentation cavity.

As the phenomena here referred to are met with in all the Vertebrata below the Protamniota, there is every probability that the tendency to their production was transmitted to the

latter hypothetical class; and this probability is strengthened by the fact that the tendency has been transmitted through the Protamniota to the Sauropsida, for in the latter division of the Vertebrata the segmentation cavity is distinguishable both in Lacertilia and Aves. In the Lacertilia it is represented by the space which in Hoffmann's account of their development is described in the following words:—"Alle Zellen sind mit Dotterkörmchen sehr stark gefüllt. Die obersten bilden schon ein eingeschlossenes Blatt, welches nur eine Schicht dich ist, und durch einen kleinen, aber deutlichen Zwischenraum vom dem darunter liegenden getrennt ist" (**22**, p. 1878). And again, in the account of a later stage, "An die peripherie liegen sie [the hypoblast-cells] in mehren schichten dicht aneinander gefügt und bilden dort den sogennanten Keimwall hier liegt der Epiblast unmittelbar dem Hypoblast auf, während mehr centralwärts beide Keimblätter durch einen deutlichen Zwischenraum vom einander getrennt sind" (**22**, p. 1881). In birds it has been recognised by Duval (**9**) as a small transitory cavity; and in both Lacertilia and Aves the archenteron appears excentrically situated within the hypoblast, its ventral wall, which is enormously hypertrophied, forming the yolk-mass.

6. As the tendency to the production of the above phenomena, so characteristic of the development of the lower Vertebrata, has been transmitted through the Protamniota to one of the great branches, the Sauropsida, which has extended from it, a priori it might be expected that a similar tendency would be transmitted to the only other branch of the parent stem—that is, to the Mammalia; and we actually find in the rat, the mouse, and the hedgehog, that both the segmentation cavity and the archenteron appear as separate and distinct cavities, the former of which disappears coincidently with the formation and extension of the latter, which is developed amidst the hypoblast as in the lower Vertebrata. In the rabbit and the bat only one cavity appears; this cavity ultimately becomes separated into two parts, the yolk-sac and the enteric canal, and presumably, therefore, it corresponds

to a modified archenteron.   The non-appearance of the seg-
mentation cavity is probably due to the rapid formation of a
comparatively large mass of hypoblast which has invaded the
region in which the cavity would have appeared under other
circumstances.   Looked at from this point of view, the ova of
the animals in question present the peculiar features of all
comparatively large-yolked ova; that is, they consist of a small
mass or layer of epiblast superposed upon one pole of a large
hypoblastic mass, and consequently in these ova, as in all ova
with a comparatively large yolk-mass, the formation of the
archenteron commences before the epiblast has extended over
the whole surface of the hypoblast.

The very early stages of the development of the shrew's ova
have not been observed; but the blastocyst of that animal is
so similar to the blastocyst of the mole that there can be but
little doubt that the changes which occur during the very early
stages of development in the ova of both these animals are very
similar.

The phenomena which have been noticed in the opossum
seem at first sight to point to the conclusion that the segmenta-
tion cavity becomes the archenteron ; but it is to be noted
that between the very early stages in which the segmentation
cavity is present and the stages of a distinct blastocyst there
is a hiatus in the specimens observed, and it is not at all im-
probable that the stages intervening are those in which the seg-
mentation cavity is obliterated and the archenteron is formed.

If the explanation I have suggested is correct, it is interesting
to note that in the hedgehog we have comparatively a small
amount of hypoblast, which is rapidly surrounded by epiblast,
and the presence of both segmentation cavity and archenteron ;
that in the rabbit, mole, shrew (?), and the bat there is a relatively
large mass of hypoblast, which is only gradually surrounded
by the epiblast, and there is no segmentation cavity ; that in
the opossum there is a relatively large amount of hypoblast,
but a segmentation cavity appears; and that in the rat and
mouse, on account of special circumstances, the epiblast does
not completely surround the hypoblast.   In other words, we

have indications that a tendency to the production of certain
phenomena, which appear in other classes of the Vertebrata,
has been transmitted through the Protamniota to the Mam-
malia, where, although it is exhibited upon comparatively
small ova, it produces, under relatively similar circumstances,
very similar effects to those met with in the larger ova of
various other Vertebrates.

The hypothesis of the nature of the various component
parts of the young mammalian blastocyst, which has been
stated in the preceding pages, agrees in so far as the ento-
dermic nature of a large portion of the blastocyst wall is con-
cerned with that which was proposed by Minot in 1885, and
which was again restated by him in the 'American Naturalist'
in 1889 ; but I cannot see any facts which support Minot's
suppositions, that the whole of the subzonal epithelium is ento-
dermic, and that therefore there is a complete inversion of
the germinal layers in the young developing mammalian ovum ;
that the whole of the inner mass in the mammalian blastocyst
is ectoderm, and that the blastocyst cavity is comparable with
the segmentation cavity of the lower Vertebrates.   On the
contrary, the evidence we possess appears to me rather to
point to the following conclusions :

(1) That the greater part of the subzonal epithelium is
entodermic, but a small portion is ectodermic.

(2) That the main part of the inner mass of the mammalian
blastocyst is ectodermic, but a small portion of it is entodermic.

(3) That the cavity of the mammalian blastocyst does not
correspond with the segmentation cavity of the lower Verte-
brates, but with the archenteron.

The Formation of the Cœlom.

In most accounts of mammalian development the formation
of the cœlomic cavity is very shortly and incompletely dealt
with, and little can be gathered from published descriptions
beyond the fact that the space appears within the mesoblast.
We do not know, however, except in the cases of a few animals,
whether it appears in the embryonic or extra-embryonic area,

or whether all portions of it, when they are first formed, are continuous or separate.

There can be little doubt that the intra-embryonic cœlom of the higher Vertebrata corresponds closely with the cœlomic cavity of the lower Vertebrates.   The differences which exist are secondary; they are due partly to altered circumstances of existence in the later periods of development, and partly to the formation of a yolk hernia which has interfered with and modified the development of the cavity in the earlier stages; but we are not in a position to say how these modifications are produced, for as yet we scarcely understand what they are, and how they appear in the ontogeny of any one animal.

Van Beneden's and Julin's researches upon the formation of the amnion in the rabbit and bat (3) have added greatly to our knowledge of the extension of the extra-embryonic portion of the body-cavity, but the accounts of this process in other mammals differ considerably from that of van Beneden and Julin.

In the sheep the cœlom arises in the extra-embryonic area, as a series of small irregular spaces in the mesoblast.   These spaces soon fuse, and a continuous cavity results.   The formation of the cœlom commences at the anterior and posterior ends of the embryonic area, and afterwards extends forwards and backwards (6).

In the cat also, according to Fleischmann (12), the formation of the cœlom commences in the extra-embryonic area, whence it afterwards extends into the embryonic region, but instead of appearing as a series of irregular independent spaces, it is from the first a tubular canal; possibly, however, Fleischmann has missed the earlier stages, for, according to Bonnet's account, the tubular cœlom succeeds the irregular spaces and results from their fusion.

In the guinea-pig (45) the cœlom appears in the extra-embryonic area before the formation of the mesoblast commences, and it is bounded at first only by epiblast, trophoblast, and hypoblast; its mesoblastic walls are formed afterwards by migratory cells, which advance over its primitive walls from

the posterior end of the embryonic area (45). According to Strahl and Carius, a portion of the intra-embryonic cœlom of the guinea-pig and rabbit is developed separately, and fuses secondarily with the general body-cavity (50 A).

According to the accounts given by Professor Fraser and Professor Selenka, the cœlom in the rat and the mouse is formed much in the same way as in the guinea-pig. These observers, however, differ from each other upon some details. Professor Fraser, in a diagrammatic representation of a longitudinal section of a rat's ovum at nine days seventeen hours, figures the distal trophoblast as only partially covered by the mesoblast, which is extending as a continuous layer. Professor Selenka considers that this is incorrect, and my observations confirm his conclusions, but they do not support his assertion that a portion of the wall of the extra-embryonic cœlom of the mouse is formed by migratory mesoblastic cells.

Duval (9) agrees neither with Fraser nor Selenka, and according to his account the formation of the cœlom in the mouse is very similar to its formation in the sheep. It commences in the extra-embryonic region behind the embryo as a series of spaces which gradually fuse into a continuous cavity.

My specimens do not confirm Duval's account, but the appearances they present are more in accord with his descriptions than with that given by Selenka, for they show that the cavity appears, as in most Vertebrates, within the mesoblast, and not, as in the guinea-pig, between the trophoblast and epiblast.

In the mouse ovum during the latter part of the eighth day, and at a corresponding period in the rat's ovum, a knob-like mass of mesoblast is projected backwards from the posterior end of the primitive streak (fig. 14, Pl. XXIV). The growth of this mass pushes the posterior end of the epiblast towards the anterior end, and thus produces the first rudimentary amnion fold (A F C). Selenka calls this mass the "Allantoisknopse" (44, 45), and states that the migratory cells which enclose the cœlom are budded off from its surface. It is not the allantois, for that organ does not appear until the latter part of the

ninth day.   It is the first rudiment of a portion of the embry-
onic, and of the extra-embryonic mesoblast.   It has already
been explained that from the lateral margins of this mass two
wing-like plates extend round the margin of the epiblast
cylinder, until they meet on the cephalic side in front of the
pro-amnion ; within these plates at the ninth day two small
flattened spaces appear.   An ovum at this period, which has
been cut into forty-eight longitudinal sections, shows a cœ-
lomic space appearing (fig. 15 A, Pl. XXIV) in the seventeenth
section, which continues to the twenty-first section.   It is
absent in the next six sections which pass through the middle
of the embryonic area, but reappears in the twenty-eighth sec-
tion, and again disappears at the thirty-second section.   The
two cavities soon meet on the caudal side, and, following the
direction of the growth of the mesoblast (fig. 15 B), meet and
fuse on the cephalic side in front of the embryonic area.
Thus a circular space which entirely surrounds the margins of
the embryonic area appears within the mesoblast.   When first
completed it is largest at the posterior end of the embryonic
area (fig. 15 B).   All parts of the inner wall of the ring-like
space are gradually carried towards each other, but with
unequal speed ; the caudal side and the portions nearest to it
advance most rapidly, consequently all parts of the inner wall
of the ring come together nearer to the cephalic than to the
caudal side of the embryo, and after their disappearance the
. ring-like space is converted into a large cavity, which inter-
venes between the amnion and the trophoblast.   As this
space increases towards the proximal pole of the ovum, the
distal portion of the trophoblast recedes before it, becomes
invaginated upon itself, and finally fuses with the proximal
portion as the cavity of the trophoblast is obliterated (fig. 19,
Pl. XXVII). As the distal portion of the trophoblast is forced out
of the cavity formed by the invagination of the yolk-sac, the
hypoblast with which it lay in contact is overspread by the
mesoblastic wall of the cœlom, which in this situation is
easily separable into two layers, an inner of flattened cells,
and an outer lying next the hypoblast, which is formed by a

series of irregular masses of nucleated protoplasm. The former of these is called by Selenka the somatopleure, the latter the splanchnopleure. The former is certainly continuous with the somatopleuric layer on the trophoblast, and also with that on the amnion; nevertheless it corresponds to the splanchnic portion of the extra-embryonic mesoblast of other Vertebrates, and it is naturally continuous in the region of the sinus terminalis with the somatic mesoblast which covers the trophoblast, and forms the inner wall of the chorion. The irregular groups of nucleated protoplasm, to which collectively Selenka applies the term Splanchnopleure, are in reality only a portion of that layer; they are the rudiments of the blood-vessels and their contents.

The extension of the cœlom towards the proximal end of the cylindrical ovum is accompanied by a simultaneous but smaller extension towards the distal end. This portion of the extension is best explained by the examination of transverse sections of an ovum in which it is occurring.

Fig. 16 *H*, Pl. XXVI, represents a transverse section through the posterior part of the primitive streak on the caudal side of the cylinder, and through the extra-embryonic area in front of the pro-amnion on the cephalic side.

In the latter situation several islets of cells containing cavities are seen; they are merely sections of the irregular margins of the extra-embryonic mesoblast and cœlom.

Laterally the cœlomic space lies between a thin somatic and a comparatively thick layer of splanchnic mesoblast. It extends from the cephalic to the caudal side, where it penetrates a short distance into the embryonic mesoblast. Twenty-five micromillimetres nearer the centre of the embryonic area the relations are the same, except that on the cephalic side of the section the pro-amnion intervenes between the two halves of the cœlom (fig. 16 *G*, Pl. XXVI). Six sections further back on the cephalic side, and further forward on the caudal side, that is thirty micromillimetres nearer the centre of the embryonic area, four sections of the cœlom appear (fig. 16 *E*, Pl. XXV). Two are on the cephalic side in the peripheral margins of the

pre-cephalic mesoblast, the other two are in the peripheral
margins of the caudal portion of the paraxial mesoblast.   As
the sections approach nearer to the centre of the embryonic
region the anterior portion of the hypoblastic ridge, from
which the chorda and the bucco-pharyngeal membrane are
formed, appears on the cephalic side of the sections.   In
fig. 16 *D*, which represents a section thirty-five micromilli-
metres from 16 *E*, the cœlom is no longer present on the left
side, and the cephalic and caudal portions of the paraxial
mesoblast are fusing.   On the right side of the section the
cephalic and caudal portions of the paraxial mesoblast are
still separate, and each contains a small cavity in its peri-
pheral margin.   In fig. 16 *C*, Pl. XXV, the two portions of
the paraxial mesoblast have just fused on the right side,
and the cœlomic space is represented by a single small
cavity.   This section is fifty-five micromillimetres from 16 *D*,
and between the two sections the cœlomic spaces in the
cephalic and caudal parts of the paraxial mesoblast unite
together.

During the latter part of the ninth day, as the cœlom is
gradually extending in the extra-embryonic area, the allantois
is formed.   It is a solid mass of mesoblast which grows out
from the posterior end of the primitive streak and extends
through the cœlom towards the trophoblast, which it reaches
and fuses with during the latter part of the eleventh or at the
commencement of the twelfth day.   It, therefore, hangs free
within the body-cavity for a period of two days.   It is at first
a solid mass of mesoblast which, according to Selenka, is
invaded, apparently about the eleventh day, by a diverticulum
from the yolk-sac.   I have been entirely unable to find any
such diverticulum, and I am quite convinced that it does not
exist.   The diverticulum which Selenka figures is nothing
more than a fold of the wall of the yolk-sac which has been
cut in an oblique section.   The hypoblast which is found
within the placenta at a later period enters that organ from
the margin of the yolk-sac, not by the allantois; but as this
peculiar feature in the development of the rat and the mouse

has already been noted in another communication (42 A) it is not necessary for me to refer to it here.

A mere glance at figs. 15 *A*, 15 *B*, and 19 might give the impression that the cœlom of the rat and the mouse commences in the extra-embryonic area, but it must be noted after the fusion of the two halves of the space on the caudal side the allantois is projected from the posterior extremity of the primitive streak between the somatic and splanchnic layers (fig. 19, Pl. XXVII). The root of the allantois is attached to the splanchnic layer and somatic layers in an area which afterwards becomes part of the ventral wall of the enteric canal (fig. 21, Pl. XXVII), and it becomes evident on reference to fig. 19 that when the point *X* at the anterior end of the embryonic area and the point *X'* at the posterior end are folded round till they meet in the middle line on the ventral surface at the closure of the umbilical orifice, a portion of the root of the allantois will be included within the body. The portion of the cœlom which surrounds the root of the allantois will be converted into a part of the peritoneal cavity ; therefore, as this is the section of the cœlom which was first formed, the cœlom in the rat and the mouse commences in the embryonic area. In this respect, therefore, it differs both from the cœlom of the sheep and the guinea-pig. It differs also from the cœlom of the sheep in commencing as two lateral spaces instead of by an irregular series of unconnected cavities, and in its extension, which is very similar to the extension of the cœlom in the rabbit, as described by van Beneden (3).

In the sheep the cephalic and caudal portions of the cœlom apparently arise separately and afterwards fuse together (6), but in the rabbit the cœlom commences posteriorly, and then extends round the outer margins of the embryonic area as two lateral wings, which meet and fuse in front of the pro-amniotic space (3).

In the rat and the mouse the cœlom commences in the posterior part of the embryonic area. After the fusion of its two primitive sections it becomes semilunar in outline, the convexity of the semilune passes backwards into the posterior

part of the embryonic area, and the horns of the crescent advance, passing forward a short distance from the outer margins of the embryonic area until they have passed its anterior extremity; then they converge and fuse in front of the pro-amnion, as in the rabbit. The posterior portions of the inner margins of the ring-like space which is thus formed pass inwards into the embryonic region, dividing the embryonic mesoblast into somatic and splanchnic layers (fig. 16 *G*, Pl. XXVI); but the anterior portions of the inner margins of the ring never penetrate the mesoblast which surrounds the bucco-pharyngeal membrane and lies at the sides of the anterior portion of the notochord, and which constitutes the " Herzanlage " of Hensen (18) or the "pericephalic mesoblast" of Fleischmann (12). This mesoblast has the same horseshoe-shaped outline in the mouse at the ninth day before protovertebral somites have appeared that it has in the rabbit with two protovertebral somites (18). In the rabbit the horseshoe-shaped mass of mesoblast is penetrated by the cœlom before the cornua of that space have met in the mesoblast in front of the pro-amnion (3, pl. xxiv, figs. 2 and 3). In the rat and the mouse the pericephalic cœlom is formed by a forward extension of the embryonic cœlom into the pericephalic mesoblast; this extension takes place simultaneously on both sides, and the two halves meet in the middle line in front of the bucco-pharyngeal membrane in the latter part of the ninth day (fig. 19 *A, PC*) some hours after the fusion of the horns of the extra-embryonic cœlom in front of the pro-amnion. In relation, however, to the general development of the embryo, the completion of the pericephalic cœlom in the rat and the mouse occurs about the same time as in the rabbit; that is, at the period when three protovertebral somites have appeared.

The pericephalic cœlom of the sheep, the rabbit, the rat, and the mouse becomes developed into the pericardial cavity; whilst in the cat, according to Fleischmann (12), it disappears. Fleischmann's plates, however, do not substantiate his statements; on the contrary, they show that instead of disappearing the pericephalic cœlom of the cat becomes expanded, and takes

part in the formation of the pericardial cavity just as in other
animals.

The Amnion.

If we accept Selenka's dictum that amnion formation is
essentially the production of a hood for the embryo (46,
p. 131), we are bound to admit that the amnion may con-
sist of only one layer, the epiblast alone, as in the guinea-
pig, or of two layers. Usually two layers are found in an
amnion fold, and they may be either epiblast and mesoblast,
as in the tail amnion fold of the rabbit, or epiblast and hypo-
blast, as in the cephalic amnion fold of the opossum. In
making this admission, however, we accept the conclusion
that amnion formation may take place in entirely different
ways in different animals, for in all the Amniota except the
guinea-pig (45) the amnion folds are formed by two layers:
the caudal fold always by somatic mesoblast and epiblast, the
cephalic fold in most, if not all cases, first by epiblast and
hypoblast, which constitute the so-called pro-amnion, and after-
wards by somatic mesoblast and epiblast. In the majority of
the Amniota a disc of epiblast rests upon the upper pole of
the ovum. As the amnion folds rise the margin of the epi-
blastic disc is gradually contracted, and it disappears finally
when its remaining portions fuse in the situation of the amnion
navel. In the guinea-pig, as in the rat and the mouse, the
epiblast is not formed as a flat disc with outspreading margins,
but as a solid mass of cells within which a cavity appears
(fig. 11, Pl. XXIII). In each of the above-mentioned animals the
cavity is at first enclosed on all sides by the epiblast; but in
the rat and the mouse, by the separation of the margins of
the epiblast, the cavity is soon opened out, becoming continuous
with a cavity in the trophoblast (fig. 13, Pl. XXIII), and the two
together correspond to the extra-embryonic space, which in
the case of the rabbit is enclosed by the fusion of the placental
ridge of epiblast with the uterine mucous membrane. After-
wards, when the mesoblast appears, the margins of the
epiblast are again carried over the dorsal aspect of the embryo
at the apices of the amnion folds until they meet and fuse at
the amnion navel. In the guinea-pig the margins of the

epiblast do not separate, and therefore the layer, instead of becoming spread out as a disc, retains its sac-like form.   The dorsal wall of the sac constitutes the epiblastic portion of the amnion, and it becomes thinned out as the cavity expands. There is no amnion navel, for there is no fusion of amnion folds ; the amnion is at first formed by a single layer of epiblast, which is afterwards covered by a layer of somatic mesoblast, and thus in its completed state it resembles the amnion of other Vertebrates.   The mode of its formation is, however, evidently secondary; it has probably been derived from the type which occurs in the rat and the mouse by the retardation of the separation of the margins of the epiblastic disc.   Amnion formation in the guinea-pig is a mere variation from the ordinary type, and we may therefore consider that all amnia are produced by the folding of two germinal layers at the same time, either the epiblast and the hypoblast together, or the epiblast and the mesoblast.   From folds of the latter kind the whole of the permanent amnion is produced in the majority of the Amniota; they may therefore be called true amnion folds, or, shortly, amnion folds.   Folds of the former class are generally transitory ; they give way and disappear before the extension of the amnion folds, therefore they have been well designated by van Beneden pro-amniotic folds.

In the rat and the mouse the tail amnion fold is the first to appear (fig. 14, Pl. XXIV).   Its production is due to the growth of the mesoblast at the posterior end of the embryonic area, and it is at first quite independent of the formation of the cœlom.   In the same manner the lateral amnion folds are formed as the mesoblast extends round the margins of the epiblastic cylinder ; and last of all the rudiment of the cephalic fold appears (fig. 15, Pl. XXIV).   But whilst the posterior and lateral folds are produced by ridges of mesoblast projecting against the margins of the epiblastic cylinder, the cephalic fold consists of two portions,—one caused by the formation of the mesoblast, a true amnion fold ; and a second, further back (*A M P.*), by a fold of the two primitive layers of the germ. It is, therefore, a pro-amnion fold, which corresponds closely

with the pro-amnion fold of the rabbit. When first distinguishable this portion of the cephalic fold is extremely short; and although it increases for a time it is, in comparison with the same area in other animals, always relatively small. In connection with this area in the ova of the mouse and the rat it is necessary to consider van Beneden's hypothesis of the inversion of the germinal layers. In discussing the formation of the pro-amnion fold in the rabbit, van Beneden has pointed out that it results from the sinking of the cephalic extremity of the embryo into the yolk-sac, which is thus gradually invaginated, and in the walls of the invagination cavity the layers are inverted, the epiblast being turned inwards towards the embryo, and the hypoblast outwards towards the chorion (3). In a similar manner a pro-amnion fold is formed in Lacertilians (20, Taf. i, figs. 3 and 4; 49, Taf. i, fig. 2), Chelonians (35), Aves (39, 48), Cheiroptera (3), Insectivora (24), and the opossum (46). The process is therefore very general, and van Beneden suggests the probability that the inversion which occurs in so many rodents is simply an accelerated pro-amnion formation. If this supposition is correct, it is evident that as the inversion of the membrane commences before the weight of the embryonic area can be supposed to have any effect in its production, it must be due to the precocious expression of an inherited propensity which tends to develop itself independently of circumstances. It is noteworthy in connection with this subject that the inverted portions of the wall of the ova which undergo typical inversion are much more extensive then the amniotic fold—that is, a portion of the false amnion is also inverted; and thus we find that the inherited tendency is not only precocious in the time of its appearance, but it also produces excessive results.

Further, the pro-amniotic area is bounded posteriorly by the pericephalic mesoblast, and in front by the inner margin of the ring of the extra-embryonic mesoblast which is formed by the fusion of the two horns of the crescentic extension of the middle layer. The pro-amniotic area is therefore intimately associated with mesoblastic formation. It is an area

immediately in front of the embryo in which mesoblastic formation is comparatively retarded. I have already shown that a bilaminar area with similar boundaries appears in the ova of rats and mice at a period which corresponds closely with that during which the pro-amniotic area of the rabbit is defined, and thus in the rat and the mouse the inherited tendency to pro-amnion formation becomes manifest long after its supposed precocious expression in the inversion of the layers. It is probable, therefore, that the early inversion of the germinal layers is not due to acceleration of development, but to other causes which have modified more usual processes of growth.

All the ova in which inversion occurs become embedded at an early period in a crypt of the uterine mucosa which is soon converted into a closed space, and in all the epiblast is covered from an early period by a layer of trophoblast. That inclusion within a uterine crypt is not, alone, sufficient to cause inversion is demonstrated by the development of the hedgehog's ovum, which is embedded in a uterine crypt at an early period, but still retains the uninverted condition (24). Two circumstances in the development of this animal are especially noteworthy: the first is that the growth of the ovum and the expansion of the uterine crypt in which it is contained are simultaneous and equal events; and the second that as the epiblastic disc expands, the trophoblast which covers it becomes thinner. In the rat and the mouse the progress of events is very different. At a very early period the margins of the trophoblast which cover the epiblast apparently become adherent to the walls of the yolk-sac on which they rest (fig. 8, Pl. XXIII); consequently during its subsequent growth the epiblast, which is thus bound down to the yolk-sac by the trophoblast, is prevented from expanding laterally. It increases as an oval mass, and produces the invagination of the dorsal wall of the sac (fig. 9, Pl. XXIII). The close apposition of the walls of the yolk-sac to the maternal tissues prevents the extension of the trophoblast round the hypoblast, and its margins become adherent to the uterine tissues. As it cannot extend over the yolk-sac, and as its pro-

liferation is rapid, probably as the result of an inherited tendency to expand rapidly over a comparatively large yolked ovum, its constituent parts become massed together in the uterine crypt into a solid rod of trophoblastic tissue (fig. 10, Pl. XXIII). During the increase of the rod its distal end pushes the epiblast before it, and thus continues still further invagination of the yolk-sac (figs. 11 and 12, Pl. XXIII). The inversion of the membranes in the rat and the mouse does not, therefore, seem to be due to the precocity of an inherited tendency, but rather to a series of modified circumstances which influence and change the normal processes of development. These circumstances are—

1. The inclusion of the ovum in a narrow uterine crypt.

2. The adhesion of the trophoblast to the wall of the yolk-sac.

3. The adhesion of the trophoblast to the wall of the crypt, and its subsequent extension in the cavity of the crypt.

4. The unequal rapidity in the increase of the ovum, and of the crypt cavity within which it is contained.

The invaginated walls of the ova of rats and mice cannot be considered as true pro-amniotic folds. They are not formed to enclose the embryonic area beneath a hood-like covering, but are the result of modified developmental conditions, and only portions of them are subsequently transformed into the true amnion folds.

The pro-amniotic area in the rat and the mouse corresponds with the pro-amniotic areas of all other Amniota except the cat, in which animal, according to Fleischmann's description (12), the pro-amniotic area is not present in the early stages, but appears later when the cephalic curvature commences, and lies immediately in front of the rudimentary head. Therefore, whether the pericephalic mesoblast disappears or not, the pro-amnion and the bucco-pharyngeal membrane must be continuous structures. This is an improbable circumstance, for the pericephalic mesoblast in all animals becomes the wall of the pericardiac cavity, and Fleischmann's figures show that the pro-amnion of the cat lies at the sides and in front of the peri-

cardial portion of the cœlom.   It seems probable that renewed observations on the development of the cat, aided by longitudinal sections of the ovum, will remove the distinction which at present is supposed to exist between the pro-amnion formation in the cat and other Vertebrates.

It has already been shown that the pro-amniotic area in the rat and the mouse is very small when it first becomes defined (figs. 15 and 15 *C*, Pl. XXIV).   It increases for a time, but is obliterated by the extension of the mesoblast between its layers before the cephalic curvature appears.   It is carried upwards in front of the embryonic area not by the sinking of the cephalic extremity of the embryo into the yolk-sac—an impossible occurrence in the rat and the mouse,—but by a folding of the yolk-sac wall which takes place in front of the embryonic area, synchronously with the formation of the mesoblast and the cœlom round the margin of the epiblast.

The amnion folds in the rat and the mouse are completed after the formation of the cœlom, and as the cœlom is most extensive posteriorly the tail amnion fold is most developed.   But all the folds are from the first continuous with each other, and the lateral and cephalic folds are mere extensions of the tail fold.   This is the case not only when they are caused by the solid mesoblastic ridge, but also when the ridge becomes hollowed by the formation of the cœlom.   The lateral folds appear before the mesoblast has become bilaminar, not after, and the laminæ of the lateral folds are not at first separate from the cavity of the tail fold, as Selenka asserts.   They therefore correspond closely with the amnion folds of the rabbit, which also commence at the caudal end and extend round the sides of the embryonic area, meeting eventually some distance in front of it where the cephalic fold is formed.   In fact, the whole of the amnion is formed by the extension of the caudal fold which embraces the embryonic area, passing gradually through the crescentic to the circular or oval form, its margin afterwards becoming contracted and finally obliterated at the amnion navel, the fold at the same time being converted into a hood.   In the rat, the mouse, the rabbit, the

lizard, and the bird, when the hood is first formed, it covers only the posterior portion of the embryonic area, and its anterior margin abuts against the anterior margin of the pro-amnion fold which covers the cephalic portion of the embryo. As the pro-amnion fold atrophies the hood-like caudal amnion extends until the disappearance of the pro-amnion, when it completely encloses the embryonic area.

In the rat and the mouse, therefore, amnion formation follows closely in the path marked out by mesoblast formation; it commences posteriorly, extends laterally, and is completed anteriorly. In the majority of other Amniota the extension of the amnion takes place in a similar manner; such differences as are met with are due to individual variations from a common plan.

So far as I am aware the general plan of amnion formation is modified to an important extent only in the human ovum and the ovum of the guinea-pig; for in the sheep, although the cœlom apparently commences as two distinct portions, the amnion folds do not rise until it has become one continuous cavity; and Bonnet says that upon its first appearance the amnion fold is not separable into distinct sections, but forms a continuous ridge round the embryonic area (6); whilst in the Chelonians (34) the peculiarities are due to interference with the completion of the process, not to any alteration of the plan.

The guinea-pig has been previously referred to, and now the peculiarities of the human ovum may be considered.

According to the typical schemata of Kölliker (26), there is a period during which the embryo with its yolk-sac and amnion lie free within the serous sheath, to which they afterwards become united by means of the allantois. His (21) has shown that this period is entirely supposititious so far as the human ovum is concerned; and he has pointed out that through the whole period of intra-uterine life the embryo never loses its connection with the chorion. He terms the stalk of connection the " Bauchstiel," and looks upon it as the direct backward prolongation of the embryo, the allantois taking

no part in its formation.   Hertwig (20) suggests that the
" Bauchstiel " is nothing more than the allantoic stalk which
has been conducted backwards to the chorion by the posterior
part of the amnion.   Hubrecht has entered upon an elaborate
explanation of its formation (24).   He suggests that in the
human ovum the epiblast disc is covered by a layer of tropho-
blast to which its margins are adherent, as in the hedgehog.
As the amnion folds rise the margins of the epiblast are
gradually separated from the trophoblast, but at one point the
connection remains permanent, and so the continuity of the
embryo and chorion is maintained.   The mesoblast growing
backwards from the primitive streak extends beneath this epi-
blastic connection.   This mesoblast, being axial, remains
unsplit until the mesoblastic connection between the embryo
and the chorion is established, and the splanchnopleuric layer
is afterwards separated.

Neither the trophoblastic covering of the epiblastic area,
nor the delayed splitting of the posterior portion of the meso-
blast, seems to be an essential factor in the production of the
conditions found in early human ova.

Commencing with the youngest ovum figured by His (21,
fig. 17 a, p. 171), in which the epiblastic disc is not covered by
the trophoblast, but is continuous with that portion of the
external layer by its margin, we may imagine that mesoblast
formation takes place early, but in the usual way — that is, it
commences posteriorly, and extends laterally round the mar-
gins of the epiblastic area to its anterior extremity.   After the
mesoblast is well formed, we may suppose that the splitting of
the layer commences bilaterally and posteriorly as in the rat
and the mouse.   As the splitting extends inwards through the
posterior part of the axial mesoblast, it separates the somato-
pleure from the splanchnopleure.   (This separation evidently
occurs at an early period in the human ovum, for from the
first the cœlom intervenes between the under surface of the
"Bauchstiel " and the posterior wall of the yolk-sac.)   Whilst
the splitting is proceeding in this region, and before the dis-
tension of the cœlom occurs, the allantoic mesoblast grows

rapidly backwards from the posterior end of the primitive streak, along the under surface of the somatopleure. In respect to its position, the allantoic mesoblast of the human ovum does not differ from the allantoic mesoblast of other animals, for in all cases this mesoblast is attached to the somatopleure, which becomes the ventral wall of the embryo from the umbilical orifice to the anterior wall of the rectum. The precocity of its appearance, before the formation of the caudal amnion fold, prevents the necessity of its growth as a free stalk through the extra-embryonic cœlom, in order that it may attain a connection with the trophoblast, for as soon as it passes the posterior limit of the epiblast it becomes at once attached to the trophoblast, therefore its extra-embryonic portion is comparatively extremely short in the early stages of the human ovum, and after the completion of the cœlom the embryo does not lie in the centre of the ovum, but close to that portion of the wall to which it is attached (21, fig. 9 *a*, p. 145).

The rapid growth of the allantoic mesoblast and its early attachment to the trophoblast interfere with the formation of the central portion of the tail amnion fold by preventing the dorsal extension of the body-cavity at the posterior end of the embryonic area. The forward extension of the cavity is not affected, and as soon as its lateral horns have fused in front of the embryonic area it rapidly increases both ventrally and dorsally. The dorsal extension is most rapid at the anterior end, consequently the cephalic amnion fold rises, and is carried backwards over the embryonic area. The lateral folds and the lateral portions of the caudal fold rise more slowly, and the closure of the amnion navel takes place at the posterior part of the embryonic area, in a situation which afterwards becomes the posterior boundary of the umbilicus. When this closure is completed the epiblast is separated from the trophoblast (His, 15, Taf. ix, figs. 1 and 3; Taf. xii, fig. 4), and the dorsal surface of the "Bauchstiel" forms part of the boundary of the amniotic sac—that portion which extends, when the curvature of the embryo is completed and the umbilical

orifice is closed, from the front of the urino-genital sinus to the umbilicus. The ventral extension of the posterior part of the cœlom is not interfered with by the early appearance of the allantoic mesoblast; therefore the separation of the splanchnopleure proceeds rapidly, not only anteriorly and laterally, but also posteriorly, and the yolk-sac is soon completely separated from the trophoblast.

If this explanation is correct there is no necessity for the trophoblastic covering of the epiblastic area which Hubrecht has supposed to be present in the human ovum, but of which there is no trace in the youngest known specimens (Reichert's, as figured by His), nor for retardation of mesoblastic cleavage in the posterior part of the embryonic region. The union of the embryo with the chorion is not maintained by means of the tail amnion, along which the allantois grows, as Hertwig suggests, but by union of the allantoic mesoblast with the chorion before the central part of the tail amnion fold is formed, and the "Bauchstiel" is not entirely independent of allantoic formation, as His asserts (21, p. 172): for although the "Bauchstiel" is not the allantoic stalk, it contains all the characteristic constituents of that stalk, the hypoblastic tube, the arteries, and veins; and it is the posterior portion of the embryonic area to which, as in all mammals, the intra-embryonic portion of the allantoic stalk is attached: further, it takes part, during the later periods of embryonic life, in the formation of the posterior portion of the ventral wall of the embryo.

The Mesoblast.

A complete consideration of the peculiarities of mesoblast formation in the rat and the mouse, and a comparison of them with the main facts which have been noted in other Vertebrates, must be deferred to a subsequent communication. For the present I merely desire to draw attention to the fact that the phenomena observable in the rat and the mouse during the development and extension of the middle germinal layer point to the conclusion that this layer is developed from the peristomal cells and from the hypoblast. The mesoblastic

formation commences in the region of the primitive streak by proliferation of the peristomal cells, and from this region the middle layer is extended by proliferation of the greater part of the embryonic and a portion of the extra-embryonic hypoblast; but the mesoblast extends into the embryonic area chiefly from behind and from the sides, the portion last formed being that which enters into the constitution of the protovertebral somites.

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EXPLANATION OF PLATES XXIII—XXVII,

Illustrating Mr. Arthur Robinson's paper on " Observations upon the Development of the Segmentation Cavity, the Archenteron, the Germinal Layers, and the Amnion in Mammals."

Alphabetical List of Reference Letters for all Figures.

AA. Allantoic artery.   *AFH.* Head amnion fold.   *AFT.* Tail amnion fold.   *AL.* Allantois.   *AM.* Amnion.   *AMC.* Amnion cavity.   *AMP.* Pro-amnion.   *AN.* Anal membrane.   *BMP.* Bucco-pharyngeal membrane.   *CAS.* Caudal side of embryonic cylinder.   *CD.* Chorda dorsalis.   *CES.* Cephalic side of embryonic cylinder.   *CHY.* Chorda hypoblast.   *COE.* Cœlom.   *D.* Distal or anti-mesometrial.   *DA.* Dorsal aorta.   *DWT.* Distal wall of tropho-blast.   *E.* Germinal epiblast.   *EC.* Cavity of epiblast.   *ED.* Ectoderm.   *HY.* Hypoblast.   *HYE.* Uninvaginated or external hypoblast.   *HYF.* Hypoblast of ventral wall of fore-gut.   *HYI.* Invaginated or internal hypoblast.   *M.* Meso-blast.   *MI.* Blood islets.   *NC.* Neurenteric canal.   *NEU.* Neural canal.   *P.* Proximal or mesometrial.   *PAM.* Paraxial mesoblast.   *PC.* Pericardial cavity.   *PCM.* Precephalic mesoblast.   *PG.* Primitive groove.   *PS.* Primitive streak.   *SC.* Segmentation cavity.   *SM.* Somatapleure mesoblast.   *SP.* Splanchnopleure mesoblast.   *T.* Trophoblast.   *TC.* Secondary cavity of trophoblast.   *TC.'* Primary cavity of trophoblast.   *TD.* Distal portion of trophoblast.   *TDW.* Distal wall of trophoblast cavity.   *TP.* Proximal portion of trophoblast.   *VA.* Vitelline artery.   *VC.* Vitelline cavity.

PLATE XXIII—

    Fɪɢ. 1.—Mesial section of the ovum of a rat at the fourth day.   × 566.

    Fɪɢ. 2.—Section [fifth of eight] of the ovum of a rat at about the fifth day.   × 566.

    Fɪɢ. 3.—Section [fifth of eight] of another ovum from the same uterus as that represented in Fig. 2.   × 566.

    Fɪɢ. 4.—Longitudinal section [sixth of eleven] of the ovum of a mouse at about the end of the fifth or in the early part of the sixth day.   × 566.

    Fɪɢ. 5.—Longitudinal section [sixth of nine] of the ovum of a mouse at the sixth day.   × 566.

    Fɪɢ. 6.—Mesial longitudinal section of the ovum of a mouse at the sixth day.   × 566.

FIG. 7.—Oblique section of another ovum from the same uterus as that represented in Fig. 6.   × 566.

FIG. 8.—Oblique section of the ovum of a mouse at the seventh day. × 566.

FIG. 9.—Mesial longitudinal section [slightly oblique] of another mouse ovum at the seventh day.   × 566.

FIG. 10.—Slightly oblique longitudinal section [eighth of fourteen] of a mouse ovum about the middle of the seventh day.   × 566.

FIG. 11.—Longitudinal section of the ovum of a mouse [eighth of seventeen] at about the end of the seventh or in the early part of the eighth day.   × 566.

FIG. 12.—Longitudinal section of the ovum of a rat [seventh of thirteen] at a period corresponding to the end of the seventh or the early par of the eighth day in the mouse.   × 566.

FIG. 13.—Mesial longitudinal section of an ovum of a rat at a period corresponding to the second half of the eighth day in the mouse.   × 89.

FIG. 13 A.—Mesial longitudinal section of the central portion of the embryonic area of a rat's ovum from the same uterus as the ovum represented in Fig. 13.   × 283.

FIG. 13 B.—Mesial longitudinal sections of the anterior and posterior ends of the embryonic area of an ovum from the same uterus as that represented in Fig. 13.   × 283.

PLATE XXIV—

FIGS. 13 C—G.—Transverse sections of the germinal area of a rat's ovum from the same uterus as the ovum represented in Fig. 13.   The positions of the sections are indicated by the lines c, d, e, f, and g respectively in Fig. 13.   × 283.

FIG. 14.—Slightly oblique longitudinal section [sixteenth of thirty] of an ovum of a rat at a period corresponding to the eighth day in the mouse. × 85.

FIG. 14 A.—Mesial longitudinal section of the central portion of the germinal area of a rat's ovum from the same uterus as the ovum represented in Fig. 14.   × 283.

FIGS. 14 B and C.—Transverse sections [slightly oblique] of the germinal area of a rat's ovum, taken from the same uterus as the ovum represented in Fig. 14.   The positions of the sections are indicated by the lines b and c respectively in Fig. 14a.   × 283.

FIGS. 14 D—F.—Transverse sections [slightly oblique] of the germinal area of a rat's ovum, taken from the same uterus as the ovum represented in Fig. 14.   The positions of the sections are indicated by the lines d—i respectively in Fig. 14.   × 283.

FIG. 15.—Mesial longitudinal section of the embryonic area of a mouse ovum at the ninth day.   × 150.

Fig. 15 A.—Longitudinal section [twenty-ninth of forty-eight] of the embryonic area of a mouse ovum at the ninth day. × 50. The ovum was taken from the same uterus as the ovum represented in Fig. 15, but it has attained a slightly more advanced stage of development.

Fig. 15 B.—Longitudinal section [slightly oblique, twenty-ninth of fifty-two] of the embryonic area of a mouse ovum a little older than the ovum represented in Fig 15 a. × 150.

Fig. 15 C.—Longitudinal section of the anterior portion of the embryonic area of the mouse ovum represented in Fig. 15. × 566.

Fig. 15 D.—Mesial longitudinal section of the centre of the germinal area of the mouse ovum represented in Fig. 15. × 283.

Fig. 15 E.—Longitudinal section [twenty-ninth of fifty-two] of the anterior portion of the embryonic area of the mouse ovum represented in Fig. 15. × 283.

PLATE XXV—

Figs. 15 F—H.—Oblique transverse sections of the embryonic area of a mouse ovum at the ninth day. This ovum is a little more developed than that represented in Fig. 15 D. The positions of the sections are indicated by the lines f, g, and h in Fig. 15 B. × 198.

Fig. 16.—Oblique transverse section of the distal end of the embryonic cylinder of a rat's ovum at a period corresponding to the ninth day in the mouse. × 320.

Figs. 16 A—E.—Slightly oblique transverse sections of the germinal area of a rat's ovum at a period corresponding to the ninth day in the mouse. Each section is five micromillimetres thick. Section 16 A is the sixth, 16 B the eleventh, 16 C the seventy-third, 16 D the eighty-fourth, and 16 E the ninety-first section from the centre of the germinal area. × 320.

Fig. 16 F.—Section through a portion of the extra-embryonic area of a rat's ovum at a period corresponding to the ninth day in the mouse, showing the formation of the mesoblast from the extra-embryonic hypoblast. × 566.

PLATE XXVI—

Figs. 16 G, H.—The ninety-seventh and hundred and first sections respectively of the embryonic area of the ovum represented in Figs. 16 A—E, Plate III. Fig. 16 G is × 85, and Fig. 16 H is × 320.

Figs. 17 and 17 A.—Mesial longitudinal sections of the central portion and the anterior end of the embryonic area of a mouse ovum at the ninth day. × 378.

Figs. 18 and 18 A.—Mesial longitudinal sections of the central portion and the anterior end of the embryonic area of a rat's ovum at a period corresponding to the latter end of the ninth day in the mouse. × 566.

Fig. 18 B.—Transverse section of a portion of a rat's ovum immediately in front of the embryonic area at a period corresponding to the latter part of the ninth day in the mouse.   × 260.

Figs. 18 C—E.—Transverse sections through the anterior portion of the embryonic area of a rat's ovum at a period corresponding to the latter end of the ninth day in the mouse.   Sections 18 C and 18 D are taken in the directions of the lines C and D respectively in Fig. 18 A.   Section 18 E is situated more posteriorly.   × 260.

PLATE XXVII—

Fig. 18 F.—Transverse section through the embryonic area represented in Figs. 18 A—E, but in the region posterior to that represented in Fig. 18 E. × 260.

Fig. 19.—Longitudinal section of the ovum of a rat at a period corresponding to the tenth day in the mouse.   × 58.

Fig. 19 A.—Mesial longitudinal section of the anterior portion of the embryonic area of the ovum represented in Fig. 19.   × 566.

Figs. 20 and 20 A.—Transverse sections through the central portion of the embryonic area of a mouse ovum at the tenth day.   The sections have passed through the area a short distance in front of the anterior end of the primitive streak.   Section 20 is the more anterior, and Section 20 a the more posterior of the two.   × 283.

Fig. 20 B.—A transverse section through the anterior end of the primitive streak of a mouse ovum at the tenth day.   × 283.

Fig. 21.—A mesial longitudinal section through the posterior portion of the embryonic area of a mouse ovum at the eleventh day.   × 185.

Fig. 21 A.—A mesial longitudinal section through the posterior end of the chorda dorsalis and the anterior end of the primitive streak of a mouse ovum at the eleventh day.   × 283.

Fig. 21 B.—A slightly oblique transverse section through the posterior part of a mouse embryo at the eleventh day.   The section passes through the posterior end of the chorda dorsalis.   × 285.

Primitive Segmentation of the Vertebrate Brain.

By

Bertram H. Waters, A.B.,
E. M. Fellow in Biology.

With Plate XXVIII.

In 1828 Von Baer (26) observed various symmetrical folds in the hind-brain of the Chick, but only recently has their segmental value been appreciated. Much later they were seen by Remak (23) and Dursy (5), the former pointing out their intimate relation to the cranial nerves. In 1875 Dohrn (6) showed the segmental relations of these folds to the mesoblastic somites, and compared them to the segmentation of an insect. In 1876 Foster and Balfour (7), and a year later Mihalkovics (20), inclined to consider them as the purely mechanical results of mesoblastic pressure; but later histological investigation of their structure seems to render such a position untenable. However, Balfour (2) says, in speaking of these constrictions in the hind-brain in the Chick, "The sides become marked by a series of transverse constrictions, dividing it into lobes which are somewhat indefinite in number. The first of these remains permanent, and its roof gives rise to the cerebellum. It is uncertain whether the other constrictions have any morphological importance. More or less similar constrictions are present in Teleostei. In Elasmobranchii the medulla presents on its inner face at a late period a series of lobes corresponding to the roots of the vagus and

glosso-pharyngeal nerves, and it is possible that the earlier constrictions may potentially correspond to so many nerve-roots." Quite recently, Béraneck (4, a) in Amphibia, and Kupffer (16, a) in Teleostean and Cyclostome fishes, have observed and treated these folds as having segmental value, the latter enumerating eight segments or metameres in the hind- and mid-brains, and noting their resemblance to similar structures in the spinal cord. In a later paper Béraneck (4, b) gives details of the neuromeres (replis médullaires) in the Chick, assigning to each a segmental value. His observations of the nerve relations to them are substantially those given by Orr (21), except in regard to the fifth, which he considers, as does Miss Platt (22), to be derived from the first and second of the hind brain neuromeres, and the result of two primitively independent nerve trunks. Moreover he does not agree with the later investigators in homologising neuromeres and myelomeres, i. e. similar constrictions in brain and cord. In 1887 Orr (21), while investigating the embryology of the Lizard, figured and described six folds in the hind-brain, five of them of equal size ; the sixth, from which the tenth nerve arose, somewhat larger. In describing them he used the name " neuromeres," a word somewhat differently applied by Ahlborn (1). Orr has observed—

(1) That each neuromere is separated from the adjacent one on either side of it by an external dorso-ventral constriction and an internal dorso-ventral ridge, each having between these limits a semicircular or half-oval appearance :

(2) That these constrictions or neuromeres are perfectly regular and opposite on both walls of the brain :

(3) That the elongated cells of which they are composed are situated radially to the inner curved surface of each neuromere, and that their nuclei are generally nearer the outer, approaching the inner surface only toward the apex of the ridge :

(4) That the cells are confined to their respective neuromeres, so that one structure does not insensibly pass into the next succeeding, but is separated from it by a more or less

sharp line of demarcation, along which the cells of both are much crowded together:

(5) That from the crest of each neuromere, i. e. the external convex surface, arises a mass of cells constituting the roots of the cranial nerves proper to that region.   From the first the fifth, from the second the sixth, and from the third the seventh and eighth. Opposite to the fourth appears the auditory vesicle. From the fifth neuromere the ninth, and from the sixth (which he observed not so clearly as the others) the tenth nerve arises.   Orr observed no neuromeres in the mid-brain, but described it as an unsegmented sweep of brain wall which was nearly equal in extent to three of the hind-brain neuromeres.   In the fore-brain he has described two, similar to those in the medulla, save that they give off no nerves. Behind the origin of the tenth nerve he found no constrictions.

I have given these points at length because they formed the incentive and the basis of the work immediately afterward taken up in this direction, and because they resulted from the first definite and systematic investigation of these extraordinary appearances.

In 1888 McClure (19) undertook the investigation of these neuromeres with a view to demonstrating the neuromeric segmentation of the neural tube throughout its whole extent. By sections of early stages of Amphibian, Reptilian, and Avian forms, he corroborated the observations of Orr, except for the origin of the abducens nerve, and added the following points:

(1) That the fore-brain neuromeres conform in every detail of structure with a typical hind brain neuromere :

(2) That the lateral walls of the spinal cord are divided into segments, which, while less distinct, are histogenetically similar to those of the medulla, and, in fact, are continuous with them and the transition gradual.   Thus in both regions they must have held similar relations to the mesoblastic somites :

(3) That from the dorsal surface of the segments of the spinal cord, or "myelomeres," as McClure has named them, the roots of the spinal nerves take their origin in the same

manner as that described by Orr for those of the medulla, or encephalomeres :

(4) That all these segmentations, whether giving origin to nerves or not, rapidly and early degenerate.

Since the preparation of this paper one has been published by Miss Platt (22) bearing upon the subject. She has developed the relation of the neuromeres to the protovertebræ, but falls into the error of confounding with the neuromeric segmentation, the so-called vesicular segmentation. She differs from Béraneck, Orr, McClure, and myself as to the relations of the nerves to their corresponding neuromeres, at least in the Chick, deriving them from the constrictions between, and not from the crests of, the neuromeres ; and holds that the internal ridge described by Orr is composed of cells which converge to the root of origin of the nerve from that region. Hence she connects the latter with both the neuromeres between which it arises, and upon this ground assumes the individuality of the seventh and eighth nerves.

I cannot agree with Miss Platt upon this point. I have never observed any such conditions even in very early stages of Chick embryos. From the fact that both McClure's observations and my own support the original statement of Orr concerning the point of nerve origin, I find her statements difficult to accept.

It will be seen that these investigations left the primitive condition of the fore-brain very doubtful and that of the mid-brain undetermined. I have attempted by the study of Fish and Amphibian embryos to confirm the points already made and to add some new ones, regarding especially the fore- and mid-brain.

The investigations, of which this paper is a résumé, were undertaken during the winter of 1889–90 in the Morphological Laboratory at Princeton under the direction of Dr. Henry F. Osborn, to whom I wish to express my deep obligation, not only for the kindly interest which enabled me to complete the work, but also for many valuable suggestions. It was intended, by the study of some low form of Ichthyopsida,

to determine the total number of segments or neuromeres, and especially to clear up the doubt in which my predecessors have left the mid-brain. The form studied was Gadus morrhua, embryos from six to eleven days' incubation being used, for which I am greatly indebted both to Mr. H. V. Wilson, of the United States Fish Commission Station at Wood's Holl, and to Dr. William Libbey, of Princeton; embryos of Amblystoma punctatum were also used by way of comparison. I greatly regret that my efforts to obtain the embryos of Petromyzon were without success, as my observations are thus limited to Teleostean forms. However, the results obtained from the investigation of young stages of the lamprey will, I hope, form a second part of this paper.

In the early stages of the Cod the small amount of cranial flexure renders a horizontal longitudinal section of the entire neuron possible, and in such sections the walls of the brain are closely approximated, there being no proper lumen anterior to the fourth ventricle, its course and extent being indicated merely by the cell nuclei. The brain in section through the ventral portion, i. e. below the forming cerebral hemispheres and cerebellum, shows a perfectly straight and narrow tube, the walls of which are in apposition but not fused, as the line of their demarcation is perfectly apparent. In the earliest stages examined (about six days) the optic diverticula were well formed, as was also the auditory pit. The side walls of the fore- and mid-brain regions, however, were perfectly regular in extent, and showed no traces of neuromeric segmentation (see fig. 1). This I found to be true of all stages under ten days' incubation, at least as far as the fore- and mid-brain regions are concerned, certain of the sections showing some of the characteristic hind-brain neuromeres. This fact is difficult to explain if, as seems probable, these segmentations are the remains, in part atavistic, of a primitive condition, on any other ground than that of the gradual abortion and disappearance of these structures. McClure has referred to the degeneration of the neuromeres, and it seems to me not unreasonable to conjecture that these constrictions,

being essentially primitive and in a state of degeneration, have gradually been more and more crowded out by the specialising brain development, and hence appear at a much later period in the ontogeny than would be expected. However that may be, their tardy appearance is a strong proof of their intimate relation to the brain itself, and affords a striking refutation of one hypothesis which has been suggested, viz. that they are the mechanical results of mesoblastic pressure· Moreover, as Miss Platt has pointed out in her recent paper, the neuromeres often appear before the corresponding proto-vertebræ, and consequently must be independent of any formative influence of the latter.

In sections of about ten days' incubation the eyes are most prominent, appearing abnormally large and apparently in advance of the other parts in development (see fig. 2). Cranial flexure is more marked, and renders section of the entire brain area impossible. However, a series taken at a slight angle from the horizontal shows the ventral portion of the brain to be a simple tube, the walls of which are of uniform thickness and separated by a considerable lumen. In embryos of this age the neuromeric constrictions seem first to become prominent, though I find them also in stages about twenty-four hours younger.

Fore-brain.

As it was my object to study the mid-brain, almost all my sections were made with reference to that region. At first I had some difficulty in satisfying myself of its extent and location, the eyes being so large relatively that they were of no service as guides to this region. Later the determination of the anterior extremity of the medulla and the posterior commissure perfectly defined its extent. The Cod brain is a long narrow area made up of closely placed small cells, round or spindle-shaped, with a clear undifferentiated border—the forming white brain substance. A short distance behind the axis of the eyes it is crossed by a broad band of white fibres, which, except for a few scattered cells, are continuous with the outer

white border (see fig. 2, *p.c.*). This I consider the posterior commissure, and that it defines the backward limit of the primitive fore-brain. This area has a peculiar club-shaped or trefoil appearance, and nothing can be seen of the brain cavity or of the canalis centralis, which appears as a faint line, except where the drawing away of the brain walls, to accommodate the budding out of the optic vesicles, forms an irregular lozenge-shaped opening into the cavity of the third ventricle (fig. 2, *op. lu.*). Directly in front of the eyes, and in apposition with the anterior extremity of the thalamencephalon, lie the olfactory vesicles (figs. 2 and 3, *ol. v.*). These I find connected with the brain by a short thick mass of cells on either side, the olfactory nerves, which even in the late stages seem to have no connection with the prosencephalon, so that for the Cod I am able to confirm the observations of Marshall (**17**, *c*) in regard to this nerve in other forms. While I can affirm nothing as to the persistence of the neural ridge to the forward extremity of the fore-brain, all my sections, even the earliest stages, show the olfactory pits lying on either side, and slightly in front of the fore-brain, and there is in no section the slightest trace of an olfactory lobe. The nerves themselves in relation and histological structure agree closely with the other cranial nerves, except that so far as I am able to observe they are developed somewhat in advance of them. My sections being longitudinal I am not able to state with certainty their precise point of origin, but I can see no reason to doubt that it is at the anterior extremity of the fore-brain. They certainly pass downward and outward and at right angles to the longitudinal axis of the head—the characteristic course, according to Marshall, of a segmental nerve. They consist of rounded or oval cells with a very few nerve-fibres. Many earlier stages show a distinct proliferation of the cells into the white substance on either side, behind and above the position occupied by the developing nerve, which seems to support Marshall's idea that these nerves have been shifted downward and forward from their original point of origin. The region of brain-wall giving rise to them shows markedly the characteristics of

a true neuromere as determined by Orr, and is, I think, the first or olfactory neuromere, as from its crest occurs the proliferation of cells already mentioned. I cannot be certain in regard to this point, as lack of early stages renders accurate determination of the boundaries of the medullary plate impossible, and differentiation between primary and secondary fore-brain extremely difficult. The point is an important one, and deserves further careful investigation.

Immediately back of what, for convenience, I have termed the first neuromere, and in a line with the forward portion of the orbit, the brain-wall on either side begins to bulge out into broad, somewhat bluntly rounded diverticula (fig. 3, *op. lu.*). These are the remnants of the optic vesicles, the distal portions having been constricted off to form the second nerves. Now, while none of my Cod sections give any reliable evidence of a neuromere at this point, I will show that in Amblystoma there is certainly a second neuromere, and that the optic diverticula hold a curiously significant and close relation to it. Behind the optic vesicles the brain assumes its narrow and uniform tract-like appearance (fig. 3). The closely compacted oval cells show little or no lumen, and are enclosed on either side by a narrow border of white cortical matter (figs. 2 and 3, *cm.*). At a point somewhat behind a line drawn through the axis of the eyes, these borders join across the brain by a narrow band of white fibres—the posterior commissure,—thus marking the junction of the fore- and mid-brain. The distance from the posterior commissure to the termination of the second neuromere is about one third the entire neural length from before backward to this point. It is not possible in this space to observe any nerve or any proliferation of cells, however slight, neither can the cells of the brain-walls be said to have any radial arrangement. However, the condition of my material is quite unsatisfactory, permitting no clear observation of cell boundaries ; and from the fact that there is just sufficient room at this point for another neuromere, and that in Amblystoma I have been able more satisfactorily to prove its existence, I have called this the third

neuromere, thus making the fore-brain contain three neuro-
meres (see figs. 6 and 7, *n*. 3). I think that this conclusion
is strengthened by the fact that both Orr and McClure assigned
to the fore- and mid-brain together five neuromeres, though
they did not definitely determine the number composing each ;
and I feel sure that investigation will confirm my observations
on this point.

Mid-brain.

Orr has stated that this region of the brain appears equal
in length to two hind brain neuromeres. McClure has made
it even longer; but I think he is mistaken in assigning to it,
as he does, though on purely speculative grounds, a third
neuromere. I think it most probable that accurate measure-
ments would make it slightly longer than Orr has estimated
it; for while I find it contains but two neuromeres, I have
also found that the segments increase in length toward the
fore-brain, those of the spinal cord, the myelomeres, being
uniform in size, but smaller than the encephalomeres, the
largest of which is the first, or olfactory. This fact may be
accounted for perhaps on phyletic grounds by the rapid de-
velopment of the forward portion of the brain. Sections of
Cod of about eleven or twelve days, the plane of section having
reached almost to the floor of the canalis centralis, exhibit a
region extending from the posterior commissure to a point
some little distance behind the eyes (see fig. 3). Within these
limits there appear two well-marked convolutions of the brain-
wall. Owing to the cranial flexure at this stage it is difficult
to obtain true longitudinal sections, but by comparison with
those of younger stages I have fairly well identified this region
as the mid-brain. The constrictions are slightly smaller than
those of the fore-brain, and rather more semicircular in shape.
The characteristic radial arrangement of cells is present, but
I have not been able to satisfy myself with regard to their
relation to nerves. The first one, at a low level, seems to give
origin to some fibres which may correspond to the third nerve ;
from the second I have observed no nerve originating. I am

convinced, however, that an osmic acid, or some other more
distinctive paeparation, would show each of these neuromeres
to be connected with a nerve-root.   In regard to the segmental
value of the two nerves of this region Gaskell (9) has said,
" Both these nerves possess within themselves structures
which appear to me to have been originally the nerve-cells
and nerve-fibres corresponding to the cells and nerve-fibres of
the stationary ganglion on the posterior root of a spinal
nerve; so that in the possession of afferent fibres with a sta-
tionary ganglion, as well as in the possession of efferent fibres,
these two nerves conform each to the type of a segmental
nerve."   Kupffer, in a recent paper (16, b), speaks of a short
fibrillar cord which springs from the ventral aspect of the
mid-brain; also of a cord springing dorsally from the mid-
brain, of the position of which as an eye muscle-nerve he is in
doubt.   Should it prove to be constant he would assign it to
the branchial system, thus establishing its primitive character.
The existence of such nerve-elements in connection with what
must now be granted to be segmental structures seems at last
to define the position of these nerves, in explanation of which
so many different opinions have been held.

    The results obtained from the preparations of Ambly-
stoma are offered mainly in confirmation of those already
stated.   They are more satisfactory as regards the enumera-
tion and location of neuromeres, less so as to the nerve origins,
the stages being so young that the latter are indefinite, and
their determination much obstructed by the abundance of
yolk spherules.   While it has been said that the neuromeric
segmentation seems to be retarded, this must be understood
as being only relatively true, and, as degeneration of the seg-
ments begins with the most anterior, early stages were neces-
sary.   The figures show a series taken as nearly as possible in
a horizontal plane through the mid-brain shortly after the
closing in of the medullary folds, the section at the lowest
level showing, in slightly oblique section, the fore-brain, the
obliquity being due to a slight amount of cranial flexure.
Anteriorly is seen the first or olfactory neuromere with the

characteristic radial arrangement of cells, which toward the crest become more numerous, more compacted, and larger; while beyond the brain itself the proliferated cells of the first nerve extend down on either side to the already well-formed olfactory pits.   The first neuromere finally loses itself posteriorly in a large but gradual inward convexity of the brain wall, which in sections at a lower level may be seen to occlude the lumen, while toward the dorsal surface they fall rapidly away from the lateral walls (figs. 5, 6, 7, and 8, *c. s.*).   These are the corpora striata.   Immediately behind these the brain-walls sweep outward again to form the well-marked second or optic neuromere.   Here there is no evidence of nerve origin; but tracing this region through successive sections from lower to higher levels, it may be seen that the optic diverticula are thrust out immediately dorsal to this segment on either side, and merge insensibly into it below (figs. 5, 6, 7, and 8).   This position occupied by the optic diverticula relative to the second neuromere is strangely homologous to that held by nerves of acknowledged segmental character to their respective neuromeres, and seems to me to point to the conclusion that the second pair of nerves, before their great specialisation, may have been perfectly comparable to the other cranial nerves, and were probably in connection with the second neuromere, and are, therefore, deserving of a place in the list of segmental nerves.   In speaking of the primitive nerve relations of Ammocetes, Kupffer (**16**, *b*) notes that of the lens of the eye to the ganglionic chain, and remarks that as the auditory organ is related to the principal ganglia, so the eye appears to belong to the epibranchial series.   Behind the optic is seen the third neuromere of the fore-brain, in connection with which here, as in the cod, there seems to be no nerve.   For this the early stage of the material may perhaps be held to account, at the same time that it affords proof of the fact that the first nerve is much in advance of the others in development.   Behind the third neuromere there appears to be a small portion of brain wall, which is either unsegmented or a part of an additional segment; for as the series is traced dorsalwards the fore-brain

runs out by very oblique sections separated by a slight interval from oblique sections of the medulla (figs. 5 and 6). The latter gradually lengthens, running into horizontal sections of the mid-brain, the extent of the latter being finally marked out anteriorly by the small round tubular process of the epiphysis, which in Amblystoma is well developed, and has considerable lumen (fig. 9, *ep.*). Within this area—bounded in front by the epiphysis, and behind by a well-marked neuromere, from which a considerable number of cells are proliferated, and which I consider the trigeminis—two fairly well outlined neuromeres may be seen, viz. the oculo-motor and trochlear. From the latter, at a high level, some cells are proliferated, which correspond in position at least to the roots of origin of the sixth nerve (see figs. 9 and 10).

Hind-brain.

McClure's investigations of the hind-brain are so satisfactory that little need be added here. I have been able to verify his results as follows :

(1) In the hind-brain of the Cod six neuromeres are to be seen, corresponding in number to those observed by him in the Lizard and the Chick.

(2) In Amblystoma only five neuromeres are to be found in the hind-brain, the sixth or abducens being absent.

(3) These neuromeres exhibit closely the characteristics already described.

I have not been able to verify McClure's statement that the vagus neuromere greatly exceeds the others in size, and I am inclined to think that the point will not prove constant, but that, as I have said, the neuromeres decrease gradually in size, i. e. in length, from the first to the eleventh inclusive. In this way only have I observed that the trigeminis exceeds the others of the mesencephalon. In Amblystoma, however, in which no abducens neuromere is apparent, the trigeminis is especially prominent, but I think owes its increased length to its fusion with the abducens neuromere, as it is about equal to two hind-brain neuromeres. This variation in the primitive

structure of Amblystoma may be explained, as McClure has suggested, by the departure of recent Amphibia from the main Vertebrate line, and the potent retarding and formative influence of a large amount of food yolk ; but, as I shall have occasion to note, it seems more rational to ascribe it, on purely phyletic grounds, to variation in position of the sixth nerve itself. In regard to the other segments and nerves of the hind-brain, I have seen nothing which would lead me to doubt the observations already made.

Béraneck has pointed out that certain of the hind-brain segments are in direct relation to certain corresponding nerves. Orr has derived the fifth, sixth (seventh, eighth), ninth, and tenth nerves by ganglionic cell masses from the dorsal surfaces and crests of the fifth, sixth, seventh, ninth, and tenth neuromeres respectively, and the sixth at a later period from the ventral surface of the sixth neuromere of the hind-brain, no nerve being given off from the eighth neuromere proper, the space adjoining it being occupied by the auditory vesicle. McClure has been able to confirm these points, with the exception of the point of origin of the abducens nerve. This he has been unable to locate exactly, but has placed it approximately between the fifth and seventh neuromeres. I have been able to observe in young Amblystoma embryos that the sixth nerve arises by a proliferation of cells from the base of the brain, ventral to and slightly in front of the root of origin of the seventh and eighth, though it has no connection with it (see figs. 11 and 12). In the Cod, which, as I have said, shows six hind-brain neuromeres, I have not been able to satisfy myself in regard to this nerve, but at least am positive that its origin is ventral to and much in front of the seventh and eighth. In other words, I am inclined to think that Orr is correct in placing its origin at the ventral surface of the sixth neuromere in the Lizard, and that it has such origin in all species in which six neuromeres are present in the hind brain, i. e. forms in which the nerve has retained more or less perfectly its primitive character ; and that its deviation from this position and gradual shifting

backward, together with the coalescence of the fifth and sixth neuromeres, has brought about the condition of affairs which may be observed in Amblystoma. That the absence of the sixth neuromere in Amblystoma is due to the degeneration of the sensory portion of the corresponding nerve I think improbable, even as a matter of conjecture as suggested by McClure, for except for the great deviation of recent Amphibia, which alone seems to be an insufficient one, there appears to be no other valid explanation of the variation. The fact remains that the nerve itself, or its representative, is present in low forms, and that in those much higher in the scale of development both neuromere and nerve have been shown to be present, and to occupy almost, if not exactly, their theoretical position.

McClure's observation in regard to the probable double origin of the seventh and eighth nerves seems to me to be well founded.

The auditory vesicle develops rapidly and comparatively early, invading all the space lateral to the auditory neuromere, and might thus easily have caused the eighth nerve to shift forward and rise secondarily with the root of the seventh, thus accounting for the absence of nerve origin from the eighth neuromere. This theoretical evidence seems to me to be further enforced by the fact that the seventh, in containing both motor and sensory elements, is clearly segmental; while the eighth, in being purely sensory, retains thus so much of its primitive character, and renders probable its posterior origin, its anterior root and its motor fibres having been lost or differentiated.

In regard to the myelomeres and spinal nerves, I have nothing to add to the observations of McClure.

In the preceding pages I have attempted to contribute a few points to the solution of the problem of Vertebrate brain segmentation. The evidence is far from being conclusive, but enough has been done to suggest the results that may finally be attained through this method of investigation. I have endeavoured to make the following points:

(1) That the fore-brain is composed of at least two well-marked neuromeres.

a. Of the existence of the first I am in doubt. The first nerve arises in the same manner, though at an earlier period than the other cranial nerves, thus indicating, however slightly, its segmental character.

b. From the second no nerve springs, but it is directly opposite to the eye, and the optic diverticula spring from its dorsal crest in a manner entirely comparable to the other cranial nerves; thus pointing to the conclusion that, though highly specialised in existing Vertebrates, it was originally not so closely identified with the brain itself, but was homologous with the other segmental nerves.

c. From the third no nerve arises, but I think it probable that still lower forms in earlier stages will show some nerve arising at this point.

(2) That the mid-brain consists of two neuromeres, from which I have every reason to think the third and fourth nerves take origin, and hence deserve to be recognised as segmental structures.

(3) That the hind brain consists of six neuromeres. In regard to this region I think the observations of McClure and Miss Platt are sufficiently satisfactory, except as regards the origin of the sixth nerve and the abducens neuromere. This nerve I have found to occupy its theoretical position when the neuromere exists; when fusion has taken place between the trigeminis and abducens neuromeres, the sixth nerve has been shifted backward toward the seventh and eighth nerves.

It seems reasonably certain that the central nervous system of the primitive Vertebrate form consisted of a series of symmetrical segments, of which those of the neuron held the same relation to the mesoblastic head segments as did those of the cord to the protovertebræ, i. e. were intersomitic; that those of the head, ten or eleven in number, gave origin to their respective nerves precisely as did those of the cord to the spinal nerves; that, in fact, the two regions were perfectly homologous in origin, character, and function. Hence from

the primitive neuron, i. e. the first ten or eleven segments, by a direct differentiation and specialisation, the complex region known as the encephalon has been evolved. That striking fact of Vertebrate embryology, viz. the rapid increase of the anterior brain region and its great differentiation, seems to account for the relatively greater size of the fore- and mid-brain segments, and their early degeneration, and for the per-sistence of those in the hind-brain, which is more primitive in character.

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EXPLANATION OF PLATE XXVIII,

Illustrating Mr. Bertram H. Waters's paper "On the Primitive Segmentation of the Vertebrate Brain."

Index Letters.

I. n., *II. n.*, *III. n.*, &c. First, second, third, &c., cranial nerves. *ap.* Apex and internal edge of neuromere. *t. c.* Thalamocœle. *m. c.* Mesocœle. *ol. v.* Olfactory vesicle. *op. lu.* Optic lumen. *c. s.* Corpora striata. *au. v.* Auditory vesicle. *c. m.* White cortical substance. *p. c.* Posterior commissure. *spt.* Neural septa. *N.* 1, *N* 2, *N* 3, &c. First, second, third, &c., neuromeres. *epi.* Epiblast. *Ep.* Epiphysis. *4th V.* Fourth ventricle. *F. B.* Fore-brain. *M. B.* Mid-brain. *H. B.* Hind brain.

All figures of sections have been drawn with the Abbey camera lucida and a Zeiss's microscope, a Zeiss's ocular No. 2 and objective A being used.

Fig. 1.—Longitudinal horizontal section of Gadus morrhua, six days' incubation, showing unsegmented fore- and mid-brain region, and hind brain neuromeres.

Fig. 2.—Same at ten days, showing olfactory region and nerve (*ol. v.*), the thalamocœli and region of optic lumen (*op. lu.*), posterior commissure (*p. c.*), the region of the third and fourth neuromeres, and probable place of origin of fourth nerve.

Fig. 3.—Same at ten days, showing olfactory region, and diagrammatically representing the third, fourth, and fifth neuromeres.

Fig. 4.—Same at eight days, showing thalamocœle (*t. c.*), and third, fourth, and fifth neuromeres with radial cell arrangement.

Fig. 5.—Longitudinal horizontal section of Amblystoma punctatum, showing the olfactory region, the supposed olfactory neuromere, and the optic neuromere, with the relation to them respectively of the first nerve and the optic lumen with the cavity of the thalamocœl (*t. c.*).

Fig. 6.—Same at a lower level.

Fig. 7.—Same at a lower level.

Fig. 8.—Same at a lower level.

The above series of the same specimen shows the gradual merging of the second neuromere into the optic diverticula (*op. lu.*), and their immediate dorsal situation. The last also shows the first nerve in relation to the most anterior (neuromere ?) segment.

Fig. 9.—Same, younger stage, shows the cavity of the epiphysis, the mesocœle (*m. c.*), the fourth and fifth neuromeres, and fifth nerve.

Fig. 10.—Same, shows same points as above, with part of sixth neuromere.

Fig. 11.—Same, older stage, shows the sixth, seventh, and eighth neuromeres; and the fifth, seventh, and eighth nerves and auditory vesicles (*au. v.*).

Fig. 12.—Same specimen at a low level, showing the sixth nerve ventral to the fifth.

The Oscula and Anatomy of Leucosolenia clathrus, O. S.

By

E. A. Minchin, B.A.,

Assistant to the Linacre Professor of Human and Comparative Anatomy,
Oxford.

With Plate XXIX.

As is well known, Leucosolenia clathrus is a sponge in which the oscula have been hitherto supposed to be conspicuous by their absence. In Haeckel's " Kalkschwämme " will be found a full description of this sponge, together with an account of all observations upon it published previously to the publication of that monograph. The following is a summary of Haeckel's description; for further details I refer to the monograph itself.

Leucosolenia (Ascetta) clathrus was first described by Oscar Schmidt,[1] in 1863, as follows :—" Grantia ramosa, ramis 1 mmtro. latis, paulum compressis, varie et irregulariter implexis. Oscula in summitate ramusculorum brevium. Spicula triradiata, radiis obtusis. Color læte sulphureus." Later[2] Oscar Schmidt corrected his former description, and stated that though he formerly thought he had found oscula with a simple lens, he was unable to find them now with a " compositum." He further stated that he was unable to find a trace of a canal system in this sponge; the trabeculæ (Balken) composing it consisted of two very different layers, an outer colourless one containing the spicules, and an inner

[1] 'Spongien des adriatischen Meeres,' Suppl. I, p. 24
[2] Op. cit., Suppl. II, p. 8.

yellowish granular mass without spicules, filling up the whole space which in Leucosolenia botryoides constitutes the ramified cavity. Then Gray made this sponge the type of a new genus, Clathrina, which he characterised by simply translating the original Latin diagnosis of Schmidt given above.

Haeckel finds that Oscar Schmidt was in error in describing the sponge as solid, and as the result of his studies he divides his Ascetta clathrus into four varieties. (1) Ascetta labyrinthus, with a single layer of endoderm-cells, and no endogastric septa or partitions. (2) A. mæandrina, with the endoderm thickened to form a stratified epithelium of several cell layers, the uppermost bearing flagella. No endogastric septa or partitions. (3) A. clathrina, with the endoderm forming a stratified epithelium as in mæandrina, and the gastric cavity divided by partitions in which the embryos develop. (4) A. mirabilis, the colony consisting partly of A. labyrinthus and partly of A. clathrina. In A. labyrinthus alone did the spicules lie in a single layer; in the other three varieties they formed several layers. The plexus of the anastomosing tubes was much looser and with wider meshes in A. clathrina than in A. labyrinthus and mæandrina, and the tubes themselves averaged a larger diameter (1—2 mm., sometimes 3—5 mm.) in the two latter varieties than in the former (where they measured 0·5 to 1 mm., seldom more). In A. labyrinthus and mæandrina the tubes were much twisted and contorted, reminding one of the gyri of the mammalian brain. In all forms alike Haeckel found the colonies completely devoid of oscula, Auloplegma forms. He also at first thought he had seen oscula, but found he had mistaken artificial openings for such. Even the Olynthus forms were without openings, Clistolynthi. Since Haeckel's monograph I know of no work dealing with the question of the lipostomy and variations of this interesting and beautiful sponge. Metschnikoff[1] describes the histology

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[1] "Spongiologische Studien II: Anatomisches über Ascetta," 'Zeitschr. f. wiss. Zool.,' xxxii, 1878-9, pp. 358—362.

with great accuracy, but says nothing of the oscula, though since he talks of " Tarrus forms " it might be inferred that he had seen them.   Oscar Schmidt's work[1] on the development of this form contains no mention of oscula.

The curious phenomenon which is usually termed lipostomy has always been a great puzzle to me.   How can a sponge exist without an osculum?   The osculum is the central exhalent opening of the whole canal system, and to it converge all the currents which enter by the pores and flow along the canals. How can so important an opening be wanting?   Haeckel attempts an answer to the question in the case of this sponge : " In the interior of the tubes the water goes in and out only by the pores."

Wishing to study the histology of this sponge, I was able, by the kindness of Sig. Lo Bianco, the well-known Conservator of the Naples Zoological Station, to go with the little steamer " Frank Balfour," of the Station, in order to collect and preserve Leucosolenia material fresh from the sea ; and in the very first specimen of Leucosolenia clathrus which came on board I saw oscula of such a size that I was perfectly astounded.   To be brief, I find that Leucosolenia clathrus in the fresh healthy condition not only has oscula, but in the full-sized specimens larger oscula than any other Leucosolenia known to me, whether from pictures or in the flesh.

The specimens of Leucosolenia clathrus may be for convenience divided into "large" and "small."   By large specimens I mean the big, full-grown colonies, often 10 cm. in length and 3 or 4 cm. in height.   By small specimens I mean the very young colonies of 5 mm. or less in extent.   Fig. 1 represents two oscula of a large specimen seen in profile, natural size ; one osculum (a) is widely open, the other (b) is partly contracted.   Fig. 2 represents three more oscula from the same specimen (which had altogether ten oscula), seen

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[1] "Das Larvenstadium von Ascetta primordialis und Ascetta clathrus," 'Arch. f. mikr. Anat.,' vol. xiv, 1877, pp. 249—263, Taf. xv, xvi.

from above.   Fig. 4 represents the whole of a small colony with three oscula, magnified five times linear.   It will be seen that the small colony forms a more or less flat plexus of narrow tubes, from which chimney-like oscular tubes arise of about 0·325 mm. in diameter.   In fact, it grows in precisely the same manner as the specimens of Leucosolenia coriacea at Plymouth, in which I observed and described a sieve membrane over the oscular opening.

If now we study the edge of one of the larger oscula with a lens by transmitted light, we see that it has a margin of about half a millimeter in width where the wall is more transparent than in other parts of the oscular tube, and a very short distance above where this more transparent part begins an opaque line can be seen running round the whole osculum (fig. 2 a, s.).

This clearer margin indicates the line at which the collared endoderm stops short, so that the clearer margin is lined with ectoderm within and without, while the more opaque part of the oscula tube is lined by collar-cells on the inner side.   The opaque line s is a muscular sphincter, by the contraction of which the osculum can be closed, and occurring alike in the larger and in the smaller oscula.

To proceed now to the study of sections and preparations. Fig. 6 a, b, c, d, represent four sections from a continuous series through one of the oscula of the sponge represented in fig. 4, after hardening in ½ per cent. osmic acid, soaking for an hour (on the slide) in picro-carmine, in order to counteract the blackening of the osmic acid, and finally staining with hæmatoxylin.   a, b, and c are three sections about the middle of the series, b being the next section after a, and c the next but one after b; while d, in which the osculum is cut tangentially, is the thirteenth section after a.   In all the sections the spicules have been carried by the edge of the razor towards the left, injuring the ectoderm a little.

Fig. 6 a shows well the general structure of the oscular wall.   The collar-cells stop short suddenly at a point, and it can be seen plainly in figs. 6 a and c that they are continued

directly by the flattened ectoderm-cells. In some sections
(6 *b*) it looks as if there was an intermediate form of cell;
but I am convinced that this is only due to ordinary collared
cells being cut obliquely, so that only their bases are seen. A
short distance above where the collar-cells stop, the sphincter
is seen projecting like a ledge into the interior. Above it the
ectoderm goes on for a considerable distance. The height of
this oscular margin, formed only of two layers of ectoderm
with some spicules and amœboid cells between, is really
remarkable.

Fig. 7 *a* represents the sphincter of one of the larger oscula
in section (the two sides of the oscula are of course not drawn
at their natural distance apart, for then they would have to be
separated by more than the length of the whole plate). 7 *b* is
another section, rather thick, from the same series, showing
the sphincter, which was a little crumpled, cut tangentially
and obliquely. This osculum had been hardened with a satu-
rated solution of corrosive sublimate in absolute alcohol, and
the sections stained on the slide with borax carmine first, and
then with hæmatoxylin, a method which I find exceedingly good
for showing the ectoderm. Fig. 8 represents a transverse sec-
tion of the sphincter of another osculum, prepared by the
osmic-picro carmine-hæmatoxylin method.

The sphincter, as can be seen, projects as a ring-like ridge
into the interior of the osculum. We shall consider its minute
structure presently. When this sphincter contracts it closes
the osculum, which then, in the large colonies, has a very
characteristic shape, which I do not know how to describe
better than by comparing it to the human breast (fig. 3). In
such a breast-shaped osculum the nipple is formed by the ecto-
dermal margin of the osculum, and at the base of the nipple
one finds the contracted sphincter. Fig. 9, *a*, *b*, and *c*, repre-
sent sections from a series through the osculum represented
in fig. 3, hardened in abs. subl.,[1] and stained with borax
carmine and hæmatoxylin.

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[1] I use this as a convenient abbreviation for a saturated solution of corro-
sive sublimate in absolute alcohol.

To return now to this sphincter. It consists of two layers of fusiform ectoderm-cells arranged tangentially, between which one finds at intervals some of the large amœboid mesoderm-cells which occur throughout the sponge, but they are by no means common in the sphincter. The point I wish to emphasize is that the contractile muscular cells are the epithelial ectoderm-cells.

The simple two-layered nature of the sphincter is apparent from the transverse sections 6 *a*, *b*, *c*, 7 *a*, 8 ; but still more so from the tangential sections 6 *d*, 9 *a*, and *c* ; less so in the thick section 7 *b*. Perhaps an even more convincing method of seeing it is to put a piece of the wall of a fresh living osculum into Ranvier's one third alcohol for twenty-four hours, and then to carefully pull the sphincter off with a needle and examine it laid out flat in glycerine, after previous staining with picro-carmine. In such a preparation one sees, by carefully focussing its surface, a layer of nuclei. If now the microscope be focussed deeper the layer of nuclei first seen vanishes, and a distinct layer of similar nuclei takes their place. There is absolutely no other cell layer but these two, unless one happens to find one of the scattered amœboid cells, which are by no means common.

These flat preparations offer the best means of studying the nature of the cells, and I find them differ on the opposite surfaces of the sphincter. On one surface they are spindle-shaped, elongated, and with distinct cell outlines. The spherical nucleus is surrounded by granules which form a fusiform figure, extending towards the two ends of the cell. Cells of this kind are shown in fig. 12. On the other surface the cells have similar nuclei, but no distinct cell outlines ; the granules are sometimes arranged in a fusiform figure, sometimes not, but are much fewer relatively. Cells of this kind are shown in fig. 11. By focussing the preparation deeper from which fig. 12 was drawn, I could see cells similar to those represented in fig. 11 ; and similarly by focussing the preparation drawn in fig. 11, I could see cells like those in fig. 12. I have tried to represent this point more clearly in fig.

13, *a* and *b*.   In the middle of each drawing is seen one of the amœboid wandering cells, one and the same cell both in 13 *a* and 13 *b*.   Now 13 *a* is drawn with the microscope at the lower focus, and shows the fusiform cells ; while 13 *b* is drawn at the upper focus, and shows the other kind of cells.   12 *a* represents one of the second kind of cells macerated out from the same preparation from which fig. 12 was drawn.   I take it that the fusiform cells are more specially differentiated ecto-derm-cells, while the other kind are more ordinary ectoderm-cells.   In transverse sections of the sphincter the cells on one surface commonly appear more rounded and project higher than on the other (figs. 7 *a*, 8).   I believe that the rounded projecting cells are the fusiform cells of the flat preparations, and the flat cells the others.   It is difficult to be certain of this point ; I infer it from the fact that in such sections the rounded cells are closer together than the flat ones.   The two kinds of cell appear to occur indifferently on one or the other side of the sphincter.   From the left side of fig. 7 *a*, it would appear as if both sides of the sphincter might, in places, be formed only of fusiform cells.

The nuclei of the cells composing the sphincter have a similar structure in both kinds of cells.   They are spherical or slightly ovate, measuring in glycerine preparations about 6·5 μ, in Canada balsam preparations (prepared in all points in the same manner as the glycerine ones) about 5·2 μ.   The nucleus rarely has one distinct nucleolus ; more usually several small ones.   In preparations hardened with Her-mann's fluid, and stained by Flemming's method[1] with saf-franin, gentian violet, and orange G., the structure of the nucleus is well shown, especially if the sections are cut very thin (4 or 5 μ).   Then the whole nucleus is seen to be filled with a fine network, which may be thickened at several nodal points, sometimes greatly at one, producing the appearance of a nucleolus.   Without entering at present into further histo-

[1] Vide his paper in the ' Arch. f. mikr. Anat.,' vol. xxxvii (1891), " Ueber Theilung und Kernformen bei Leukocyten, und über deren Attractions-sphären," p. 296.

logical details, I will state merely that these nuclei exactly resemble in size, structure, and appearance the nuclei of the remaining ectoderm, and differ in precisely these three points from the nuclei of the endoderm, still more so from the nuclei of the amœboid mesoderm-cells. The granules of the cells appear when carefully focussed as round black spots, but when the microscope is a little high or too low they appear as minute black rings round a central clear spot, and in sections often look not unlike fibrillæ cut transversely, which of course is not the case.

Thus, to recapitulate, this sphincter is composed of two layers of ectoderm, with a few scattered amœboid cells between, and the contractile cells are the ectodermal epithelium. Thus, in one of the simplest existing types of sponges, I have arrived at the same result as Topsent,[1] who in his work on the Clionidæ, finds that the ectodermal or endodermal "cellules de revêtement" are the contractile elements. The sphincter of the oscula of Leucosolenia clathrus is an especially favorable object in which to study this question, as the cells are so large compared with the minute cells of siliceous sponges, and the sphincter itself can be so easily prepared out or cut into sections. We have in this sphincter perhaps the most primitive type of muscle-cell in the animal kingdom; it can hardly be called a myo-epithelial cell, it is still a simple ordinary epithelial cell.

Various authors[2] have described muscle-cells lying in the mesoderm; and until Topsent wrote, I think I am right in saying that muscular cells in sponges were regarded as mesodermal. There is no reason why, in a highly differentiated sponge, muscle-cells originally forming part of an epithelium should not become more specialised and sink into the meso-

[1] "Contributions à l'étude des Clionides," 'Arch. de Zool. expér. et gén.,' tome v bis, Suppl. (1877—1890), p. 24, et seq.

[2] Vide Sollas's article "Sponges," 'British Encyclopædia;' "Monograph of the Tetractinellida," ' "Challenger" Reports,' p. 42; von Lendenfeld, "Beitrag zur Kenntniss des Nerven- und Muskel-systems der Hornschwämme," 'S. B. k. pr. Akad. Wiss.,' Berlin, Nov. 12th, 1885.

derm. But the muscle-cells described by Topsent and in this paper make it, I think, to say the least, extremely probable that all muscular cells in sponges are of epithelial origin.

Dr. von Lendenfeld has published,[1] at divers times and in divers places, a classification of the Cœlenterata into Mesodermalia (sponges), in which the principal organs are derived from the mesoderm; and Epithelaria (other Cœlenterates), in which the principal organs are derived from the epithelia.

What are the principal organs of a sponge? I presume the ciliated chambers, skeleton, genital products, and the various kinds of muscle-cells, gland-cells, nerve-cells, &c. The skeleton certainly appears to be mesodermal, as far as we can judge, and perhaps also the genital cells. On the other hand, the ciliated chambers are almost certainly endodermal, and the muscle-cells of epithelial origin. There does not appear to be the slightest reason why the nerve-cells, so often described by von Lendenfeld, should (if they exist) be of mesodermal and not of ectodermal origin, as in other groups of animals; and the same may be said of their gland-cells. Thus it appears that the only principal organs of a sponge which can with any certainty be said to be of mesodermal origin are the connective-tissue system and the generative elements.

To return, however, to our oscula. We have in this sphincter a mechanism for closing the osculum, and in the sieve membrane over the oscula of Leucosolenia coriacea we have, I do not doubt, a structure which can be employed for a similar purpose, since Auloplegma forms of the latter sponge are so common. I look upon this as a good instance of two structures physiologically similar, but morphologically quite different. In my paper on the sieve membrane[2] I explained it as probably arising as a breaking through of the gastral cavity to the exterior in several places during the formation of the osculum, and hence as consisting of ecto-

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[1] 'Monograph of the Horny Sponges' (London, 1889), p. 889; 'Proc. Zool. Soc.,' London, 1866, p. 566; 'Biol. Centralbl.,' ix, 1889, pp. 113—127, &c.

[2] "Note on a Sieve-like Membrane across the Oscula of a Species of Leucosolenia, &c.," 'Quart. Journ. Micr. Sci.' (n. s.), Part 2, January, 1892.

derm externally and endoderm internally. I see as yet no reason why I should depart from that opinion. On the other hand, it can hardly be doubted that the sphincter here described arises as a simple ingrowth of ectoderm, and consists of this layer only on both faces. In the young forms the sphincter shows only one or two cells on either face in transverse section (fig. 7, *a, b, c*), while in the older ones it consists of a great number lying side by side (figs. 7 *a*, 8, 11, 12, 13), so that it evidently grows with the osculum. I have not yet found an osculum devoid of a sphincter, but it is very probable that the young Olynthus would have none.[1]

In specimens of this sponge fresh from the sea the oscula were, as I have said, exceedingly conspicuous.[2] How is it these oscula have not been found before? I selected on my first collecting trip several large specimens of the sponge with widely open oscula, and put them into a separate vessel in sea water. What was my astonishment, however, when I got back to the Zoological Station, to find no trace of oscula in any of my specimens, not even an elevation to mark where they had been! The thin delicate walls of the sponge had completely collapsed, and the whole presented a shrivelled appearance, as different from the beautiful outlines and transparent yellow colour of the fresh living sponge as anything could be imagined. On a second occasion I selected another very fine specimen, and put it in a separate vessel, and brought it back with great care, changing the water several times on the way home. It was, however, of no avail; it arrived in the same shrivelled condition. The only indication that these sponges

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[1] Since Haeckel observed only Clistolynthus forms it is possible that even the Olynthus has a sphincter. On the other hand, it would be quite possible for an Olynthus to contract itself completely without any special sphincter. Vide Metschnikoff's figure of a Clistolynthus of Ascetta blanca in longitudinal section, 'Zeitschr. f. wiss. Zool.,' xxxii, 1878–9, Taf. xxii, fig. 9.

[2] I cannot but express my astonishment that Haeckel did not see them, since he tells us in his monograph (p. 33) that he found this sponge growing in great quantities in a little bay (San Clemente) on the south side of the Spalmadori Cliffs on the coast of Lesina in 1871, and collected in a short time several hundred small and large colonies.

had ever had oscula was that the anastomosing tubes converged towards the points where the oscula had been.

These specimens, after being a few days in the aquarium, recovered slowly from their drooping condition, like a plant that has been transplanted.   The tubes became rounded and of a healthy appearance, and sent out diverticula, which grew often to 10 or 12 mm. in length, and attached themselves to the side of the vessel.   Such diverticula occur in the natural condition also (see fig. 2).   From the places where oscula had been breast-shaped eminences raised themselves, which were normal closed oscula like fig. 3.   Sometimes a small opening would appear in the " nipple," but only once did I observe in my aquarium that one of my specimens opened out a large normal osculum.   Specimens with closed oscula like fig. 3 are of frequent occurrence in nature, and I have often observed them in specimens fished up fresh on the steamer.   I went on three separate occasions to the only locality where this sponge occurs abundantly in the Gulf of Naples—a very sheltered grotto near Capo Miseno,—and the following short journal of observations may be of interest:

Oct. 2nd.—A fine bright day, the water smooth and clear. All the specimens had wide open oscula.

Oct. 8th.—The weather as before.   All the specimens had open oscula, and on this occasion I preserved fresh from the sea the colony from which figs. 1 and 2 are taken.   In one large specimen I observed a closed osculum.

Oct. 19th.—The sea was smooth, but the day was cloudy, and the water in the grotto was turbid, so that when I dived it was difficult to see the sponges clearly under water.   There had been scirocco and bad weather previously.   Every specimen examined had closed oscula.

Since this date the weather has been so bad and the sea so rough that the steamer has been unable to put out, and so my observations are extremely incomplete ; but they give one at least the suspicion that the state of the sea and weather influence the sponge, and cause it to contract or open : and, indeed, one can hardly wonder that it should be so.   Leu-

cosolenia clathrus, in the widely expanded condition, is one of the most delicate organisms known to me, the least touch being sufficient to break or tear it. If it is even lifted out of the water the tubes and oscula collapse. In the whole Gulf of Naples it is only known to occur in profusion in one grotto. This is a kind of natural tunnel running through a rock peninsula, and putting a small bay in communication with the sea; but the tunnel runs through obliquely, and meets the shore-line at an angle which is acute towards the open sea: hence the waves can never break into it with much force, and it is exceedingly sheltered. But even here it might well be imagined that the sea would be too rough for this delicate animal. When the sponge is contracted, however, it is very much firmer and stronger, and can be handled with more safety. I noticed that the sponges brought home on Oct. 19th with closed oscula did not droop in the same manner as those brought home on Oct. 2nd and Oct. 8th, but remained healthy and firm. A specimen in a similar contracted condition would be much more able to withstand the force of the sea than one expanded.

It is not, however, only the oscula that can contract, but the tubes can also contract very greatly. In fig. 5 is represented, magnified four diameters, a small piece cut off a specimen which had been growing in my aquarium for a month, and which is still quite healthy. It has fairly attached itself on all sides, and is continuing to send out processes, many of which can be seen in the figure. This sponge, when it first came under my notice, was a specimen like that in figs. 1 and 2. After it recovered from the transplantation it several times completely expanded, and it was in this specimen that I saw the only completely open osculum I have ever seen in the aquarium. The manner in which the sponge had alternate periods of expansion and contraction was noteworthy. It would frequently be widely expanded one day and contracted the next. I could find no cause for these expansions and contractions; only about the last week in October the weather became very much colder, and a chilly tramontana blew for

some days.   The sponge contracted completely when the cold
weather began, and has not expanded since ; but it is still
perfectly healthy, as shown both by the histology of sections
from portions of it, and by its continuing to grow and send
out processes.

If we compare fig. 5 with figs. 1 and 2, it is obvious that the
tubes have shrunk to about one eighth of their former dia-
meter.   Imagine now a Leucosolenia tube, with its walls
composed of ectoderm externally, jelly containing a single
layer of spicules and a few cells, and most internally a con-
tinuous, closely packed lining of collared cells.   If this tube
contracts greatly what must be the result?   There can be no
longer room for the collar cells to form a single layer, and the
spicules will also be closely packed, probably into several
layers.

Figs. 15 *a* and *c* represent two sections from a series
through some partly contracted tubes.   The spicules now
form at least two layers in the much-thickened mesoderm,
and the collar cells are arranged in a stratified epithelium, of
which the uppermost only bear flagella.   In some places the
endoderm is thrown into folds (fig. 15 *c*).   In other words, we
have before us Haeckel's variety Ascetta mœandrina.

Mr. Bidder, in his recent review of Dendy's ' Monograph of
the Victorian Sponges,'[1] has written (p. 628), " In these Aus-
tralian sponges (Calcarea Homocœla) there appears to
occur none with a many-layered endoderm.   This structure,
observed by Haeckel, and since universally discredited, cer-
tainly appears in Ascetta clathrus."   I must say that a
many-layered endoderm as a normal feature of sponge ana-
tomy is to me as inconceivable as that a sponge should be
permanently without an osculum.   In every preparation I
have made of this sponge in the expanded condition I find a
single-layered endoderm.   On the other hand, if the sponge
be sufficiently contracted, a many-layered endoderm does and
must occur.   One usually finds it in preparations made from

[1] ' Quart. Journ. Micr. Sci.,' vol. xxxii, part 4, October, 1891, pp. 625—
632.

sponges living in the aquarium, and also in freshly preserved sponges which are contracted.

Fig. 14 represents a section taken at random from a series through the piece represented in fig. 5. Here the contraction has reached almost its limit. The spicules form in places as many as five layers (in the section figured the razor has displaced them a little, in a direction passing from the north to the south of the drawing), and the endodermic layer is now so thickened that the lumen of the tubes is reduced to series of narrow lacunæ. In some places the tube is even solid, as Oscar Schmidt described originally. It is evident from Oscar Schmidt's figure of the sponge that he had to do with a very contracted specimen. In almost every respect the sponge agrees with Haeckel's Ascetta clathrina, both in external form and in anatomy. It is true that the compartments (Fächer) are not separated from one another by " exoderm " (i. e. mesoderm), covered on both sides with endoderm; but if a specimen with folded endoderm, as in fig. 15 b, were to completely contract, that might be the case. It is true also that the compartments do not contain embryos, but that, I suppose, would depend on the time of year at which the sponge was observed.

Thus, to recapitulate : Haeckel's Ascetta labyrinthus is the ordinary expanded condition of this sponge, but with closed oscula, like the piece shown in fig. 3. His Ascetta mœandrina is the same a little contracted, as in fig. 15. Ascetta clathrina is the sponge in an extreme state of contraction, as in figs. 5 and 14. Finally, Ascetta mirabilis is this sponge partly expanded, partly contracted.

In the walls of the tubes also there are no elements to which the contraction could be due except the ectoderm-cells ; and to the great power of contractility I attribute the fact that the ectoderm[1] in this sponge is, as Metschnikoff observed, so

[1] Mr. Bidder has recently described (loc. cit., p. 628) the ectoderm of this sponge as consisting of the mushroom-shaped cells described by Metschnikoff in the Olynthus (Clistolynthus!) form of Ascetta blanca. I do not wish at present to enter into histological details, which I hope to do in another

exceedingly distinct.   I find the very greatest difference in this respect between Leucosolenia clathrus and L. coriacea occurring at Plymouth.

To sum up the results obtained :

Leucosolenia clathrus is not permanently lipostomous, but has very large and distinct oscula.

These oscula are provided with a sphincter by which they can be completely closed for a time, apparently as a protection against unfavorable external conditions.

Haeckel's four varieties of the sponge are only different states of contraction, and are no more zoological varieties than a polyp with contracted tentacles is a variety of a polyp with expanded tentacles.

The many-layered endoderm is also only a temporary condition, the mechanical result of the contraction of the whole sponge.

The contractile elements in all cases are the flattened ectodermal epithelium.

In conclusion, it is my pleasant duty to express my thanks to the staff of the Naples Zoological Station, and especially to my kind friend Sig. Cav. Lo Bianco, without whose help this work could never have been done.

NAPLES; November 10th, 1891.

ADDENDUM.

WHILE the above was in the press, a work by von Lendenfeld has appeared, entitled "Die Spongien der Adria.—I. Die Kalkschwämme" ('Zeitschr. f. wiss. Zool.,' Bd. liii, Heft 2, pp. 185—321, Taf. viii—xv ; and Heft. 3, pp. 361—433), containing a detailed account of Ascetta clathrus (pp.

paper, but as my figures might be thought to be erroneous I will only say that in freshly preserved material of the sponge the "Metschnikoff's cells" only occur sparingly, the predominant form of the ectoderm being flattened epithelium ; and I have almost conclusive evidence to show that the "flask-shaped cells" are only the contracted condition of the flat cells.

210—217, Taf. viii, fig. 4; ix, figs. 27—37). The author divides the sponge into four forms, which he terms A, B, C, and D, rejecting Haeckel's varieties, "since these forms appear to arise one from the other in the course of the post-embryonal development." Form A consists of a mass of anastomosing tubes, 1—5 mm. in diameter, the walls of which have pores and contain numerous stellate connective-tissue cells, but no large granular elements; the endoderm forms a single layer. In Form B the tubes are only 0·3—1·5 mm. in diameter, and form a flat spread-out creeping network. Pores are rare, and the "zwischenschicht" (mesogloea) contains, besides stellate cells, large granular, spherical or irregular cells; the endoderm is many-layered. Form C is similar externally to Form B, but has no pores in its walls; the same large granular cells occur as in B, and the endoderm is many-layered but more closely packed than in the latter form. Form D consists of a flat network of trabeculæ, 1·5—3 mm. thick, in which no pores were to be found; in the mesoderm large granular cells were not observed, and the endoderm fills up the interior of the tubes, leaving only irregular lacunes. All the forms agree in having no oscula visible to the naked eye, and are reticulate Auloplegmas. The author believes that the many-layered endoderm and the closing of the pores is connected with the ripening of the eggs (see p. 217). Dr. von Lendenfeld has made a considerable advance in rejecting Haeckel's varieties, but is nevertheless far from a correct explanation of the different forms, which are nothing more than different states of contraction of the sponge. Thus his fig. 30 (Taf. ix), representing a section of Form D, is in all essentials completely similar to my fig. 14, which is taken from a series of sections through the piece of sponge shown in fig. 5. This sponge, as above described, when first collected was like the specimens shown in figs. 1 and 2, and after completely contracting, frequently expanded again to this form. I have recently observed a similar contraction in another sponge, an Ascon of a beautiful orange-red colour, but with the spiculation of Ascetta primordialis, which when

brought in by the fishermen was widely expanded, with large open oscula.   In a few hours it contracted completely, the tubes shrinking to perhaps one tenth of their former diameter, and having no visible oscula.   The following morning, being placed in a current of pure sea water, it again expanded to its former dimensions and opened its oscula ; but the current being stopped, it slowly contracted again.   In the evening I again placed it in the circulation, and the next morning it was expanded a third time, though not in all parts, the tubes furthest removed from the oscula being to a certain degree contracted.   Some of its oscula opened completely, others were closed and breast-shaped, but at least visible ; whereas, in its completely contracted state, it was impossible to see that the sponge had ever had oscula.   On the strength of these so oft-repeated observations, I cannot but state my disbelief that any Ascon (or any sponge) is permanently lipostomous ; and I have no doubt that where von Lendenfeld has described Auloplegma forms, e. g. in Ascetta spinosa (op. cit., p. 203), he has simply overlooked the oscula, as he has certainly done in Ascetta clathrus.   The large granular cells in Forms B and C admit of a very simple explanation ; they are simply closed pores, which the author has overlooked in Form D, where they are equally common.   Other points of histology I hope to criticise in another place.   I will only draw attention to the statement (p. 190), that in sponges the " skeleton forming, sexual, and muscular cells are formed in the mesoderm, and are not of epithelial origin " (compare also the account of the "zwischenschicht," pp. 398—405).   After what I have already written, this statement requires no further comment.

Naples ; 1st March, 1892.

DESCRIPTION OF PLATE XXIX,

Illustrating Mr. E. A. Minchin's paper on "The Oscula and Anatomy of Leucosolenia clathrus, O. S."

PLATE XXIX.

All the sections of the sphincter have been drawn so that the inner (gastral) face of the sphincter looks towards the south, the outer face towards the north side of the plate.

The following letters are for all the figures.

ect. Ectoderm. *end.* Endoderm. *a. m.* Amœboid mesoderm-cell. *mes.* Jelly (mesoglœa) containing spicules. *s.* The muscular sphincter of the osculum.

Fig. 1.—Two oscula from a large colony, preserved fresh from the sea in 70 per cent. alcohol, in profile view. One of them (*a*) is widely open, the other (*b*) half closed. Natural size.

Fig. 2.—Three more oscula from the same colony viewed from above. Natural size.

Fig. 2 *a.*—The osculum of Fig. 2 viewed as a transparent object, magnified about three diameters. It is very slightly contracted.

Fig. 3.—A closed osculum. Natural size.

Fig. 4.—A small colony with three oscula, magnified five diameters.

Fig. 5.—A piece of a colony in a very retracted condition, the Clathrina clathrus of Oscar Schmidt, the Ascetta clathrina of Haeckel.

Figs. 6 *a, b, c, d.*—Four sections from a series through one of the oscula of the colony represented in Fig. 4 : 6 *a*, a section near the middle of the series ; 6 *b*, the next section after 6 *a*; 6 *c*, the next section but one after 6 *b*; 6 *d*, the thirteenth section after 6 *a*. Magnified 350 times. Osmic half per cent., picro-carmine, hæmatoxylin.

Figs. 7 *a* and *b.*—Two sections from a series through a large expanded osculum like those in Figs. 1 and 2 : 7 *a*, a median section ; 7 *b*, a thick tangential section. × 330. Abs. subl., borax carmine, hæmatoxylin.

Fig. 8.—A median (transverse) section of the sphincter of another large open osculum. Osmic half per cent., picro-carmine, hæmatoxylin. × 330.

Figs. 9 *a, b, c.*—Sections from a series through the closed osculum in Fig. 3 : 9 *a* and *c*, tangential section ; 9 *c*, a detached muscle-cell. Abs. subl., borax carmine, hæmatoxylin. × 330. (In one place the section 9 *a* is slightly broken.)

Fig. 10.—Nuclei from a series of sections through the sphincter of an expanded large osculum. Hermann's fluid, safranin, gentian violet, orange G. Zeiss, compens. ocular 8, apochr. F.

Fig. 11.—Surface view of a relaxed sphincter of the osculum of a large colony. One third alcohol, picro-carmine, glycerine preparation. × 500.

Fig. 12.—Surface view of another similar preparation. × 500. 12 a, an isolated cell from this preparation. The upper (north) limit of Fig. 12 represents the natural free edge of the sphincter.

Figs. 13 a and b.—Two views of another preparation similar to that from which Figs. 11 and 12 are drawn : 13 a, drawn with the microscope at the lower focus; 13 b, with the upper focus. × 430.

Fig. 14.—Section from a series through the piece of sponge represented in Fig. 5. × 70. Abs. subl., borax carmine, hæmatoxylin.

Figs. 15 a, b, c.—Sections from a series through a contracted sponge. 15 b is a portion of 15 a more highly magnified to show the many-layered endoderm. Abs. subl., borax carmine, hæmatoxylin. 15 a and c × 120.
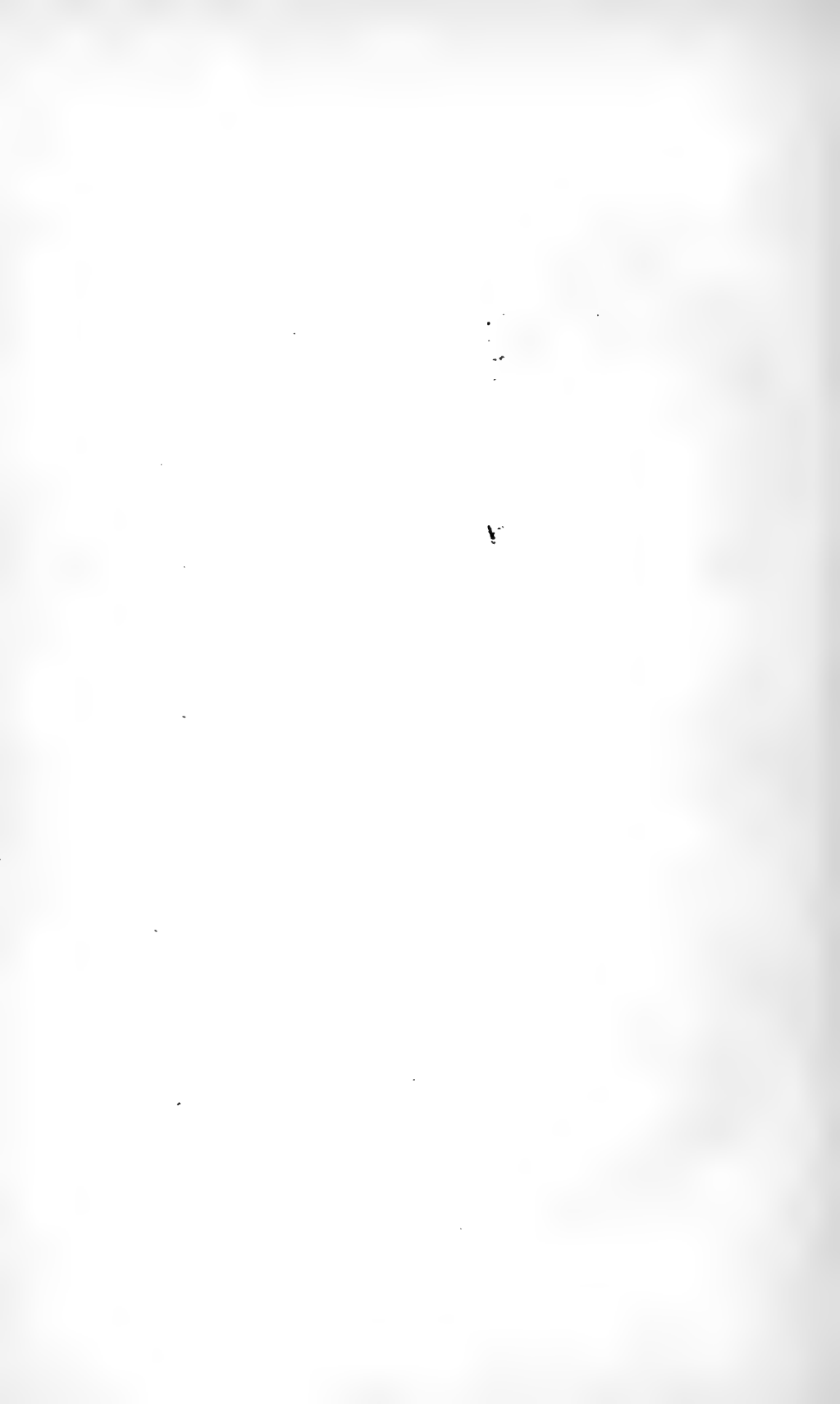
Researches into the Embryology of the Oligochæta.
No. I.—On Certain Points in the Development of Acanthodrilus multiporus.

By

Frank E. Beddard, M.A.,
Prosector of the Zoological Society of London.

With Plates XXX, XXXI.

TABLE OF CONTENTS.

Our knowledge of the development of the Oligochæta is at present based only upon that of a few indigenous forms. Indeed, Lumbricus (including Allolobophora and Allurus), Enchytræus, Rhynchelmis, and Criodrilus are the only types which have been investigated by modern methods. The present paper is the first of what I hope will be a series of memoirs dealing with exotic forms. In addition to Acanthodrilus multiporus, which is treated of in the present communication, I have a few embryos of Acanthodrilus novæ-zelandiæ, which will form the subject of a later com-

munication. Mr. W. W. Smith, of Ashburton, New Zealand, to whom I am deeply indebted for these and many other specimens, has kindly promised to collect other cocoons for me. They travel very well in a living condition. I have already had two sets of cocoons belonging to a new genus of aquatic Oligochæta (Pelodrilus), the anatomy of which I have described in the 'Transactions' of the Edinburgh Royal Society; but unfortunately the tubes in which they were packed were broken in transit. As the cocoons of this species are common and easy to find I have great hopes of getting more.

During August of last year I received a large quantity of cocoons of Acanthodrilus multiporus containing living embryos in various stages of development. The cocoons had been carefully packed in damp moss in a small tin box, which was enclosed in a large biscuit tin, filled with damp moss and covered over with several layers of paper, so as to render it quite air-tight.

Besides the living cocoons I received about two dozen ripe embryos, which had been extracted from the cocoons by Mr. Smith, and preserved with corrosive sublimate.

I shall presently describe the cocoons themselves, but it will be as well to state at once my reason for believing that they belong to the species Acanthodrilus multiporus. It is necessary to do this, as the cocoons were not deposited by the worms in captivity, but were found in the soil upon the margin of a lake. Hence there might appear to be some little doubt as to the species to which the cocoons belong.

Acanthodrilus multiporus was originally described by myself six years ago from material kindly supplied by Professor T. J. Parker, F.R.S. I have since obtained abundant additional material from Mr. Smith.

One of the most prominent features in the structure of this species is the complete duplication of the dorsal blood-vessel; this peculiarity is, it is true, to some extent shared by another New Zealand Acanthodrilus—A. novæ-zelandiæ; but there are numerous other characters which render it quite im-

possible to confound the two species.   Besides, the dorsal
vessel is not completely double in Acanthodrilus novæ-
zelandiæ.   The two tubes, as I have already pointed out
(7), in the latter species become fused at the septa;
whereas in Acanthodrilus multiporus they retain their
distinctness at the septa as elsewhere.

In Acanthodrilus multiporus the prostomium is small,
and does not divide the buccal segment; the anterior segments
are so much divided into annuli that their limits are almost
impossible to map by a superficial inspection of the worm;
there are also internal structural differences of importance,
particularly in the nephridial system, between the two spe-
cies.

The transparent cocoons allowed the dorsal vessel to be
quite plainly visible, and it showed the complete double cha-
racter which has just been referred to; the mature embryos
extracted from the cocoons showed the other external charac-
ters which serve to discriminate the species.   As the cocoons
were all perfectly similar I have no doubt that they all belong
to this species.

Mr. Smith informs me that " the cocoons were found on the
edge of a swamp, which is now quite dried up owing to the
long drought; in other seasons there are generally several feet
of water covering the spot where we obtained them.   I ob-
tained a few (some of the more advanced) among the loose
shingly soil, from which the water had subsided."

§ The Cocoon.

Our knowledge of the cocoons of earthworms is at present
almost confined to the indigenous Lumbricidæ.   A very good
summary of our knowledge upon this point, together with
many new observations, is to be found in Professor Vejdovsky's
' Entwickelungsgeschichtliche Untersuchungen,' Heft i.

The only exotic form which has been as yet described is
Megascolex cœruleus, of which I myself have given an
account (8).   It is clear that the cocoon of that species, as
Vejdovsky has pointed out, departs in no essential particular

of structure from the cocoons of Lumbricus and Allolobophora.

Neither do the cocoons of Acanthodrilus multiporus. It seems clear, therefore, that the formation of this structure is pretty much the same in all these genera, and that the various glandular structures—such as the atria, with which Acanthodrilus is furnished and Lumbricus is not—have nothing to do with the manufacturing of the cocoon. No doubt the clitellum alone is concerned in its production.

The cocoons have a length of about half an inch. They are regularly oval in form, and have a slight stalk-like projection at either end, such as occurs in Lumbricus.

The colour is yellowish brown ; the empty cases have a clear transparent brown colour.

The cocoon is formed of two perfectly distinct membranes which are readily separable after it has been cut open. Vejdovsky has remarked that this is the case also with Lumbricus, particularly L. rubellus, where the outer layer forms a remarkable projection longer than the cocoon, and to which particles of soil adhere.

The cocoons vary somewhat in size. In a cocoon from which the worm has escaped, a circular orifice at one end marks the place at which the young worm made its exit.

The cocoon never contained more than a single embryo ; even in the youngest stages which I examined (fig. 20A) there was but one embryo. As I opened about fifty cocoons, I feel justified in considering that this species may be characterised by the fact that the cocoon only contains a single embryo ; it is probable that there are a number of eggs originally enclosed in each capsule, but it is evident that they must disappear early.

Among the Lumbricidæ one to three embryos seem to be the rule ; but in Allolobophora fœtida there may be as many as six. I found two embryos in a cocoon of Megascolex cœruleus (8).

In fig. 20 are represented the sizes of the embryos of the various stages which I examined ; the youngest is of course A,

while D is a worm ready to leave the cocoon, and showing the principal blood-vessels.

Unfortunately I have very little material belonging to the younger stages; I have only one specimen of stage A, and three or four of B. But considering the length of time which elapsed between the collecting of the cocoons and their arrival at the Zoological Gardens, I may regard myself as extremely lucky in having such a comparatively large series of the younger stages.

Besides the embryos in the cocoons, there were about half a dozen young worms which had been hatched during the voyage.

It seems evident, therefore, that the development of this species may take a considerable time.

The case arrived at the Zoological Gardens on August 11th; it was packed for transmission at latest on June 23rd; a period of seven weeks therefore intervened.

The rate of development also must differ considerably, as Wilson (15) has pointed out in the case of Lumbricus; but the shortest time, so far as I can judge by the evidence afforded by the specimens sent me, can hardly be less than five or six weeks. This is about double the period which Wilson found in two species of Lumbricus. It was always possible to detect an "addled" cocoon by observing whether it did or did not float in water: healthy cocoons with living embryos inside them always sank to the bottom; if the cocoons floated on the surface of the water it was a sign that the embryo was dead.

I have occasionally met with Nematodes in the interior of earthworms' cocoons, as have other observers. In two cocoons of Acanthodrilus multiporus with partially decayed contents I found not Nematodes, as I at first thought, but several examples of an Enchytræid not yet identified : a curious circumstance was that the majority of these small Annelids were dead,—infected, I suppose, by the decaying matter round them. I saw another example on the moss, which is probably the habitat of the species. This family of

Annelids has not yet to my knowledge been recorded as occur-
ring in New Zealand.

The characters of the albuminous fluid filling the cocoon
have been shown by Vejdovsky to vary somewhat in different
species. Particularly noteworthy is the fact that in Allurus
tetraeder this fluid is perfectly clear and transparent; in
this there is a resemblance to Rhynchelmis. I have else-
where pointed out that this earthworm Allurus, largely
aquatic in habit, resembles many aquatic genera in the large
size of the eggs, and in the shortness of the sperm-ducts, which
open on to the exterior in front of the oviducal pore. The
coincidence of these three characters in an aquatic Oligochæte
belonging to a group which is so characteristically terrestrial
seems to require some explanation, which, however, does not at
present appear to be obvious.

In Acanthodrilus multiporus, as in Lumbricus
rubellus, &c., the albuminous fluid filling the cocoon is
milky and opaque; it is readily washed out of the cocoon by
water, and there was thus no difficulty in extracting the
embryos.

§ Development of Nephridia.

Stage A.

The youngest embryo which I have is rather younger than
that figured on pl. xviii, fig. 51, of Wilson's memoir (No. 15).

It has hardly acquired a vermiform shape (see fig. 23), and
is 2 mm. in length, being, therefore, rather smaller than the
embryo of Allolobophora fœtida referred to. The
stomodæum had not yet joined the mesenteron, and I could
find no proctodæum, which here, as in Lumbricus, is a late
formation.

In this embryo the anterior half-dozen pairs of nephridia
were fully developed and no doubt functional. They are
distinctly paired structures, and lie on either side of the
gut or stomodæum as the case may be.

Each nephridium is furnished with a well-developed funnel

which is placed on the anterior side of the intersegmental
septum, behind which the nephridium lies.   The funnel is
ciliated, and the nephridium, where it communicates with it,
has a remarkably wide lumen, and is ciliated.   The funnel lies
close to the nerve-cord, but the external apertures of the
nephridia are more dorsal in position; they open in the neigh-
bourhood of the lateral setæ.

Fig. 21 represents a single nephridium, which happened to
be displayed in a fortunate section for nearly the whole of its
course.   The nephridia are present to the number of a single
pair per segment, but the anterior pair (the "mucous glands"
of the adult worm) occupy two segments.

These, however, like the following ones, have a single
funnel, which is placed on the anterior side of a septum (see
fig. 22), which seems to represent the septum dividing Segments
ii and iii.   In this region of the body, however, from the
earliest stage the septa have not the regular arrangement that
they have posteriorly.   The first pair of nephridia open
close to the stomodæal aperture; the funnels neces-
sarily lie behind this point, hence from the very
first this anterior pair of nephridia is anomalous,
and so far unlike those which follow.   It would be very
interesting to ascertain whether this difference holds good
from the first formation of the nephridia; unfortunately this
is one of the many questions in the embryology of Acantho-
drilus multiporus which I have no means of solving at
present.

Figs. 17 and 18 represent two successive sections which
illustrate the position of a nephridium.   In fig. 17 the external
aperture is seen, which lies dorso-laterally; in fig. 18 the
funnel is shown.   The first figure shows more of the anterior
extremity of the body, owing to the somewhat awkward
position in which the worm was embedded.   The developing
setæ of Segments ii and iii belonging to one of the ventral
pairs are seen on the left side (fig. 18); they fix the position of
the funnel which is seen to lie between them on the anterior side
of a septum.   The enormously distended cavity which lies

below the alimentary tract is the ventral blood-vessel; on the opposite side is the brain (*br.*). The stomodæum is easily recognisable from the mesenteron by the different character of its epithelium ; as far as I could ascertain the lumen of the stomodæum was not continuous with that of the mesenteron.

Whether the anterior two segments contain provisional nephridia or not, I am unable to say ; this is, unfortunately, another of the many questions in the embryology of Acantho-drilus for the solution of which I do not possess material. I am inclined, however, to think, as will be pointed out later, that the nephridia of the first two segments have no pre-cursors.

Stage B.

The size of embryos belonging to this stage is indicated in fig. 20 B. I have only had three embryos for study, one of which was slightly larger than the others, but not in a more advanced condition of development.

These embryos are completely vermiform in shape, but the anterior end is still considerably the thicker, and dwindles gradually towards the tail end.

Fig. 22 represents a section through the first four segments of an embryo belonging to this stage. The setæ are numbered consecutively, the first seta (*I*) corresponding, of course, to the 2nd segment. The first pair of nephridia seems to occupy three segments, but the irregular arrangement of the muscular bands attaching the pharynx (stomodæum) to the parietes make it a little difficult to be very certain upon the point. Both the funnel (*f.*) and the external pore (*N. p.*) were quite obvious at this stage ; the funnel lies, as before, in the 2nd or 3rd seg-ment. The external orifice of the nephridium is just within the stomodæum. There is, in fact, no particular change from the conditions characterising the last stage.

Stage C.'

Embryos of this stage measure rather more than half an inch in length ; they are, of course, completely vermiform in shape.

The nephridia are considerably advanced as compared with those of the earlier stages, but are still very far from showing the characteristics of those of the adult Acanthodrilus.

The first pair occupy the first four segments, which correspond to the extent of the stomodæal invagination; these segments are divided by irregularly running septa, and are only recognisable by the setæ.   Apparently, therefore, there is an important difference between this stage and the last in respect of the number of segments occupied by the "mucous gland."   This difference is, however, only apparent; it is caused by the concrescence of the "mucous gland" with the following nephridium.   I have carefully followed out the first and second pairs of nephridia, and I can nowhere find a break of continuity between them, such as is quite obvious in the two earlier stages.   The original distinctness of the two nephridia is still shown by the presence of two funnels, occupying precisely the position of the earlier stages.   I detected the external orifice belonging to the posterior nephridium; the whole organ also opens anteriorly as in the earlier stages, but in this stage much further within the buccal cavity.   Moreover the actual orifice is now formed by an outgrowth of a tube from the buccal cavity lined with columnar cells, continuous with those which form the lining of that cavity.   The shifting backwards of the aperture of the nephridium appears to be related to the shifting backwards of the cerebral ganglia and of the circumœsophageal commissures; the point of opening and the position of those commissures are close together, opposite to the setæ of the 2nd segment.   The first nephridium extends back beyond the third seta, and therefore appears to occupy four segments.

Fig. 16 represents a portion of two segments of an embryo at this stage, showing the body-wall, the ventral blood-vessel, and the intersegmental septa.   The nephridia belonging to the segment are not shown in their entirety; only the funnel and the proximal end of a tubule are indicated.   The rest of the nephridium forms a compact and dense coil of tubules, with very thin walls and a comparatively wide lumen.   Fig. 2 illus-

trates a portion of the nephridium (*n.*) of two consecutive seg-
ments; the anterior nephridium—that at the bottom of the
figure—is the end of the "mucous gland." *N* represents the
duct of the nephridium following this; the duct is of greater
calibre than the tubule from which it arises, and is always, as
is shown in the figure, made up of denser—at any rate more
darkly staining—protoplasm in the centre. Very constantly
radial strands of this denser layer of protoplasm immediately
surrounding the lumen were given off, reaching the periphery.
The funnels of the nephridia of worms belonging to stage C
have undergone a remarkable change, illustrated in figs. 16
and 19. The funnel is still more or less funnel-shaped—that
is to say, it contains a lumen; but the cells composing it have
lost their cilia, and have begun to proliferate, forming (see
fig. 19) several layers of cells. The section of the nephridium
following the funnel has no lumen. The nuclei of the cells are
arranged alternately, now on one side, now on the other.

Embryos just before hatching (Stage D).

I always found it possible to tell from an examination of
the cocoon whether the contained embryo was nearly or quite
ready to make its way out. After washing off the earth the
chitinous layers, particularly when the outer one had been
peeled off, were sufficiently transparent to show the principal
blood-vessels, and even the outline of the worm in parts.

At this stage the worm almost entirely fills the cocoon, there
being but little room to spare; what room there is, is occupied
by the remains of the albuminous fluid, which has undergone
no changes visible to the eye. This fact rendered it somewhat
difficult to extract the embryos without injury from the scissors
or knife employed in cutting through the cocoon.

The embryo, just before leaving the cocoon, measures about
two inches in length. It is of a milky-white colour, and cer-
tain of the principal blood-vessels show up very conspicuously.

The dorsal blood-vessels are usually visible from end to end
of the body, and they are separated by a very considerable in-
terval. Between them a third trunk may be frequently made

out; this is the supra-intestinal vessel. Anteriorly about four pairs of hearts can be seen, and the ventral vessel is quite evident, running throughout the whole length of the body.

The absence of any pigment in the integument is not so remarkable, since this species appears to have hardly any traces of integumental pigment at any time of its life.

The nephridia have acquired their definitive form, with many external apertures in each segment. The mucous glands form a coiled mass of tubules lying on each side of the pharynx and œsophagus; they are chiefly massed in the segment behind that which contains the pharynx: I presume, therefore, that, had I been able to examine a more complete series of embryos between these and those of stage C, a further fusion of the nephridia to form the mucous gland could have been traced. The aperture of this complex nephridial gland into the stomo-dæum has moved still further back, still retaining its relation to the circumœsophageal commissure, in front of which it opens, and beneath which it passes from the glandular coils. Fig. 9 shows the tube (*Nephr.*) which leads to the stomodæal orifice close to the latter and in front of the commissure (*N*). I mentioned, in describing the nephridia of stage C, that the external aperture of the second pair of nephridia, which fuse with the first pair to form the mucous gland, still persist. In stage D there are numerous apertures by which the mucous gland opens on to the exterior; these commence upon the very first segment of the body.

§ Anal Nephridia.

The adult Acanthodrilus multiporus is, as I have recently pointed out (9), provided with anal nephridia which open into the hindermost section of the gut; there are also in the same segment integumental nephridiopores, and the tubes leading to the latter could be traced into connection with the tubes leading to the pores that communicate with the interior of the rectum. In these segments there are, moreover, numerous cœlomic funnels, which seem to be wanting in the rest of the body of the adult, though, as I have already men-

tioned, they are present (a pair to each segment) in the embryo. The anal nephridia are a secondary development. In stage C there appeared to be no difference between the nephridia of the posterior segments of the body and those lying in front; but in older embryos they were quite distinguishable on account of their greater size : in the earliest stage at which I observed them the openings into the gut had been already formed, but they are less numerous than in the adult worm ; I counted three on each side, which seemed to me to be arranged symmetrically. In describing these anal nephridia I suggested that it would in all probability be found that they opened into the proctodæum. I believe now that the section of the gut into which the nephridia open is not proctodæum ; it can hardly, therefore, be supposed that their primitive opening was on to the outside of the body, and that the orifices were invaginated along with the proctodæum. These nephridia occupied in all five segments, not the last five of the body, but some little way in front of the end of the body. They are chiefly developed on the septa, beside which their ducts pass on the way to the enteric opening. On the other hand, ducts also lead to the body-wall, and open on to the exterior, as I have already pointed out for the adult. One point in the minute structure of the tube seems to be peculiar. I have not observed the same thing in nephridia from other parts of the body. This, which is illustrated in fig. 6, concerns the relations between the nephridial tubes and their peritoneal covering. Ordinarily, as in A and B, fig. 6, the peritoneal coat (p.) closely invests the nephridial tube (n.); but very frequently, as shown in C and D, the peritoneal coat was widely separated from the nephridial tube by a space containing a coagulable fluid, the presence of which is indicated in the drawing. The existence of this fluid shows that we have not to do here with an effect produced by a reagent. The nephridial tubes illustrated in the figure have not yet acquired a lumen.

§ Perivisceral Fluid and Corpuscles.

The cœlomic corpuscles of Annelids—particularly of the Oligochæta—have been so thoroughly investigated by Professor Kükenthal (17), that I have found little to add to his excellent descriptions.

Nevertheless there are some points connected with the numbers and condition of the corpuscles at different stages of development which are worth recording.

The corpuscles themselves may be, as Kükenthal has pointed out, of two kinds—(1) those with secreted granules, and (2) those without such granules.   Both kinds are furnished with a nucleus.

In the youngest stages (viz. A and B) only the small lymphoid cells without granules are present; these cells are very darkly stained by borax carmine.   Fig. 4 illustrates three such cells isolated by teasing up an embryo of stage B in glycerin, whose tissues had been fixed by osmic acid.   These cells show every possible condition of amœboid movement; in some cases, as shown in the figure referred to, they absolutely bristle with pseudopodia; at the other extreme are roundish or " bipolar " cells.   It seemed to me that the intersegmental septa were the principal stations at which these cells originated, of course from the peritoneal layers covering the septa.

In embryos of this age there was no fluid in the cœlomic cavities that could be recognised.   In stage D the body-cavity was almost completely filled with granular corpuscles, which represent a further development of the small non-granular cells (see fig. 3).

§ Larval Sense-organ.

None of the embryos of Acanthodrilus showed any ciliation over the general body surface; even the youngest embryo was apparently too far advanced in development to show any ciliation, with the exception of a patch at the anterior end, to which I shall refer immediately.   As this embryo is older than a Lumbricus embryo in which the ventral ciliated

band has disappeared (cf. Kowalevsky, 18, pl. vii, fig. 26), the fact is not remarkable. In Allolobophora, however (Vejdovsky, 1, pl. xvii, fig. 9; pl. xviii, fig. 9), the ciliation of the ventral surface persists later.

In fig. 18 of Pl. XXXI is represented a longitudinal section through the first few segments of the youngest embryo. Just above the stomodæum and in front of the cerebral ganglia is a group (S) of three or four cells bearing long cilia: as I have not been able to compare these cilia with those which probably, as in other genera, form a band along the ventral surface of the embryo, it is impossible to say if they differ in any way; but by comparing them with the figures given by the various authors who have studied the development of Lumbricus, Allurus, and other Oligochæta, I should imagine that they will prove to be much longer and stronger cilia. Furthermore, the epiblastic cells which bear them are specialised; they differ from the neighbouring cells in their greater size.

In several Oligochæta the ventral ciliated band extends on to the dorsal surface of the stomodæum—in Lumbricus trapezoides, for example. Vejdovsky also figures (1, Tab. xvi, fig. 4) cilia upon the dorsal lip of the stomodæal invagination in Allurus teträeder, and in an earlier embryo (Tab. xvi, figs. 5, 11) of Allolobophora fœtida. This embryonic condition is preserved in the adult Æolosoma and in Ctenodrilus (if we are to regard that worm as an Oligochæte, which cannot be safely done until its genitalia are known): in both these genera the ciliation is limited to the ventral surface as in the embryo Lumbricid, which is an interesting point of similarity. But in none of these cases is there any specialisation of the cells upon the prostomium which bear the cilia; if such a group of cells existed in Lumbricus as I figure in Acanthodrilus multiporus, it could hardly, I think, have been overlooked; so many competent observers have studied Lumbricus with all the improvements of modern methods, that so obvious a structural fact could not well have failed to be noticed by one or other of them. I can discover nothing in the plates of Kowalevsky, Kleinenberg, Vejdovsky, or

Wilson at all like the larval sense-organ of Acanthodrilus, and therefore feel almost certain that it does not exist in the worms investigated by those authors.

A sense-organ of this or any kind does not seem likely to be of the least use to any embryo which undergoes direct development within a cocoon, and is not hatched until mature. At the same time Wilson has described in the embryo of Allolobophora fœtida a structure which may, he thinks, have a sensory function. The ventral lip of the stomodæum in that worm becomes expanded and thickened, and is consti-tuted by a mass of elongate fusiform cells, which is strikingly like a taste bud. This body is compared to the larval sense-organs of Clepsine. As it is found in A. fœtida only, and not in L. communis and L. terrestris, where its presence is correlated with a tough and jelly-like albumen filling the cocoon, Wilson thinks that the group of cells in question plays a part in the digestion before absorption of this albumen; it is difficult, he thinks, to understand the presence of a sense-organ so highly developed in one worm and its absence from closely allied forms. This structure is figured (15, fig. 82) by Wilson and also by Vejdovsky (1, pl. xv, fig. 5) for the same species. In any case the position of this sense-organ or glandular organ is totally different from that of the larval sense-organ of Acanthodrilus; the one is ventral, the other dorsal. The sense-organ of Acanthodrilus can hardly be a digestive organ of any kind. Whatever it is, it is transitory; I could find no trace of it in later stages. I do not think, how-ever, that it is of any use to the larva; it is difficult to under-stand that any sense-organ could be, which appears and disappears at so early a period in the development of the embryo. This sense-organ appears to me to be com-parable to the larval sense-organ of the Chætopod larva and of other worm larvæ. Such larval sense-organs, as is well known, occur in many free-swimming larvæ; they are not limited to the Chætopoda Polychæta; the Pilidium of Nemertes, for example, possesses such an organ. There is therefore no need, supposing that my comparison be justified,

to bring forward this structure as further evidence of a close affinity between the Polychæta and the Oligochæta; but the fact does appear to favour the idea of, at any rate, the former existence of a free-swimming larval stage among the Oligochæta, though not necessarily to be found in any living genus. It is true that the development of very few genera of this family is known; but the formation of a cocoon is apparently so unusual that we may suppose it to be a very old characteristic of the group, possibly so old as to embrace the ancestors of both Oligochæta and Hirudinea. The enclosure of eggs within a cocoon does not, it may be admitted, seem favorable to the idea of a free-swimming larva. In only one existing group is there any evidence of a free-swimming Oligochætous larva. Lankester has described and figured (**19**, p. 642, pl. xlviii, fig. 4) the young of Acolosoma quaternarium, which is somewhat unlike the adult, and is more extensively ciliated. Vejdovsky (**2**, p. 165) has remarked upon the resemblance of this larva to that of a Chætopod.

There are several species of Acanthodrilus which are aquatic—for instance, "Mandane stagnalis" of Kinberg; but I do not wish to urge the possibility even of there being a free-swimming larva in any of these. All that I desire to point out is that, so far as we at present know, the development of the group Acanthodrilus is the only type in which the traces of a formerly existent (?) free-swimming stage are to be found. This is so far an argument for the conclusion that Acanthodrilus comes nearer to the ancestral Oligochæte than such a type as Lumbricus does; other facts appear to me to point unmistakably towards the same conclusion.

§ Stomodæum and Proctodæum.

In the youngest embryo the stomodæal invagination is already fully established, but it does not open into the mesenteron; the opening is formed in the next stage in an embryo hardly larger than the youngest, but the aperture of communication (see fig. 24) is narrow. The narrow aperture between the stomodæum and the mesenteron is still further reduced by a

fringe of long cilia shown in the figure referred to. These cilia belong to the endoderm cells, and are only developed upon the first five rows of cells or thereabouts; behind this point there is no ciliation at all, nor is the stomodæum ciliated. The ciliated cells at the neck of the mesenteron do not differ in any way from the other cells of the mesenteron. There is an abrupt break between the cells of the stomodæum and those belonging to the mesenteron, which is quite obvious even when the sections are examined with a low power; the stomodæal epithelium is more stained. The cells of the mesenteron are laden with spherules and are not stained, excepting, of course, their nuclei. The cells of the stomodæum are columnar, but much shorter than the cells of the mesenteron.

The second half of the stomodæum has a muscular invest-ment of fibres of some thickness; this extends for a very short distance on to the mesenteron, and gradually disappears. The peritoneal investment of the stomodæum is much thicker than that of the first part of the mesenteron. The stomodæum occupies the first four segments of the body.

The proctodæum is developed much later; it had not been formed in embryos of stage C. It is of much less extent, and in transverse sections (fig. 10) is seen (*p.*) to lie dorsally of the mesenteron.

§ Epidermis of Mature Embryo.

In embryos ready to leave the cocoon the epidermis contains some peculiar cells. Here and there in longitudinal sections through the anterior segments the epidermis appears, when examined under a low power, to be perforated; under a high power the arrangement of the epidermic cells at these points presents the following appearance. Above the perforation lies a large round-cell with a conspicuous nucleus, and below this cell is the cavity already spoken of. The cuticle is bulged out above the cell. There is no great regularity in the arrangement of these cells; they occur sometimes anteriorly upon the segment, sometimes posteriorly. The only struc-

tures known to me in an earthworm with which they may
possibly be compared are the peculiar integumental cells of
Urochæta; but these latter lie at the base of the epidermis
instead of at the summit as in Acanthodrilus.   One is
usually inclined ·to regard a modified epidermic cell as a
sense-organ, but there is no evidence that the bodies in
question have a sensory function.   On the other hand, they
cannot be compared with the gland-cells of the epidermis.
The cavity lying beneath each cell is very curious, and fits
with the possibility of their being dioptric media ; they are not,
however, specially transparent, judging at least from their
appearance when preserved.   At present I think the nature of
the cells must be left unsolved.

§ Gonads.

The development of the gonads in Lumbricus has been
studied by Bergh (11), who has made known some interesting
facts as to the number originally present.   The adult Lum-
bricus has two pairs of testes depending from the anterior
septum of Segments 10 and 11, and a single pair of ovaries
occupying a corresponding position in Segment 13.   In the
embryo, however, the 12th segment possesses a pair of gonads
also, which appear never to advance beyond a very rudimentary
stage.

I find in Acanthodrilus that there are originally four
pairs of gonads present, which are in Segments 10, 11, 12, and
13; but these, instead of being attached to the front wall of
their segment, as in Lumbricus, are attached to the poste-
rior wall.   This is the case with the adult worm, as I have
satisfied myself by the dissection of a large specimen.   Acan-
thodrilus annectens, therefore, is not the only example
known of this anomalous condition.   The gonad of the 12th
segment in Acanthodrilus is, however, at first as fully
developed as any of the others; it contains, as they do, large
germinal cells, with the peculiar nuclei which characterise
these cells.   These nuclei (fig. 14) are limited by a distinct
and darkly staining membrane, against which are closely

pressed a layer of·small darkly staining bodies, the nucleoli, which are thus arranged in a peripheral layer, and are not found in the interior of the nucleus.

The presence of four gonads in the embryo is interesting, inasmuch as one genus of Oligochæta has always two pairs of testes and two pairs of ovaries. I refer to Phreoryctes, where the gonads occupy the same segments as in the embryo Acanthodrilus. The presence of two pairs of egg-sacs in Perichæta aspergillum and other species is evidence in favour of the original presence in that worm also of two pairs of ovaries; and I have elsewhere sought to show that in Eudrilus there are also two pairs of ovaries corresponding to the two pairs of testes. In Lumbriculus it is possible that the same thing occurs, but the genitalia of this worm have not as yet been thoroughly described, though Professor Vejdovsky has promised us an account of them.

The gonads appear at a comparatively early stage in the development of the worm. I first observed them in stage C, but as they are in this stage of, comparatively speaking, large size, they must be apparent even earlier. I could not, however, detect them in stage B. It should be remarked, however, that this stage is evidently separated by a considerable gap from stage C, and I have nothing intermediate between the two.

In stage C the gonad (fig. 12) is placed close to one of the nephridial funnels in which the lumen still persists, and to a certain extent the cilia also; but the duct immediately connected with the funnel has lost the lumen which it possessed in stage B, and has become solid. The gonad is in each case situated ventrally to the funnel. It is formed by a rounded elevation of cells which closely resemble the peritoneal cells covering the septum. I cannot say anything about the condition of the cells forming the gonads, as the histological details were not very clear : this was due to the preservation of the specimen in saturated solution of picric acid, which does not appear to be a good reagent for the preservation of these embryos. It is almost unnecessary to say that the four

gonads were absolutely indistinguishable from each other in their shape and relations to the septum, body-wall, and nephridial funnel; neither were there any ascertainable differences in their minute structure.

In stage D the gonads have become much more evident, and, indeed, they hardly differ in minute structure from those of the newly hatched worm. Figs. 13 and 14 illustrate the gonad and a portion of the genital ducts. In fig. 14 the gonad is shown attached to the front surface of the septum (*spt.*), and a portion of the genital duct (*vas def.*) is seen behind the septum. The gonad is frayed out into a number of processes. In fig. 13, which is a few sections further forward, the commencement of the funnel of the generative duct is shown (*funn.*); the gonad is seen to be partly attached to the funnel. Fig. 15 represents a section through one of the gonads of another individual; the " protova " (*g. c.*) are peculiar in shape, being prolonged into a stalk; round these lie accessory cells (*p. c.*), which do not, I imagine, develop into sexual products.

§ Genital Ducts.

The development of the genital ducts in Lumbricus has been studied by Bergh (11), who has arrived at the following results, which I state for the purposes of comparison with Acanthodrilus.

Bergh's results in the main confirm those of Vejdovsky upon certain aquatic genera (2). The first part of the duct to appear is the funnel, but this is not developed until after the young worm has left the cocoon. Previously to this the gonads have been formed, but only nephridia are to be found in the genital segments. When the funnels do put in an appearance they are formed beside the nephridial funnel, but are perfectly distinct from it though in actual contact (Bergh, 11, pl. xxi, figs. 19, 21). The ciliated epithelium of the nephridial funnel is figured by Bergh as totally distinct from the, at first, non-ciliated epithelium of the genital ducts. Although no actual observations were made, Bergh is inclined to confirm Vejdov-

sky's statement that the ducts themselves are formed as out-
growths from the funnel.

In Acanthodrilus multiporus I have found certain points
of agreement with these results, but also important points of
difference.

In the first place the genital ducts appear much
earlier in Acanthodrilus than in Lumbricus. I have
first met with the funnels in embryos of rather more than half
an inch in length (stage C). They were prominently developed
in embryos of an inch in length (still some way from being
ready to emerge from the cocoon). In the recently hatched
worm the funnels are ciliated, and are furnished with a con-
siderable length of duct. Fig. 1 represents two funnels, those
of the 10th and 11th segments, in an embryo of stage D. In
this stage I should observe that the funnels correspond-
ing to the four gonads are equally well developed,
and it would be impossible to predict that that of the 12th
segment was destined to atrophy subsequently. I could,
furthermore, detect no difference between the ovi-
ducal and spermiducal funnels, such as Bergh mentions
and illustrates. The funnels show no signs of ciliation, but
are somewhat folded. The gonad, as already mentioned, is in
contact with its funnel, and there is a large blood-vessel to the
upper side; the cells of the funnel could be easily distinguished
from those of the neighbouring regions by the large size of
their closely crowded nuclei. The septum was greatly
thickened in the neighbourhood of the funnel, but thin just
below the funnel itself; at this point it is traversed by a solid
cord of cells with large nuclei, surrounded by peritoneal cells
with smaller nuclei. The continuity of this rod with
the funnel was quite plain; it is the commence-
ment of sperm-duct or oviduct, as the case may be.
This rod passes obliquely and without any flexure to the body-
wall; there it is continuous with a coiled tube in
which a lumen could be in parts detected; the whole
structure, in fact, bears an unmistakable resem-
blance to a nephridial tubule. In the figure referred to

it will be noticed that in the case of the 10th segment the straight rod is prolonged for a very short distance beyond the point where it gives off the coiled tubule *(nphr.)*; this appears to indicate a commencing separation between the nephridium and that part of it which is metamorphosed into sperm-duct. Bergh, as well as Vejdovsky, has noticed that the commencing vas deferens is a solid structure which only later acquires a lumen; it might be imagined, therefore, that my supposed nephridium is nothing more than the solid condition of the vas deferens or oviduct; but the connection with it of a coiled tube possessing an intra-cellular lumen and agreeing point for point with the nephridia elsewhere negatives this supposition.

In the 7th, 8th, and 9th segments there are small rudimentary funnels attached to the posterior wall of their segments. These funnels are not ciliated, and consist of but few cells—indeed, the expression " funnel " is hardly applicable to them; each is, however, continuous with a tube passing through the mesentery which is identical in appearance with the tube connected with the funnels of Segments 10—13. This tube is also solid; it has a thick coating of peritoneal cells, and ultimately passes into a coiled tube with an intracellular lumen. In the 9th segment the straight part of the tube (see fig. 11) gives off a branch which perforates the body-wall, and nearly, but not quite, reaches the exterior (I traced it into the circular muscular layer). In the segments lying behind the 13th the same arrangement could be observed, but though the septa were perforated by nephridial tubules there was nothing that could be called a funnel upon the opposite side, only a few cells presenting an irregular arrangement.

It seems to me impossible to avoid the conclusion that these structures correspond from segment to segment, and that therefore the funnels of the sperm-ducts and oviducts, as well as the commencement of the ducts themselves, are formed out of a section of the nephridia of their segments. In describing the development of the nephridia I have mentioned that the nephridial funnels and the tube which is immediately connected with them very soon become

solid and lose their cilia ; this condition is seen in the newly
hatched worm, where, however, there is hardly a trace of the
funnels. It is, therefore, perhaps surprising to find that four
out of these funnels commence to grow again and a second
time acquire cilia, serving as the funnels of the genital ducts in
the adult worm. This led me to suspect that the funnels
might possibly be new structures, and that the original nephri-
dial funnel might be present and connected with the nephridial
tubule which I have described as the commencing sperm-duct or
oviduct. I should remark, however, first of all that the loss of
cilia in a cell (Protozoa) is not necessarily a prelude to de-
generation ; on the contrary, it is sometimes followed by a
specially marked activity of division. Besides, the solidifica-
tion of that portion of the nephridial duct which is connected
with it might be looked upon in the same light, as it is un-
doubtedly at first furnished with a wide lumen which is
ciliated. But the outgrowth of a tube from this, which I
have figured (fig. 11) as nearly reaching the exterior, is
hardly an indication that the structure in question is really
degenerating. There seems to be no doubt, however, that,
with the exception of those in Segments 10—13, the original
nephridial funnels do ultimately disappear, or are represented
by the merest traces.

On the hypothesis that the structures which I have identified
with the nephridial funnels of Segments 10, 11, 12, and 13,
are really new structures, and not the persistent funnels, some
traces of the latter should be forthcoming. I could not, how-
ever, detect any such traces. Yielding this point for the
moment, it would be sufficient as an argument for the time
being if there were no clear connection between the large
funnels of the genital segments and the nephridial tubule ;
this connection, however, is quite unmistakable.

It seems, therefore, difficult to explain the connection of the
funnels of the genital ducts with nephridia except on the hypo-
thesis, supported by other facts, that the funnels in question
are the persistent and enlarged nephridial funnels. I do
admit, however, that this is by no means necessarily a proof

that the distal sections of the genital ducts are formed out of these nephridia.

There are some facts, nevertheless, which are in favour of this view.

In the young worm that has just escaped from the cocoon the genital ducts are more advanced in development, though still imperfect. This statement, however, only applies to the ducts of the 10th, 11th, and 13th segments; those of the 12th, though not much smaller than they were during the last stage, are, by comparison with those of the remaining genital segments, rudimentary.

The latter have become ciliated. The epithelium is here, arranged several layers deep. Each funnel communicates with a short duct, which has acquired a lumen plainly intra-cellular; the duct passes somewhat obliquely to the body-wall, but without any windings, and penetrates for a short distance into the longitudinal muscular layer. It is particularly noticeable that at this stage it is impossible to distinguish, except by their position, the sperm-ducts from the oviducts.

Where the sperm-duct or oviduct comes into contact with the parietal peritoneum it is in close relation with a part of the nephridium; but, as far as I can make out, there is no actual communication between nephridium and genital duct. The most careful search failed to reveal any nephridial funnel in Segments 10, 11, 12, and 13, other than the genital funnels, nor did the nephridial tubules in these segments approach very nearly to the septum, as if there were such a funnel present which I had overlooked.

On the other hand, in the 8th and 9th segments, at any rate, a nephridial tubule (solid, with no lumen) passed up from the body-wall to the septum, and, perforating it, terminated on the opposite side in a group of cells, which is no doubt a rudimentary funnel. Careful measurements showed that the position of this nephridial tube and funnel in the 8th and 9th segments is precisely the same as that occupied by the genital duct and funnel in each of the four following segments. This looks very much as if the solid rod communicating with the

genital funnels in the earlier stage were really metamorphosed into the proximal part of the genital duct; at any rate, there was no trace in the young worm of these solid rods, except in the segments in front of and behind the genital segments, other than the straight genital duct.

The temporary occlusion of the lumen during the development of the vasa deferentia and oviducts marks the change in their function from excretory tube to efferent ducts of the genital products. It is, however, also shown that the nephridial tubes are destined to undergo this physiological change, though in a less degree; in a less degree because the funnel, which possesses an obvious lumen in the youngest stages (Pl. XXXI, fig. 18), never seems to recur to this condition, any more than does the immediately following section of the tubule. It has appeared to me, however, that in the adult worm part of the nephridia had regained the lumen temporarily obliterated. In any case the occlusion and reopening of the lumen does occur with the genital ducts. This phenomenon seems to be connected with the growth of the tubes; although remarkable, it is not without parallel. Balfour (3) discovered that in Elasmobranchs the œsophagus during development became solid, and then reacquired a lumen. "The solidification of the œsophagus," he remarks (3, p. 218), "belongs to a class of facts which are curious rather than interesting, and are mainly worth recording from the possibility of their turning out to have some unsuspected morphological bearings." More recently (16) Dr. Milnes-Marshall and Mr. Bles have observed precisely the same phenomenon in the developing frog. The solid œsophagus is "a constant feature in tadpoles of from $7\frac{1}{2}$ mm. to about $10\frac{1}{2}$ mm. in length."

These observers have not referred to a paper by de Meuron (14), in which a similar temporary closure of the œsophagus is described in the chick.[1]

[1] I am indebted for this reference to Professor Howes.

§ Homology between Genital Ducts and Nephridia in the Oligochæta.

The homology between genital ducts and nephridia has been a question of interest in Annelid morphology for some years. But the evidence in favour of the homology, as regards the Oligochæta, has hitherto rested upon comparatively few facts; indeed, some of the arguments that have been used were based upon inaccurate observation. Claparède pointed to the absence of nephridia in the genital segments of the aquatic genera as evidence that the genital ducts were the slightly altered nephridia. Vejdovsky, however, discovered that the nephridia are at first present in the genital segments of these worms, but that on the maturation of the genital products and the development of special efferent ducts they disappear. Some years ago Lankester (20) put forward the ingenious suggestion that in earthworms (Lumbricus was at that time the only type known anatomically) the genital ducts represented the sole remaining traces of a set of ventral nephridia corresponding to the persistent dorsal nephridia. This view subsequently received a good deal of support from the researches of Perrier into the anatomy of earthworms. Perrier discovered that in some genera the dorsal set of nephridia were retained while the apertures of the genital organs were related to the ventral setæ, showing, therefore, an interesting reversal of the conditions in Lumbricus. Later (6) Perrier found that in Plutellus the nephridia actually alternated from segment to segment, which of course was a further confirmation of the view that originally two pairs of nephridia existed in each segment of the body in earthworms. Mr. Benham's discovery (4) of a form—Brachydrilus—actually possessing two pairs of nephridia in each segment would have clinched the matter had it been made a little earlier, but would at the same time have necessitated some alteration in the hypothesis as formulated by Lankester, for in Brachydrilus the genital segments also contain two pairs of

nephridia. However, my own discovery of numerous external pores in each segment of Acanthodrilus multiporus did away with some of the difficulties which had been raised by Perrier. The whole subject has been recently treated in an exhaustive fashion by Dr. Eisig in his magnificent treatise upon the anatomy and physiology of the Capitellidæ; his own observations upon the Capitellidæ led him to confirm the view that nephridia and genital ducts in the Oligochæta are homologous. As I disagree with Dr. Eisig's view that the Capitellidæ are near akin to the Oligochæta, I should regard his very important observations as only furnishing an argument from analogy comparable to Mr. Sedgwick's discovery of the complete homology between the genital ducts and nephridia in Peripatus.

The study of the embryology of Lumbricus has thrown considerable doubts upon the justice of the view that the genital ducts have a relation to the nephridia. Dr. Bergh, who is one of the latest investigators of the group, has not positively denied any such homology (except in the case of the spermathecæ), but his observations have shown the complete independence in development of the sperm-ducts and oviducts from the nephridia. The former arise in the first place at a considerably later date than the latter, and have no relation to them; the septa which bear the funnels of the reproductive ducts are also perforated by a nephridium ending in the usual fashion in a funnel. Dr. Bergh, however, admits that the proved presence of more than a single pair of nephridia in a single segment of Annelids does away with the force of any arguments which his facts might be supposed to lend against the view that the genital ducts are modified nephridia.

Dr. Otto Lehmann (27), who has lately reinvestigated the question from the point of view of the development of Lumbricus and Allolobophora, entirely confirms Bergh as to the perfect independence of the vasa deferentia and oviducts from nephridia; but he states that the nephridia of the genital segments become degenerate on the appearance of the vasa

deferentia (p. 332: "Hier finden sich beim jungen Tiere in den Genitalsegmenten aufangs nur Segmentalorgane, die mit der Entwickelung der Geschlechtsorgane degenerieren "). This is a matter that requires confirmation, since Bergh distinctly states that there is no such degeneration. Lehmann gives no figures of the development of these organs. The paper concludes with a brief summary of the arguments for and against the view that the genital ducts and nephridia are corresponding structures. The arguments against lose some of their weight by reason of the statement that no instance is known of the presence of numerous nephridia in a single segment in the Oligochæta (!). This paper was published two years after I found that Acanthodrilus multiporus is furnished with multiple nephridia, and communicated the discovery to the Royal Society.

Dr. Benham, in discussing this question (5), also admits the probable homology, which he regards as fairly evident in the case of the oviduct. As to the sperm-duct, it is suggested that the modification which the nephridium has undergone is—(a) a fusion of a series of nephridia, (b) a disappearance of a part of nephridia, (c) a shifting of the position of the pore. " In the somites in which the ciliated rosettes are, the external extremity of the nephridium has disappeared; in the somites carrying the male pore the funnel region of the nephridium is absent, whilst in the intervening somites both these regions have aborted, and a fusion of these various parts has taken place to form the more or less elongated duct."

Professor W. B. Spencer (22) writes decidedly against any homology between genital ducts and nephridia. The first objection which he puts forward is the inter-cellular duct of the vasa deferentia and oviducts, and the intra-cellular duct of the nephridia. This objection would of course apply equally well to the homology between the nephridia of Oligochæta and those of many Polychæta; and, as a matter of fact, I show in this paper that an intra-cellular duct is, in the case of oviduct and vas deferens, actually converted into an intercellular duct. Another difficulty is " the presence of the perfectly

distinct ducts running side by side in Megascolides."
Another objection urged by Mr. Spencer is one which I also
urged myself; it is that in Perichæta, which has a primitive
excretory system, the genital ducts are fully as specialised as
in other types where the nephridia are greatly modified.   He
concludes that in all Terricolæ the genital ducts have no con-
nection with the nephridia, and are not to be regarded as
nephridia specially modified to serve the purpose of conveying
genital products to the exterior.

M. Perrier had found that in Anteus the sperm-ducts are
actually represented by nephridia, a condition analogous to
that of Æolosoma to be mentioned immediately.   But in view
of the possibility that in Anteus the sperm-ducts run em-
bedded in the body-wall as in Acanthodrilus, and may
therefore have been invisible on dissection only, it must be
considered that this instance requires further study before the
facts can be definitely accepted as a contribution to the ques-
tion of the homologies between genital ducts and nephridia.
Besides, in Rhinodrilus, which comes extremely close to
Anteus, if it be not actually congeneric, vasa deferentia of the
ordinary type are unquestionably present.

An important contribution to the question of the homolo-
gies of the genital duct with the nephridia is contained in a
memoir upon the genital organs of Æolosoma by Dr. Štolc
(23).   As Dr. Štolc has favoured me with an English abstract
of his paper I give a fairly full account of it, as there must be
comparatively few persons who can read the original.

The testis is situated in the 5th segment, the ovary in
the 6th; the gonads appear to be unpaired, and are situated
between the ventral blood-vessel and the ventral body-wall.
The spermathecæ vary in number from one pair to three,
and are in Segments 3, 4, and 5.   There is a tendency for
only one spermatheca in each of these segments to be deve-
loped.   The clitellum is developed only on the ventral side
of the sexual segments.   On the median ventral surface of the
6th segment is the unpaired oviducal pore; as a pair of
nephridia also exist in this segment, it is not plain that the

pore represents the rudiment of nephridia. There are no special sperm-ducts present; only the nephridia of the 6th segment seem to be slightlye nlarged. Dr. Štolc observed the spermatozoa to pass out of the body by all the nephridia, especially by those of the 6th segment, which are figured in his paper as slightly different from the rest. During the sexual period the nephridia in certain segments disappear either wholly or in part. There appears to be no formation of genital setæ; these at least are not figured.

M. Roule has discovered something of the same kind in Enchytræoides minimus (26). In this worm the first eight segments contain no nephridia at any time; those of the 9th, 10th, and 11th segments appearing only to disappear when the sexual organs are developed. The 12th segment possesses no nephridia, and it is not until much later that the sperm-ducts arise where the nephridia should have been; they are not preceded by nephridia, but may be regarded perhaps as equivalent to a pair of nephridia somewhat delayed in their development. Inasmuch, however, as there is no conversion of undoubted nephridia into undoubted genital ducts, the homology in this case could only be regarded as probable.

My own facts, which have been described in some detail above, seem to show that in Acanthodrilus multiporus, at any rate, the funnels and a portion of the actual ducts themselves of the vasa deferentia and oviducts are formed out of the nephridia. Balfour's suggestion ('Comp. Embr.,' vol. ii, p. 617) that "in the generative segments of the Oligochæta the excretory organs had at first both an excretory and a generative function, and that, as a secondary result of this double function, each of them has become split into two parts, a generative and an excretory," is thus confirmed in a way, which is to me most unexpected. We have in each segment to begin with a single pair of nephridia; in the genital segments a portion of these nephridia is used to form the genital ducts, the rest retaining its excretory function.

I have not had material which permitted me to trace the growth of the vasa deferentia to their point of opening on to

the exterior in Segment 18.   I imagine, however, that there is little doubt but that they continue to grow backward until this point is reached.

I do not think that Benham's suggestion of a fusion with portions of nephridia lying in Segments 12—18 will prove to be correct.   In any case I have seen no evidence of the commencement of any such process, which ought to be apparent in my latest stages; on the other hand, I have evidence as to the growth of the blind end of the developing sperm-duct into the body-wall for a certain distance.

It is important to remember that this subdivision of the excretory organ into a genital and excretory portion does not commence until after the excretory organ has acquired more or less the complicated structure that it has in the adult; the distinctively paired character of the embryonic nephridia has been to some extent lost by the development of numerous external pores in each segment. This fact must be duly borne in mind in attempting to account for the apparent differences in the development of the genital ducts in Lumbricus, where all observers agree that there is no actual connection with the nephridia.   I have described in Perichæta the existence in each segment not only of numerous nephridiopores, but of numerous funnels also. Acanthodrilus multiporus agrees with Perichæta in having numerous nephridiopores in each segment, not so numerous perhaps as in Perichæta, but still considerably more than an opening to each seta; but I have never succeeded in finding a corresponding number of funnels except in the case of the " anal nephridia " described above (see also No. 9); the persistent rudiment of the larval funnels is all that appears to exist in the anterior segments of the body, and even that may sometimes vanish.   I am inclined, therefore, to think that Spencer was after all right in believing the acquisition of numerous funnels to be secondary, and to be derived from a condition where there were no funnels.

Now it seems impossible to doubt that the genital ducts in Acanthodrilus are perfectly homologous with those of

Lumbricus; the converse supposition is incredible, although the development appears to be so different. In Lumbricus the pronephridia become the persistent nephridia after but little change; there is no "flame-cell," as in Rhynchelmis (Vejdovsky); in Acanthodrilus multiporus the pronephridia are converted into the nephridia by the degeneration of the funnel and by the multiplication of the external pores; it is after this has occurred that the genital ducts are split off. I can only reconcile this mode of development with that shown in Lumbricus by the supposition that the persistent nephridia in that type are not the exact equivalents of the paired nephridia of the young Acanthodrilus, but that the breaking up of the nephridium is slurred over, only showing traces of its former existence in the funnels and the proximal portion of the genital ducts. In this case the apparent simplicity of the nephridia in Lumbricus, and their apparent correspondence with an early stage in the development of the nephridia of Acanthodrilus will be delusive. If, on the other hand, it be proved that the nephridia of Lumbricus have never advanced beyond the stage which characterises the embryo Acanthodrilus, some explanation will have to be offered of the different modes of development of the genital ducts in the two types.

The whole matter is evidently not ripe for solution at present, and the problems involved grow more difficult as fresh facts are accumulated.

§ Remarks upon the Nephridia in the Oligochæta.

The facts recorded in this paper have an obvious bearing upon the question of the evolution of the excretory organs in the Oligochæta. But before discussing how far these facts affect current theories upon the matter, it will be useful to clear the ground by briefly describing the structure and development of the nephridia in other Oligochæta.

Lumbricus and Allolobophora.—We owe our knowledge of the development of the excretory system in these types principally to Kleinenberg (24), Vejdovsky (1, 2), Bergh (12),

and Wilson (15).   I leave out of consideration the larval ex-
cretory organs, which consist of epiblastic cells in the neigh-
bourhood of the mouth, known as "Schluckzellen," for they
appear to have no relation to the excretory system of the
adult.

In the young embryo there are a pair of provisional excretory
organs, " pronephridia " they have been termed by Vejdovsky.
They have also been termed the head kidney.   These organs
appear in very young larvæ (younger than the earliest stage
of Acanthodrilus described by me) as a pair of delicate
ciliated tubes lying on the dorso-lateral walls of the archen-
teron, and, according to Wilson, who could find neither ex-
ternal nor internal orifice, actually embedded in the walls of
the archenteron.   Lehmann (21) found no aperture into the
cœlom ; the canal runs along nearly the whole length of the
very young embryo, and opens by a pore at about the middle
of its length.   In later stages only the anterior portion appears
to be present ; it runs from the head-cavity backwards and
opens on to the 4th segment ; in this embryo were three pairs
of definitive nephridia.   The head kidney runs in the body-
cavity ; anteriorly it terminates in a single large cell traversed
by several canals.

Vejdovsky's account is different ; according to him the aper-
ture is anterior in position, and lies (cf. his tab. xix, fig. 15,
pn.) just behind the brain upon the 1st segment of the body ;
therefore it occupies two segments, and the permanent
nephridia, which develop before the pronephridium disappears,
only commence in the 3rd segment.   The position of the ex-
ternal pore, it should be observed, does not correspond with
that of the permanent nephridia.

In Rhynchelmis, again, there is a pronephridium of the
same kind on each side of the body, i. e. a single pair which
occupy the first two or three segments ; these, however, are
figured as opening below and to the side of the cerebral ganglia
(cf. Vejdovsky's tab. xii, figs. 12, 13, 15, *pr.* or *pn.*).

In Criodrilus Bergh (12) finds that the pronephridia open
posteriorly on the sides of the body ; they end blindly in front

in the head-cavity. Otherwise their structure appears to be that of the pronephridia of Lumbricus and the other types studied.

The differences between Criodrilus and the other types are remarkable, and not at all easy to understand.

But, apart from this genus, there is considerable evidence in favour of regarding the pronephridia as homologous (homo-dynamous) with the permanent nephridia. The permanent nephridia only commence after the pronephridia. The difference in form of the pronephridia may be correlated with their early appearance, and with the needs of the larva. The fact that they occupy two segments is more puzzling. Vejdovsky explains it for Rhynchelmis (1, p. 290) by the incomplete formation of the septa at the date of the appearance of the pronephridia; they are thus able to extend further back, being uninter-fered with by the formation of the intersegmental partitions.

In the aquatic Oligochæta—in many of them, at least—a number of pairs of nephridia after the first pair also disappear, which is further evidence for regarding the whole series of nephridia, including the first pair, as homodynamous.

In Rhynchelmis the pronephridial stage is marked by the formation of a closed cell with a single flagellum; this after-wards develops into the funnel; there is no such change in Lum-bricus. The pronephridia are directly converted into the permanent nephridia.

The correspondence between the pronephridia of the 1st segment ("head kidney") and the succeeding nephridia is strengthened by the observations recorded in the present con-tribution. In the youngest embryo at my disposal a pair of nephridia lie in the first two segments, and open on to the exterior just within the mouth-cavity. These nephridia do not degenerate as development advances, but become more com-plicated, and remain as the mucous glands. Now the embryo in which these nephridia are in the simplest stage of develop-ment, resembling in almost every particular the nephridia which follow, is apparently not older than embryos of Lumbricus with functional head kidneys. In both cases, it

may be remarked, the nephridia occupy two segments; but the position of the funnel, as well as of the external pore, is different. In the development of Acanthodrilus multiporus I have pointed out that the apertures of the mucous glands gradually shift backwards with the brain until they are placed well within the buccal cavity, instead of only just within it : it is quite possible that in an earlier stage still they are outside of it. If the aperture belongs to the prostomium it is perhaps inevitable that with the ingrowth of the stomodæum they should come ultimately to lie within the buccal cavity. The prostomium of these embryos, as of the embryo Lumbricus, is inconspicuous ; but fig. 22 looks very much as if the aperture of the first pair of nephridia is in reality upon the prostomium. In this case the apparent difference in position between the nephridiopores of the first pair and of those which follow can be understood.

I think that there can be no doubt as to the homology between these nephridia and the provisional first pair of Lumbricus; and they are certainly not different from the nephridia which follow, except that they occupy two segments. I have referred to the difficulty of comparing the first pair of pronephridia to those which follow, by reason of the fact that they occupy two segments in Lumbricus; and also to Vejdovsky's suggestion that they are able to extend backwards through the incompleteness of the septa. The same explanation will suit Acanthodrilus perfectly well, so far as these facts are concerned.

Later on a complication ensues in the fusion of the first and second nephridia, the compound organ having two funnels.[1]

[1] In connection with this I may refer to the mucous gland of Urochæta; I described that organ as extending through several segments (which Perrier first pointed out), and as furnished with several funnels. Dr. Rosa has lately pointed out (25) certain discrepancies between my descriptions and figures. On again looking into the matter I find that Rosa is right, and that the mucous gland opens on to the anterior margin of the first setigerous segment ; and that an interval of a segment occurs between the pore of the mucous gland and that of the first nephridium, i. e. the latter is upon the third setigerous

The connection thus formed between the first and second nephridia recalls the statements of Hatchek with regard to the development of the nephridia in Polygordius.   I have lately pointed out that in a Eudrilid Libyodrilus (10) such a connection is soon established between successive nephridia; but in all these cases the paired nephridia are developed first, and the connection is brought about later.   There are, however, plenty of analogies which might support the view that there has been an acceleration in the development of the nephridia, the connecting duct being developed later and later until it ceased to appear at all.

However, it is in any case evident that the "pepto-nephridia" of Acanthodrilus are compound structures representing the two pairs of nephridia of the first three segments. It is quite possible that the corresponding organs in other earthworms have a similar history.   It is very noticeable that in earthworms which have no pepto-nephridium the nephridia do not commence before the third segment;[1] in Lumbricus this is the case, and in Urobenus, Acanthodrilus, and Rhinodrilus.   Microchæta appears to be an exception; but the segmentation of this form is not yet definitely made out.

In these worms, therefore, I should expect to find a "head kidney" lying in the first two segments of the larva, while in forms which have a pepto-nephridium I should expect that this organ will prove to be a permanent "head kidney" plus the following one or two nephridia.

The next important fact of general interest to be noticed about the development of the nephridia in this Acanthodrilus is their paired condition in the embryo.

The facts that I have been able to make out in the ontogeny

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segment.   Dr. Rosa has suggested that I have accidentally referred to the mucous gland a funnel which should belong to the following nephridium.   I would point out, in reply, that it is very possible that the development of the first pair of nephridia in Urochæta is like that of Acanthodrilus; in this case the existence of more than one funnel would be not surprising.

[1] Later, of course, in such forms as Pontodrilus, but never before that segment.

of the nephridia are apparently at variance with the views which I have up to the present maintained with regard to the phylogenetic development of these organs in the Oligochæta.

There can be no possibility of doubt that in Acanthodrilus multiporus the embryo is at first furnished with paired nephridia—one pair to each segment,—with the funnels opening into the segment in front of that in which the main part of the nephridium lies. This seems to indicate that Oligochæta with paired nephridia are in this respect more primitive than such forms as Acanthodrilus multiporus and Perichæta, where the nephridia are furnished with numerous irregularly arranged external pores.

I suggested originally that the diffuse condition of the nephridia in the latter types was the more primitive, and that the paired arrangement could be derived from this by reduction. Professor Spencer strengthened this view, though differing from myself in certain details. Dr. W. B. Benham has also argued in favour of the primitive nature of the excretory organs in Perichæta. Others have, however, declined to accept this theory of the evolution of the excretory organs in the Oligochæta. Dr. Eisig has treated the question at great length in his work upon the Capitellidæ, naturally basing his arguments principally upon the Capitellidæ themselves. These worms were the first in which the presence of many nephridia in a single segment was made known (by Dr. Eisig). Since the many nephridia per segment of the adult worm are preceded by a single pair in each segment, it appears reasonable to conclude that the former is the more primitive condition.

Dr. R. S. Bergh, who has recently contributed a series of valuable memoirs to the settlement of these questions, has nothing to say in favour of the position taken up by Spencer and myself. Indeed, the facts in the development of Lumbricus, which he has done so much towards elucidating, would hardly allow him to be of our opinion. Dr. Bergh begs to be excused from refuting all the arguments used by us, for the reason that they carry their own refutation. In so far as concerns the longitudinal duct of Polygordius

and Lumbricus Dr. Bergh is very possibly right, but I must object to his curt dismissal of the arguments to be derived from the " network " of Perichæta with the remark, " Gewisse anatomische Verhältnisse (Existenz eines 'nephridialen Netzwerkes' bei Perichæta und bei einigen anderen hochdifferenzirten Regenwürmern), die ohne Weiteres, als " ursprünglich Einrichtungen gedeutet werden." In the first place, " einigen " ought to be replaced by " vielen ;" and in the second place, to state that Perichæta and those other forms in which the " plectonephric " condition occurs are highly differentiated is, at least, begging the question. Finally, neither Spencer nor I assumed the primitive character of the nephridia in these types " ohne Weiteres."

At the same time I freely admit that the observations recorded in the present paper, added to those of Bergh, Kleinenberg, and Wilson, have shaken considerably the position which I have taken up in regard to the phylogenetic development of the nephridia in the Oligochæta.

They seem to show that paired nephridia in this group are earlier than the irregular network. It must be remembered, however, that the permanent nephridia of Lumbricus cannot be the precise equivalents of the nephridia of the embryo Acanthodrilus; the reason for this is that the permanent nephridia of Lumbricus are developed out of the pronephridia. It is true that in Lumbricus there appears to be no break in continuity ; the pronephridia are converted into nephridia by a gradual series of changes. In Rhynchelmis, however, the pronephridial stage is marked by the single " flame-cell " which afterwards, by division, develops into the funnel. In Acanthodrilus there is a break between the pronephridia and the permanent nephridia; this is marked not only by the disappearance of the funnels, but also by the occlusion of the lumen of the tubules. This would seem to imply a temporary cessation of function or at least an alteration of function in the nephridia.

It is interesting to notice that the enormous development of perivisceral corpuscles loaded with secreted granules occurs

after the early stages in which the funnel is present and functional; whether there is any connection between these two facts I do not know. To a certain extent, therefore, the nephridial system of the adult worm is a new formation, for which, however, the nephridia of the embryo furnish the material. It is not a simple increase in size and complication of structure which converts the nephridia of the embryo into those of the adult. There is first of all a temporary cessation of function (?) in a part of the nephridium—the portion nearest to the funnel—which is produced by the disappearance of the lumen ; this part of the nephridium, as well as other parts, then grows actively, and a fresh series of apertures to the exterior are formed. There is quite as much change from the nephridia of the earlier stages to those of later stages as from the " pronephridia " (Vejdovsky, 1) of Lumbricus to the definitive nephridia. I have pointed out in this paper that the mode of development of the genital ducts in Acanthodrilus, as compared with Lumbricus, seems to show that the paired nephridia of the larval Acanthodrilus cannot be regarded as the exact equivalents of the permanent nephridia of Lumbricus. It seems, therefore, still possible to believe that, as regards earthworms at any rate, a more diffuse arrangement of the nephridia has preceded the paired regular arrangement ; at the same time it appears to be necessary to assume that originally the paired arrangement existed, and that the diffuse condition of the nephridia was a subsequent modification of this. But in my opinion it must still be proved that the paired nephridia of Eudrilus, and other earthworms whose development is unknown, are homologous with the paired nephridia of the larval Acanthodrilus.

§ Summary of the more Important New Facts contained in this Paper.

(1) Nephridia.—In the youngest embryos these are paired tubes opening on to the exterior by the lateral setæ, and each

provided with a funnel which opens at the segment in front. They are present in all the segments of the body, but there is only one pair of nephridia to the first two segments. These latter open on to the exterior at the commencement of the stomodæum. Later the second pair of nephridia, and afterwards one or two other pairs, become connected with the first pair, and collectively constitute the "mucous gland;" the stomodæal opening of this gland has moved further within the stomodæum. The funnels of the nephridia, except those belonging to Segments 11—14, become rudimentary, and numerous secondary external apertures are formed. The anal nephridia are a comparatively late formation; they appear to open into the mesenteron, not into the proctodæum.

(2) Gonads.—There are four pairs of gonads, which up to a certain point develop equally; later the gonad of Segment 12 degenerates.

(3) Genital Ducts.—Four pairs of genital ducts are developed; they are formed out of the nephridial funnels and a short section of the following tube : the genital ducts belonging to the 12th segment degenerate.

(4) The young embryo is furnished with an unpaired sense-organ consisting of a few large ciliated epidermic cells to one side of the stomodæal aperture; this is not recognisable in later stages.

(5) In the epidermis of the very advanced embryo there are peculiar cells which may possibly be sense-organs; the cells lie immediately beneath the cuticle, and are separated by a space from the basal membrane of the epidermis.

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EXPLANATION OF PLATES XXX and XXXI,

Illustrating Mr. Frank E. Beddard's paper, "Researches into the Embryology of the Oligochæta." No. I. "On Certain Points in the Development of Acanthodrilus multiporus."

FIG. 1.—Longitudinal section through a portion of two genital segments of an advanced embryo.

FIG. 2.—Longitudinal section through part of Segments 2 and 3 of an embryo of "Stage C." *n*. Nephridia. *N*. Duct of posterior (upper in the figure) nephridium. *Ep*. Epidermis. *M*. Circular muscular layer. *M'*. Longitudinal muscle layer.

FIG. 3.—Cœlomic corpuscles of an advanced embryo in various stages.

FIG. 4.—Cœlomic corpuscles of an embryo of "Stage A," more highly magnified.

FIG. 5.—Transverse section through ventral blood-vessel of newly-hatched worm, illustrating the way in which the hearts communicate with that vessel.

v. b. Ventral blood-vessel. *m.* Mesentery, by which it is attached to œsophagus. *v.* Valves in hearts.

Fig. 6.—Section through a portion of anal nephridia. *A, B.* Two tubes, one cut transversely, the other longitudinally, in which the peritoneum (*p.*) is slowly adherent to the nephridial cells (*n.*). *C, D.* Two other tubes, in which the peritoneum (*p.*) is separated by a space containing a coagulable fluid from the nephridium itself (*n.*). In every case the nephridial tubules have not yet acquired a lumen.

Fig. 7.—Transverse section through two nephridial tubules to illustrate the specialisation of the protoplasm of the cells into a central and peripheral portion.

Fig. 8.—Transverse section through longitudinal muscle-layer of newly-hatched worm. *b.* Muscular fibres imbedded in a faintly granular matrix. These are closer together, and have a columnar arrangement in that part of the longitudinal muscular layer which lies nearest to the circular muscles. *a.* Cavities in the gelatinous connective-tissue matrix.

Fig. 9.—Section through part of buccal cavity of advanced embryo. *Ep.* Epithelium of buccal cavity. *N.* Circum-œsophageal nerve-ring. *neph.* Duct of " mucous gland."

Fig. 10.—Transverse section through posterior region of the body of an advanced embryo. *Nephr.* Anal nephridia. *Neph′.* Their ducts leading to exterior of body ; the apertures into the alimentary canal are not shown.

Fig. 11.—Longitudinal section through a portion of the 9th segment. *Ep.* Epidermis. *M.* Circular muscle-layer. *M′.* Longitudinal muscular layer. *s.* Septum. *f.* Rudimentary funnel of nephridium, which lies behind the septum. At *n* a duct leads towards the exterior.

Fig. 12.—Gonad (*gon.*) and nephridial funnel (*funn.*) of one of the genital segments of an embryo of Stage C.

Fig. 13.—Gonad (*gon.*) and funnel (*funn.*) of newly-hatched worm.

Fig. 14.—Gonad (*gon.*) and commencing vas deferens (*vas. def.*) of the same individual. *Spt.* Septum.

Fig. 15.—Gonad of an embryo of Stage D. *g. c.* Germinal cells. *p. c.* Peritoneal cells.

Fig. 16.—Longitudinal section through a portion of the anteriorly-placed segments of an embryo of Stage C, showing nephridial funnel.

Fig. 17.—Two anterior segments of an embryo of Stage A, showing the nephridia. The segments are numbered. *neph.* External orifice. *f.* Funnel.

Fig. 18.—Anterior end of body of same embryo. *S.* Larval sense-organ. *br.* Brain. *Stom.* Stomodæum. *Mes.* Mesenteron.

Fig. 19.—A portion of Fig. 16, enlarged to illustrate the structure of nephridial funnel, which has lost its cilia and is commencing to degenerate.

FIG. 20.—Embryos of the various stages described in the text, of the natural size.

FIG. 21.—Nephridium of Stage A, displayed for nearly its whole course. *f.* Funnel. *s.* Seta sac.

FIG. 22.—Section through head end of embryo of Stage B. *N. p.* External orifice of mucous gland. *f.* Funnel of the same. *ph.* Pharynx. The segments are numbered, the figures being opposite to the setæ.

FIG. 23.—Embryo of Stage A.

FIG. 24.—Longitudinal section through function of stomodæum and mesenteron in embryo of Stage C. *f.* Peritoneal cells. *c.* Cilia, covering a few cells of the mesenteron.

On the Innervation of the Cerata of some Nudibranchiata.[1]

By

W. A. Herdman, D.Sc., F.L.S.,
Professor of Natural History;

AND

J. A. Clubb,
Assistant in the Zoological Laboratory, University College, Liverpool.

With Plates XXXII, XXXIII, and XXXIV.

A FEW years ago (1889) one of us read a paper at the Newcastle-on-Tyne meeting of the British Association "On the Structure and Functions of the Cerata in Nudibranchiata," in which these dorso-lateral processes were regarded as being probably epipodial outgrowths. In other papers[2] published since, we have compared the conditions of these structures in various genera of Nudibranchs, and have tried to show that they are all modifications of simple lateral epipodial ridges. Garstang also, in papers[3] published about the same time, came independently to the same conclusions from the examination of a somewhat different series of forms.

Pelseneer[4] and others, however, have lately contended that

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the so-called "epipodia" of molluscs are not in all cases of the same nature ; and that the epipodia of (e. g.) Aplysia and of the Pteropods are the edges of the ventral surface of the foot proper, and are, therefore, not really homologous structures with the epipodial processes of an ordinary rhipidoglossate Gastropod, such as Trochus, as they are not outgrowths from an "epipodial line" extending from the region of the tentacles on the back of the head along the sides of the body posteriorly and supplied with nerves from the pedal ganglia, as it was originally defined by Huxley.[1] For such independent structures (as in Pteropods and Aplysia) Pelseneer suggests that the name "parapodia" given by von Jhering should be employed. We have already protested against the use of this term in a new sense, and Garstang has, therefore, proposed lately the substitute "pleuropodia" to indicate such dorso-lateral projections as those of Aplysia and many other Opisthobranchiata (including the cerata of Nudibranchs) which have had doubt thrown upon their epipodial nature.

On the other hand, Lacaze-Duthiers and others of his school have lately regarded the epipodia as pallial outgrowths innervated from the pleural ganglia; but Pelseneer has conclusively shown that, in Trochus and other Rhipidoglossa at least, the processes in question are supplied by epipodial nerves which arise from the dorsal part of the pedal ganglia, or the pedal ganglionic cords, or at the anterior end from the cerebro-pedal connectives, and that therefore they are entirely pedal in their nature.[2]

Consequently it has seemed to us of importance to determine the nerve-supply to the cerata in several different types of Nudibranchiata, with the view of settling whether or not these

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[1] 'Phil. Trans.,' 1853, pp. 40, 47, &c.

[2] In a third note ('Bull. Scient.,' t. xxiii, p. 437), issued August 18th, 1891, and which reached us some time after this paper had been written, and read at the British Association meeting, Pelseneer not only distinguishes between the "epipodia" and "pleuropodia," as explained above, but also separates off from both the cerata of Nudibranchs, as being "processes of the dorsal integument always innervated by the pleural ganglia." This is not in accordance with the facts as demonstrated by our observations detailed in the following pages.

processes, be they "epipodia" or "pleuropodia," are truly pedal structures, like the lateral processes in the Rhipidoglossa, in the sense of being innervated from the pedal centres.

The present knowledge of the matter from literature, restricting the question now to the cerata of Nudibranchs, is as follows.

Alder and Hancock,[1] in their admirable anatomical plates, figure, in the case of Tritonia, large nerves to the dorsal integument, which are shown arising from the "branchial" [pleural] ganglia, while the pedal ganglia give off only the nerves to the foot proper, and two small nerves to the skin of the sides of the body. In Goniodoris they show practically the same arrangement, and also in the case of Fiona. In Eolis, however, large nerves from the pedal ganglia are shown supplying the skin of the sides of the back where the cerata arise.

Bergh, in his important series of detailed papers on the minute anatomy of these forms, figures what we take to be epipodial nerves in various Nudibranchs. In his " Beiträge z. Mon. d. Polyceraden," No. 1,[2] he describes in the case of Euplocamus croceus the " n. pallialis " arising from the visceral part of the cerebro-visceral ganglionic mass, and giving off branches to the cerata. Again, in his " Die Cladohepatischen Nudibranchien " he describes[3] the " n. pleuralis " (or "lateralis "), evidently the same nerve as the " pallialis" mentioned above, as arising in Eolids from the pleural part of the cerebro-pleural ganglia.

Vayssière figures[4] in Marionia (a form closely related to the common Tritonia) a nerve from the pleural portion of the cerebro-pleural mass on each side, and also an accessory smaller

[1] " Monograph of the British Nudibranchiate Mollusca,' Ray Society, 1845—1855.

[2] ' Verh. d. k. k. Zool. Bot. Ges. Wien,' Bd. xxix, 1879, p. 599.

[3] ' Zoolog. Jahrb.,' Bd. v, 1890.

[4] ' Atlas d'Anatomie comparée des Invertébrés,' Paris, 1888, pl. ix, fig. 3. Vayssière has also described (' Arch. Mus. Marseilles,' t. ii, p. 96) the nerves supplying the large " parapodia" in Notarchus and Aplysia as arising from the lateral posterior borders of the pedal ganglia.

nerve from the pedal ganglion on each side, going to the lateral integument from which the cerata arise. On the right side the latter nerve supplies also the reproductive aperture and neighbouring parts. Finally, Pelseneer, in his last note which we have just received, states that in all cases the cerata are supplied by the pleural ganglia, and he gives as an example Janus (Antiope) cristatus.[1]

The results of these former investigations, then, depending entirely, we believe, upon minute dissection, are puzzling, and seem sufficiently contradictory to indicate the need of corroboration or correction; so we have set ourselves to trace all these nerves afresh by means of serial sections of such of the types as we could obtain, with the results given below.

The species we have sectionised and examined are Poly-cera quadrilineata, Ancula cristata, Dendronotus arborescens, Hermæa dendritica, Facelina coronata, and Tergipes despectus. Most of the specimens were procured alive either from the Liverpool Marine Biological Station on Puffin Island, or from Hilbre Island near Liverpool. They were killed and fixed with Kleinenberg's picric acid, stained with picro-carmine, passed through graduated alcohols, embedded in paraffin, and cut with the Cambridge rocking microtome. For the specimens of Hermæa dendritica which we used we are indebted to the kindness of Mr. W. Garstang, M.A., now Berkeley Fellow at the Owens College, Manchester. These specimens were collected near Plymouth, were plunged for a moment, while alive, into glacial acetic acid, were then transferred to a saturated solution of corrosive sublimate for half an hour or so, after which they were put through increasing strengths of alcohol up to 90 per cent. in the usual way. They were afterwards stained and sectionised like the other forms.

We shall describe the facts we have been able to make out in regard to the origin and distribution of the nerves in each form separately under the headings of the genera, beginning with those in which the cerata seem to be in their simplest

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[1] 'Bull. Scientif.,' t. xxiii, p. 440.

condition, and working up to those in which they are more complicated.

POLYCERA.

In **Polycera quadrilineata** the cerebral and pleural ganglia are completely fused to form a cerebro-pleural mass on each side, of which the anterior part is cerebral and the posterior pleural. The "epipodial"[1] nerves are found arising, one on each side, from the ventral and posterior part of this mass (Pl. XXXII, fig. 2, *ep. n.*), i. e. distinctly from the pleural ganglia ; and they eventually run along the sides of the back to supply the ceratal lobed ridges found in this species.

In one of our series we have a specimen cut into 300 sections, and we find, on the right side of the body, the epipodial nerve arising, as above described, in the 91st section, in the region of the reproductive aperture (see Pl. XXXII, fig. 2), and then running forwards[2] through ten sections to the 81st, in the region of the eyes (Pl. XXXII, fig. 1, *ep. n.*), where it suddenly bends round ventrally, and can then be traced backwards through the same series of sections (81 to 91).

In the 90th and 91st sections (Pl. XXXII, fig. 2) the cut end of the nerve is seen lying dorsally and externally to the point of origin from the ganglion, but free in the body-cavity. It can be traced back in this same free condition to about the 120th section, when it sinks gradually into the meso-dermal body-wall. The nerve then continues to pass backwards in a dorso-lateral position, lying just over the lateral edge of the ovo-testis, which overlies the liver in this part of the body (Pl. XXXII, fig. 3, *o. t.*). In about the 160th section the ceratal (epipodial ?) ridges begin to be more prominent, and a few sections further on (No. 165) a branch is seen arising from

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[1] Throughout the present paper we shall continue, in the anatomical descriptions, to call these nerves epipodial, without considering now the question of whether the parts they supply are to be regarded as "epipodial," "pleuropodial," or not "podial" at all.

[2] Possibly in consequence of the retracted position of the nerve collar, due to contraction of the animal at death.

the nerve, and running upwards dorsally into one of the two large lobes or projections on the ridge (see Pl. XXXII, fig. 3, *ep. n.'*), while the main nerve continues its course backwards in the body-wall at the base, giving off small branches, which are distributed to the ridge above. The first large dorsal or ceratal branch from the main nerve is found in the anterior part of the branchial region, and is therefore nearly in the middle of the length of the body. Our epipodial nerve is probably nerve 7 of Alder and Hancock's Fam. I, pl. xvii, fig. 12, arising from the "branchial" ganglia, and going to the skin of the back.[1]

Figs. 2 and 3 on Pl. XXXII incidentally show some other points, especially, in fig. 2, the cartilages and muscles of the odontophore (*cart., m.*) and scattered teeth of the radula (*r.*), the glands of the foot (*f.*) the blood-spaces of the body-wall (*b. s.*), and the reproductive vestibule (*r. a.*) ; and in fig. 3 the ovo-testis (*o. t.*), the liver (*l.*), the branchiæ (*br.*) with their blood-spaces, and the structure of a ceras with its large unicellular glands in the ectoderm.

ANCULA.

In Ancula cristata the pleural ganglia are distinct from the cerebral (Pl. XXXII, figs. 4, 5, and 7).

In a specimen cut into about 500 sections we find in about the 106th section or so from the anterior end six distinct ganglia (the cerebral, pleural, and pedal pairs) surrounding the œsophagus (Pl. XXXII, figs. 4 and 5). A few sections further back the cerebrals disappear, and then (in the 113th section, Pl. XXXII, fig. 6) the epipodial nerves are found arising from the dorsal edge of the pleural ganglia. They run dorsally and outwards and then posteriorly, lying free in the body-cavity.

Soon after leaving the ganglion each epipodial nerve gives off its first branch dorsally. This branch enters the mesoderm of the dorsal body-wall, and can be traced back through over

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[1] In this same figure Alder and Hancock show on the left side a small accessory nerve (8) to the side of the body, arising from the pedal ganglion.

100 sections to one of the first pair of cerata, which it enters (in 231st section in this series), and is then distributed.

Fig. 8 (Pl. XXXII) shows the main nerve (*ep. n.*) in a compartment of the body-cavity, the dorsal branch (*b.*) in the body-wall, and twigs from the latter (*c.*) passing up into the ceras above. In about the 240th section (see Pl. XXXII, fig. 9) the epipodial nerve, still in the body-cavity, gives off a small branch, which is distributed to the lateral body-wall. In the 258th section the branch to the second ceras arises. The epipodial nerve has now become more dorsal in position, and it soon leaves the body-cavity and sinks into the body-wall, where it gives off small branches to the dorsal integument and to the branchiæ. In about the 312th section the branch of the third ceras is given off, and the main nerve, now very small, runs back a little further, and then breaks up into the delicate twigs which supply the fourth and fifth pairs of cerata.

In another small specimen of Ancula cristata we find the epipodial nerves arising from the dorsal surfaces of the pleural ganglia, just posterior to the cerebrals (see Pl. XXXII, fig. 7, *ep. n.*) on both sides in the 96th section. The nerves then run back in the body-cavity to section 130, where they give off their first dorsal branches to the first pair of cerata.

Some of the sections of Ancula show the histology of the ganglia well (see Pl. XXXII, figs. 5, 6, 7). There is a connective tissue sheath, which becomes thicker where a nerve leaves, and is then continued along the nerve (see fig. 6, *p. n.* and *sh.*). The large nerve-cells are distributed round the periphery of the ganglion, while the centre is occupied by small cells and interlacing delicate nerve-fibres.

DENDRONOTUS.

Bergh[1] states that in Dendronotus arborescens the cerata are supplied by nerves arising from the pleural ganglia; but we find in addition an interesting anastomosis of a branch

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[1] "Die Nudibran. d. 'Willem Barents,'" Bijdragen tot de Dierkunde, 'Nat. Artis Mag.,' Amsterdam, 13ᵉ Afl., 1886, p. 28.

from the pedal with the pleural element of at least a part of the epipodial nerve (see Pl. XXXIV, figs. 26 and 27).

We give on Pl. XXXIV, fig. 27, a diagram constructed from a series of twenty consecutive sections (Nos. 277 to 296), and some others, so as to show from the left side the pleuropedal anastomosis, and the origin of the dorsal and lateral epipodial nerves. It shows the origin of a nerve (a) from the pleural ganglion, and one (b) from the pedal; both run backwards for a few sections, and then b gives off a branch (c) which gradually works up dorsally (still connected with b by its connective-tissue sheath in sections 286—289) until it comes in contact with (section 290) and eventually joins (section 296) a, the nerve from the pleural ganglion. Previous to this anastomosis, however, a gives off (section 291) a dorsally directed branch (d), which therefore contains no admixture of pedal element. In this way three nerves are formed (seen in section 296) on each side of the œsophagus :—the dorsal one (d.), which is purely pleural in origin ; the ventral one (b.), which is purely pedal ; and the middle one (a. + c.), which is partly pleural and partly pedal. It is with the first and last of these that we have to deal ; we may call them, from their positions further back in the body, the dorsal and the lateral epipodial nerves (see Pl. XXXIV, fig. 27).

Both of these nerves, on each side, go to the cerata. They can be traced back for some distance lying freely in the body-cavity, at first the dorsal and lateral being close together, and then becoming widely separated (seen at a point about fifty sections behind where the nerves become distinct). About thirty sections further back still (in section 371) the four nerves begin to approach, and then to sink into the body-wall, where they take up definitely dorsal and lateral positions respectively. They run in this condition through a great number of sections. In about the 440th section we find a branch arising from the lateral epipodial nerve, which passes, in the next few sections, upwards dorsally to enter the first ceras where it breaks up (see Pl. XXXIV, fig. 26). This is now between 160 and 170 sections posterior to the point of

origin of the primary pleural and pedal nerves from the ganglia, and is immediately below the most anterior pair of cerata.

The dorsal epipodial nerves also give off small branches to the cerata and to the neighbouring integument, and then the four nerves continue their course backwards to supply the more posterior cerata (see Pl. XXXIV, fig. 26).

In regard to the pleuro-pedal anastomosis described above, it is interesting to note that Pelseneer has found that a junction (the cervical plexus) between nerves having distinct origins from the pleural and the pedal ganglia is effected in Pneumonoderma, and other gymnosomatous Pteropods,[1] and also in Aplysia.[2]

HERMÆA.

In Hermæa dendritica (the opportunity of examining which we owe to the kindness of our friend Mr. Garstang) we find the epipodial nerve arising from the ventral external edge of the pleural ganglion. The anterior part of this nervous system is bilaterally symmetrical (see Pl. XXXIII, fig. 20, which shows the cerebral, the buccal, and the anterior ends of the pedal ganglia surrounding the œsophagus), but posteriorly it becomes very unsymmetrical, as was noticed by Bergh,[3] and as figs. 21 to 25 show.

The flattened expansion of the lateral edge of the body at the base of the cerata in this form is largely occupied by the lobes of the ovo-testis, their ducts, and the ducts of the hepatic cæca (Pl. XXXIII, figs. 20 and 25).

The origin of the epipodial nerve is shown in fig. 21, which is two sections posterior to fig. 20. The nerve turns upwards dorsally at once (fig. 22, *ep. n.*). It is surrounded by a very distinct connective-tissue sheath, which is seen connecting the pieces of the nerve in fig. 23. When it reaches the level of the

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[1] 'Arch de Biologie,' t. vii.

[2] "Report on the 'Challenger' Pteropods," part 3, 'Zool. Chall. Exp.,' part lxvi, pp. 43 and 88; also 'Bulletin Scientif.,' 1888, p. 195.

[3] "Beit. z. Kennt. d. Æolid.," viii, 'Verhdl. Zool. Bot. Gesell. Wien,' 1885, p. 5.

top of the œsophagus, and the dorsal surface of the base of the cerata, the nerve splits into two branches, a dorsal and a ventral (fig. 24, *ep. n.*), which proceed outwards into the cerata respectively above and below the lobes of the ovo-testis.

The otocyst lies between the pleural and the pedal ganglia, just underneath and internal to the origin of the epipodial nerve (fig. 21, *o. c.*).

Fig. 20, besides giving the general relations of nervous system, body, and cerata, shows the enormous number of glands (fig. 20, *gl.*) embedded in the anterior part of the foot.

TERGIPES.

Fig. 17 on Pl. XXXIII gives a transverse section of Tergipes despectus to show the relative sizes and positions of the few large cerata and the small body. It is a little behind the middle of the body, posterior to the stomach and nervous system, and the greater part of the cavity of the body is occupied by the very large lobes of the ovo-testis.

The only nerves which we find going to the cerata in this species arise from the pedal ganglia. Fig. 10 (Pl. XXXIII) shows part of a section in which the pedal ganglia connected by their commissure lie under the œsophagus ; the cerebrals are no longer visible, they are in sections further forward. The epipodial nerve is seen on the left side arising from the dorsal surface of the pedal ganglion, and running outwards under a part of the stomach which occurs here. The next two sections (figs. 11 and 12) show the nerve passing outwards to enter into close relations with the pleural ganglion of that side, from which, however, it remains perfectly distinct. On reaching the lateral body-wall the nerve turns upwards and runs dorsally (Pl. XXXIII, figs. 13 and 14) between the stomach and the body-wall towards the base of one of the cerata (Pl. XXXIII, figs. 15 and 16), to which it gives off a branch and then passes on backwards in a dorso-lateral position.

These sections of Tergipes despectus show well some other points in structure. The lobes of the ovo-testis (figs. 17

and 18) contain ova and spermatozoa in various stages of de-
velopment. In most cases single lobes are not hermaphrodite,
but occasionally both ova and developing spermatozoa are to be
found in the same lobe. In fig. 18 the upper lobe is a purely
female one and the lower is entirely male, while the small piece
of a third seen at the left side contains large and small ova and
also spermatic cells.

Glands are very abundant in the integument of this minute
species. Fig. 17 shows the large mass of ovate glands above
the foot, and the numerous small glands scattered all over the
surface of the cerata; these latter are seen more highly magni-
fied in figs. 13 and 19. The connection between the hepatic
cæca in the cerata and the median portion of the liver in the
body is seen in fig. 17 on the right side, and the opening of the
hepatic cæcum into the cnidophorous sac at the apex of the
ceras is shown in fig. 19. This opening is surrounded by
muscle-fibres which encircle the lower half of the cnidophorous
sac. The cnidocysts are large and distinctly nucleated
(Pl. XXXIII, fig. 19, *cn. c.*), and the cnida are of elongated
ellipsoidal form.

Eolis (Facelina).

In Eolis (or Facelina) coronata we find that, as Alder
and Hancock showed long ago for Eolis papillosa, the chief
nerves to the cerata arise from the pedal ganglia; but there is
also, on one side at least, a smaller accessory epipodial nerve
which is pleural in origin.

The numerous large cerata arise from the body in Facelina
coronata in clumps (Pl. XXXIV, figs. 28 and 32). This is espe-
cially well shown in fig. 28, where on the left side of the figure
the section shows a large basal projection from the body common
to half a dozen cerata. In this basal mass we find muscle-
bundles and connective tissue, the ducts from the hepatic cæca,
and the epipodial nerves going to the cerata. This basal region
of a clump of cerata is separated off from the body proper by a
line of longitudinal and oblique muscle-fibres (Pl. XXXIV,
figs. 28 and 31, *musc.*), through which the nerves have to pass.

The chief epipodal nerve (figs. 28 and 29, *ep. n.*) is found to arise from about the middle of the dorsal and external edge of the large pedal ganglion on each side, and to curve outwards and ventrally (fig. 29). After a short course it passes through the muscular layer of the body-wall and is distributed to the clumps of cerata (fig. 28).

In addition to the main epipodial nerve we have found also, on the left side only, a small nerve which arises from the ventral and posterior part of the cerebro-pleural mass, just below the eye (see Pl. XXXIV, fig. 28, right side of figure), and runs ventrally (fig. 28, *acc. ep. n.*) till it gets opposite the middle of the buccal mass, and then passes outwards through the layer of muscle-fibres (see fig. 31, *acc. ep. n.*) to reach the base of a clump of cerata. This nerve may, therefore, be regarded as pleural in its nature; it is distinctly anterior in its origin to the main epipodial nerves springing from the pedal ganglia, and so far as we can find it supplies only the most anteriorly placed clump of cerata on its own side.

There can be no doubt as to the ganglia from which the main epipodial nerves arise. Not only do their relations to one another and to the other ganglia and to the œsophagus (see Pl. XXXIV, fig. 29) show clearly that they are the pedals, but we have also traced from them the ordinary pedal nerves (fig. 31, *p. n.*) going to the foot. These pedal nerves arise from the ventral surface of the ganglia two or three sections in front of the origin of the main epipodial nerves, and two or three sections behind where the small accessory epipodial nerve arises from the left pleural. They are in the same section with the otocysts (Pl. XXXIV, fig. 31, *o. c.*) which lie on the upper surface of the pedal ganglia.

These figures of Facelina coronata show some other points of interest. The numerous sections of the cerata show the relations between the cnidophorous sac and the hepatic cæcum (fig. 28); and the opening of the latter into the ducts in the body (figs. 28 and 31, *n. h. cæ.*), and also the position of the cnida in distinct cnidocysts, which are epithelial cells turned in from the ectoderm on the apex of the ceras, as we have shown

in former papers.[1] The relations of the buccal mass, odonto-
phore, and œsophagus to the nervous system, and the constitu-
tion of the body-wall are also shown. Figs. 29 to 31 show the
histology of the ganglia. The largest nerve-cells, as in other
forms, are here on the surface. Fig. 30 shows the posterior
surface slice of the right cerebral ganglion from the next section
to that drawn in fig. 29. It is entirely composed of large poly-
gonal cells with huge reticulated nuclei, each of which has a
single very distinct nucleolus, which, again, sometimes has a
distinct central spot (as in the cell marked *n. c.* in fig. 30).
Fig. 29 shows on the left side at the top the next section of that
cerebral ganglion, passing forwards, and it is also nearly all large
cells ; while the pedal ganglia below in the same figure, and
also in fig. 31, show that the central parts of the ganglia are
composed of small rounded or fusiform cells and interlacing deli-
cate nerve-fibres. These fibres and the small cells come to the
surface where a commissure or a nerve leaves the ganglion (see
pedals of fig. 31).

CONCLUSION.

We have shown, then, by the examination of this series of
types, that instead of the cerata of Nudibranchs being always
innervated by the pleural ganglia, as Pelseneer supposes,[2] or
always supplied by pedal nerves, as we had expected to find
when we commenced the investigation, there are, in fact,
various arrangements of the nerve-supply. The dorso-lateral
processes of the body-wall which we call cerata may be sup-
plied entirely by the pleural ganglia (e. g. Polycera and
Ancula), or chiefly by the pleural with a small supply from
the pedal by means of a pleuro-pedal anastomosis (Dendro-
notus), or entirely by the pedal ganglia (Tergipes), or chiefly
by the pedal ganglia with a small independent accessory supply
from the pleural (as in Facelina).

If, then, we take the nerve-supply as a sure indication of

[1] ' Quart. Journ. Micr. Sci.,' vol. xxxi, p. 41; and ' Trans. Liverpool
Biological Society,' vol. iv, p. 131.

[2] ' Bulletin Scientif.,' t. xxiii, p. 439, Aug. 18th, 1891.

homology, we arrive at the remarkable result that these processes of the body-wall are not all of the same nature; and that whereas in Tergipes, and possibly also in Facelina, they may be considered as pedal in origin, and as homologous with the epipodia of an ordinary rhipidoglossate Gastropod, such as Trochus (where the epipodial ridges and processes are supplied, according to Pelseneer, by nerves arising from the dorsal part of the pedal ganglia), in Polycera, Ancula, and others, they must be regarded as totally distinct structures of pallial[1] origin. This seems to be a clear case of reductio ad absurdum.

We have in a former paper tried to show that these processes, whether ridges or papillæ or lobes, parieto-cerata as in Dendronotus and Tritonia, or hepato-cerata as in Eolis and Doto, are all really modifications of the same thing; and although it might conceivably be argued that the parieto-cerata (pleural) of Ancula might be different in their nature and origin from the hepato-cerata of Eolis (mainly pedal), still no one would be likely to suggest that the cerata of Tergipes (pedal) and of Hermæa (pleural) are not homologous structures. And in addition there are the intermediate conditions found in Dendronotus and in Marionia,[2] linking together the purely pedal and the purely pleural methods of inner-vation.

Consequently we are inclined to consider that in this case the nerve-supply cannot be taken as a sure indication of the homology, and that possibly the innervation has undergone modification in accordance with changes in the position, size, and relation to other organs of these ceratal processes in the Nudibranchiata. The cerata, which we still regard as homologous structures throughout the series of Nudibranchiata, must, from their great differences in size, shape, colours, sting-ing properties, and contents, be of very varied importance to

[1] I. e. from the integument dorsal to the foot, and supplied by the pleural ganglia, whether there is a distinct "pallium" present or not.

[2] Where, according to Vayssière, the main epipodial nerves are pleural; but there are also smaller accessory nerves from the pedal ganglia.

their possessors; and it may readily be imagined that when such modifications have taken place as led to the appropriation of important organs like large blood-sinuses, huge hepatic cæca, and cnidophorous sacs, it would not be unlikely for nerves in the neighbourhood to be diverted from their original positions and become drawn up into the cerata.

The condition of Facelina, where there is what may be the commencement of a pleural supply, and in Dendronotus, where the anastomosis may be the remains of an original pedal supply, suggest at least the possibility of the following as an explanation; viz. (1) that these ceratal outgrowths may be truly epipodial, homologous with the epipodia of Trochus, starting at first as pedal structures supplied with nerves from the pedal ganglia; and (2) may have secondarily acquired, possibly as the result of changes in form, position, and relations to other organs, a supplementary nerve-supply from the adjacent integumentary nerves arising from the pleural ganglia; and (3) this supplementary supply, while remaining subordinate in Facelina, may in other forms have gradually come to supplant the original epipodial (pedal) nerves, which (on this view) have now completely disappeared in such forms as Polycera and Ancula, and are only represented in Dendronotus by the pleuro-pedal anastomosis.   This is, however, only a suggestion, which we do not feel able to support or press further at present. We may possibly be able to get evidence for or against it from the examination of the nerve-supply in some additional forms of Nudibranchs, which we hope soon to undertake.

If, then, the cerata of Nudibranchs cannot all be said to be true epipodia innervated by the pedals, we have shown, at least, (1) that it is equally impossible to regard them all as pallial outgrowths supplied by the pleural ganglia, and (2) that possibly they may have been epipodial in origin, although there is now in some a connection with pleural nerves.

EXPLANATION OF PLATES XXXII, XXXIII, and XXXIV,

Illustrating Messrs. W. A. Herdman's and J. A. Clubb's paper "On the Innervation of the Cerata of some Nudibranchiata."

The figures were drawn from the sections, as seen under Swift's 1-inch (× 50) and $\frac{1}{6}$-inch objectives (× 300), with the occasional use of Zeiss's $\frac{1}{12}$-inch oil immersion for the more minute details. The following abbreviations are used for the reference letters :

b. m. Buccal mass. *b. w.* Body-wall. *br.* Branchiæ. *b. s.* Blood-sinus. *b. c.* Blood-corpuscles. *buc. g.* Buccal ganglion. *cer. g.* Cerebral ganglion. *c.* Hepatic cells. *c. t.* Connective tissue. *c. m.* Circular muscles. *cn. c.* cnidocyst. *cn. s.* Cnidophorous sac. *ep. n.* Epipodial nerve. *e.* Eye. *ec.* Ectoderm. *f.* Foot. *gl.* Glands. *h. c.* Hepatic cæcum. *l. m.* Longitudinal muscles. *l.* Liver. *m.* Muscle-fibres. *n. c.* Nerve-cell. *n. sh.* Nerve-sheath. *œs.* Œsophagus. *o. t.* Ovo-testis. *o. c.* Otocyst. *p. g.* Pedal ganglion. *pl. g.* Pleural ganglion. *p. n.* Pedal nerve. *r.* Part of radula. *r. a.* Reproductive aperture. *ren.* Renal organ. *st.* Stomach. *sh.* Sheath of ganglion or nerve.

PLATE XXXII.

Fig. 1.—Transverse section of Polycera quadrilineata at level of eye (section 81 of series), showing relation of cerebral ganglia (*c. g.*) to buccal mass and body-wall, and the anterior loop of the epipodial nerve (*ep. n.*). × 50.

Fig. 2.—Another transverse section of same series, ten sections further back, in region of reproductive aperture, showing cerebro-pleural and pedal ganglia, with the origin of the epipodial and of various pedal nerves, and also the epipodial nerve in section in its course backwards. × 50.

Fig. 3.—Dorso-lateral part of another transverse section of the same series (No. 166), in the region of the branchiæ, to show the epipodial nerve in the body-wall over the edge of the ovo-testis, and a branch of it entering one of the ceratal processes. × 300.

Fig. 4.—Transverse section (No. 106) of Ancula cristata, showing the three pairs of ganglia surrounding the œsophagus. × 50.

Fig. 5.—Part of adjoining section, showing the ganglia more highly magnified. × 300.

Fig. 6.—Part of section (No. 113), six further back, showing the origin of the epipodial nerve (*ep. n.*) from the dorsal edge of the pleural ganglion. The cerebrals are not present so far back. × 300.

Fig. 7.—Part of similar section of another specimen (section No. 96), showing the origin of the epipodial nerve on each side, from dorsal edge of pleural ganglion immediately behind the cerebral, a small piece of the posterior end of the cerebral ganglion being present on the left side. × 300.

Fig. 8.—Upper half of transverse section (No. 231 of same series as Figs. 4, 5, and 6) in the region of the first pair of cerata, showing the main epipodial nerve in the body-cavity, its first branch in the dorso-lateral body-wall, and twigs going up from that into the ceras on the right side. × 50.

Fig. 9.—Diagram of Ancula cristata, from the left side, showing the origin and distribution of the epipodial nerves.

PLATE XXXIII.

Fig. 10.—Part of a transverse section of Tergipes despectus, showing the origin of the epipodial nerve from the dorsal surface of the pedal ganglion. × 300.

Fig. 11.—Part of the next section, showing the epipodial nerve lying under part of the stomach-wall where it runs outwards from the pedal towards the pleural ganglion. × 300.

Fig. 12.—Part of the next section, showing the epipodial nerve lying in contact with the pleural ganglion. × 300.

Fig. 13.—Adjoining section, showing epipodial nerve passing from close to pleural ganglion to inner surface of body-wall, so as to pass up to ceras above. × 300.

Fig. 14.—Next section (54th from anterior end), showing nerve passing up dorsally between stomach and body-wall. × 300.

Figs. 15 and 16.—Neighbouring sections to last, showing branches of nerve passing up to ceras. × 300.

Fig. 17.—Transverse section of the whole body of Tergipes despectus behind the middle, to show the relative sizes of cerata and body, and the junction of hepatic cæca with liver in body. × 50.

Fig. 18.—Part of the ovo-testis in same region of body as last section, showing both ova and spermatozoa in various stages. × 300.

Fig. 19.—The tip of one of the cerata in the same region, showing the communication between the hepatic cæcum and the cnidophorous sac. × 300.

Fig. 20.—Transverse section of Hermæa dendritica, near the anterior end (Section 61st of the series), showing the relations of the cerata to the body, and the cerebro-pedal and buccal ganglia surrounding the œsophagus. × 300.

Fig. 21.—Part of the second section, further on, showing the origin of the epipodial nerve from the ventral part of the cerebro-pleural ganglion. × 300.

Fig. 22.—Part of next section, showing the epipodial nerve free from the ganglia. × 300.

Fig. 23.—Part of next section, showing the epipodial nerve turning up dorsally; the nerve is in part out of the plane of the section, but the connective-tissue sheath connects the pieces. × 300.

Fig. 24.—Part of next section, showing the epipodial nerve at the base of one of the cerata dividing into a dorsal and a ventral (rather the larger) branch, the latter of which runs down round a lobe of the ovo-testis. × 300.

Fig. 25.—Part of the next section (No. 67), including the basal part of one of the cerata, showing the dorsal and ventral branches of the epipodial nerve. × 300.

PLATE XXXIV.

Fig. 26.—Diagram of Dendronotus arborescens, showing the origin and distribution of the dorsal and lateral epipodial nerves.

Fig. 27.—Diagrammatic scheme of the anterior part of the nerves in last figure, showing in lateral view their origin from the ganglia, their branches, and the anastomosis between a and c, the result being that the lateral epipodial nerve has a pedal element in it, while the dorsal epipodial nerve is entirely pleural in origin.

Fig. 28.—Transverse section of Eolis (Facelina) coronata in the region of the eye, showing on one side, below the eye, the point of origin of the accessory epipodial nerve from the ventral part of the cerebro-pleural ganglion ; and also showing a part of the accessory epipodial nerve free from the ganglia. This also shows well on the other side the common base to a clump of cerata; the hepatic cæca and cnidophorous sacs are seen in the cerata. × 50.

Fig. 29.—Part of a section a little further back, more highly magnified, showing structure of cerebral and pedal ganglia, and the chief epipodial nerve arising from the latter. × 300.

Fig. 30.—Surface section of cerebral ganglion from section adjoining last, showing the large superficially placed nerve-cells, with reticulated nuclei. × 300 (enlarged).

Fig. 31.—Part of a section through region of the otocysts, showing an ordinary pedal nerve ($p. n.$) arising from the pedal ganglion, just a few sections in front of where the chief epipodial nerve ($ep. n.$ in Fig. 29) arises from the same ganglion. This section also shows the accessory epipodial nerve on the other side ($acc. ep. n.$) passing through the muscular layer of the body-wall to reach the base of a clump of cerata. Figs. 28 and 31 show the course of this nerve from the ganglion to the point of distribution to the cerata. × 300.

Fig. 32.—Diagram of Facelina coronata, from left side, showing the origin and distribution of the epipodial nerves.

Notes on Elasmobranch Development.

By

Adam Sedgwick, M.A., F.R.S.,
Fellow and Lecturer of Trinity College, Cambridge.

With Plate XXXV.

CONTENTS.

1. On the Formation and Growth of the Elasmobranch Embryo.

My observations on this subject, which were made upon the genera Scyllium and Raia, have led me to conclusions which differ in some respects from those of previous observers. In some of the points with regard to the tail I have been anticipated by Schwarz ('Zeit. f. wiss. Zool.,' Bd. xlviii, p. 191), Kowalevsky, and Kastschenko ('Anat. Anzeiger,' 3); but as Schwarz's account—excellent though it is—does not go over the whole ground, and Kastschenko's is without figures, while Kowalevsky's is inaccessible, being published in Russian, I have thought it worth while to treat the matter fully.

As is well known, the blastoderm attains a certain size before any trace of the embryo is visible, spreading by a uniform growth at all points of its circumference over the yolk. At Balfour's Stage A, however, the first trace of the embryo appears as a slight thickening at one point of the circumference of the blastoderm. This point is usually regarded as the hind end of the blastoderm. This is not quite correct, for it

really becomes the front end of the future embryo.　After the appearance of the embryonic rim the blastoderm still continues to spread over the yolk by a uniform growth of all parts of its circumference, but in the centre of the embryonic rim a slight indentation appears.　This indentation shares in the uniform growth of the blastoderm edge, and advances over the yolk equally with the rest of the embryonic rim and general edge of the blastoderm.　As the embryonic rim travels away from the point of its first appearance, the surface of the blastoderm so formed—that is to say, the surface of the part of the blastoderm extending between the point of first appearance of the embryonic rim and the embryonic rim at any given moment of its growth—is slightly elevated from the rest of the blastoderm, and traversed by an inconspicuous longitudinal median groove.　This raised part of the blastoderm soon becomes marked off by two ridges, which in front, *i. e.* at the point which marks the site of the first appearance of the embryonic rim, are continuous with one another, while behind they are continuous with the parts of the embryonic rim which bound the indentation.　These portions of the embryonic rim are more markedly swollen than the rest, and form the " tail swellings " of Balfour.　This elevated part of the blastoderm is the medullary plate, and the shallow groove traversing it marks the line of growth of the indentation above referred to.　These points are all illustrated by my fig. 1, which represents the embryo at a stage where the indented embryonic rim has grown back a considerable distance from the point of its first appearance.　Various stages in the process may be seen in Balfour's figures[1] of Stages B, C, D, and in Schwarz's figs. 1 and 2.　The indentation of the embryonic rim is always placed at the hind end of the groove which marks the centre of the medullary plate.　This groove is a transitory structure, and soon disappears ; its importance consists in the fact that it indicates the line of growth of the indentation of the embryonic rim.　(It is conterminous in

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[1] ' Monograph of Development of Elasmobranch Fishes,' pl. vii ; pl. viii of the Memorial Edition.

extent with the notochord, though the notochord beneath the front part of it is not at first developed.)

It must be clearly understood that the growth of the whole edge of the blastoderm has so far been a uniform one. The indentation in the embryonic rim advances equally (after its first establishment) with the more prominent parts of the embryonic rim called the caudal swellings. There is no reason to suppose that this advance of the indented part of the embryonic rim is due to the fusion of the divergent caudal swellings. On the contrary, there is every reason to suppose that the indented part of the embryonic rim advances by growth of its own substance, just as do the other parts of the edge of the blastoderm.

After a certain time the caudal swellings and the part between them begin to grow more rapidly than the adjacent portions of the edge of the blastoderm, and come to project beyond the latter like a kind of tongue overhanging the yolk (fig. 2). This appears to happen at about the time when the medullary groove is closing in its anterior part to form the medullary canal.

At the same time the edge of the blastoderm remote from the embryo has continued its rapid growth. It is only the edge of the blastoderm next the embryo in which the growth is retarded. The result of this is that the posterior projecting part of the embryo lies in a kind of bay of the edge of the blastoderm. Fig. 2 is drawn from an embryo at a stage when this bay was but little marked.

I now wish the reader to concentrate his attention upon the projecting tongue which will form the under part of the embryo. Its sides, which are part of the edge of the blasto-derm, bend ventralwards and towards each other.[1] It consists on its dorsal face of the medullary plate ectoderm, which has become folded so as to form the neural canal (in fig. 2 the neural canal is established in the front part of the embryo, but widely open at the hinder end of this projecting tongue). At

[1] A good figure of this is given by His in the ‘Zeitschrift f. Anatomie u. Entwick. Gesch.,’ 1877, pl. vii, fig. 6.

its edge, which is part of the general edge of the blastoderm, the ectoderm is continuous with the endoderm which forms the under side of the tongue. A good idea of the appearance of a transverse section through this tongue is given by fig. 1 *b*, pl. x,[1] of the 'Elasmobranch Fishes' (Mem. Ed.). The hinder end of the tongue is of course notched, and the notch is continued forwards along the line of the groove above mentioned as occupying the centre of the medullary plate, as a slit which actually completely perforates the blastoderm, so as to lead into the space between the endoderm of the tongue and the yolk. This is shown clearly in fig. 3, and at a later stage in fig. 4. Whether this slit is due to a bilobed backward growth of the notched portion of the embryonic rim, the growth at the middle point, *i. e.* at the bottom of the notch, ceasing—in other words, to an emphasising of the notch already present—or whether it arises as a secondary perforation of the medullary plate and endoderm along the line of the groove before mentioned, I am unable to say; but I think it is due to the former.

While these changes have been taking place—and I must now refer back to fig. 2—the sides of the projecting tongue become bent ventralwards and towards each other until they meet or nearly meet in the ventral middle line. Now two important structural results, which should be noted and understood, follow from this bending: (i) the two angles formed by the junction of the edge of the blastoderm in the embryonic region with the edge of the blastoderm in the non-embryonic region—the angles, one of which is marked *a* in fig. 2, become closely approximated ventrally beneath the embryo; and (ii) a space is enclosed on the ventral side of the embryo, which space is lined by endoderm, and opens ventrally to the exterior through a slit formed by the contact of the ventrally bent edges of the tongue, and dorsally into the neural canal by the slit in the medullary plate. This space[2] is the hind

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[1] Old edition, pl. ix, fig. 1 *b*.

[2] A section of the tongue in this stage in front of the neurenteric slit is shown in Schwarz's fig. 16.

gut, and the two slits which are continuous with one another round the hind end of the embryo are portions of the blastopore. By the time that the two angles marked a and the edges of the embryonic part of the blastoderm have come into contact ventrally, the non-embryonic edges of the blastoderm adjacent to the embryo have grown backwards over the yolk to form the bay mentioned by Balfour. The two sides of this bay, which it will be remembered are portions of the edges of the blastoderm, come to lie close together on the yolk beneath the tail of the embryo. For a little time they remain unfused, and the yolk is still freely exposed between them in a linear streak.[1] This slit, which is bounded by the edges of the non-embryonic part of the blastoderm of the two sides, is a part of the blastopore, and is continuous, passing along the hinder side of what will be called the umbilical stalk, with the portion of the blastopore leading into the hind gut and extending along the ventral side of the tail. This last portion is, as we have seen, continuous with a dorsal portion which leads through the medullary plate into the medullary canal.

The last part of the blastopore to be mentioned is the so-called yolk-blastopore, described by Balfour in the ' Elasmobranch Fishes,' p. 81 (Mem. Edition, vol. iii, p. 296), and in the ' Comparative Embryology,' 1st ed., ch. iii, p. 52.[2] The lips of this portion are continuous with the lips last mentioned as running back on the yolk parallel to one another, and ventral to the tail of the embryo.

To recapitulate : the blastopore of Elasmobranchii is at the present stage—i. e. the stage immediately before closing—an elongated narrow slit, slightly dilated in front, where it lies on the floor of the medullary canal (fig. 3) and more dilated behind (Balfour's yolk-blastopore, ' Comparative Embryology,' vol. ii, ch. iii, fig. 30 b). Between these two limits it takes the course of a reversed letter S, as shown in the adjoining woodcut, where its lips are represented unfused.

The anterior part, a b, perforates the floor of the medullary

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[1] Again see Schwarz's fig. 16, $d.o.$

[2] Mem. Ed., vol. iii, p. 63.

canal, and is dorsal; this is continuous round the end of the tail, *b c*, with a ventral part, which extends forwards along the ventral side of the tail, *c d*, as far as the yolk-stalk, along which it passes, *d e*, to continue backwards along the yolk, *e f*, as the slit-like non-embryonic part of the blastopore, which passes behind into the more dilated and posterior part of the so-called yolk-blastopore.
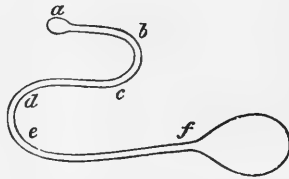
Shortly after this stage the blastopore completely closes, excepting one point in its dorsal portion, which persists for some time as the neurenteric canal.

Balfour, as is well known, was the first to compare the primitive streak of the Amniota to "the linear streak in Elasmobranchii, formed by the coalesced edges of the blastoderm which connect the hinder end of the embryo with the still open yolk-blastopore" ('Comparative Embryology,'[1] 1st ed., vol. ii, ch. xi, p. 240); and he also says, in the same place, that "the passage at the front end of the primitive streak [the neurenteric canal] is the dorsal part of the blastopore, which in Elasmobranchii becomes converted into the neurenteric canal." But he never, either in the chapter quoted or in his account of the actual development of Elasmobranchs in ch. iii, p. 52, describes the ventral embryonic part of the blastopore (woodcut, *c d*) which connects together the linear streak on the yolk, *e f*, with the dorsal part of the blastopore, *a b*.[2] In fact, he says (ch. iii, p. 52), "It is interesting to notice that, owing to the large size of the yolk in Elasmobranchs, the posterior part of the primitive blastopore becomes encircled by the medullary folds and tail swellings, and is so closed long

[1] Mem. Ed., p. 288.

[2] This part of the blastopore is clearly recognised and figured by Schwarz.

before the anterior [what I have called posterior] and more ventral part, which is represented by the uncovered portion of the yolk."

I have dwelt at some length upon this point because Balfour's description of the Elasmobranch blastopore has always bothered me, in that it does not show the connection between the yolk part of the blastopore—the linear streak—with the dorsal part; and also because I wish to present a slight modification of the comparison which Balfour made between the primitive streak of the Amniota and the linear streak on the Elasmobranch yolk. Balfour does not say that the two structures are homologous; he expressly guards himself from this. He says ('Comparative Embryology,' 1st ed., vol. ii, ch. iii, p. 51), "A linear streak [my woodcut, *ef*] formed by the coalesced edges of the blastoderm is left connecting the embryo with the edge of the blastoderm. This streak is probably analogous to (though not genetically related with) the primitive streak in the Amniota" (the italics are mine). But he undoubtedly does compare the primitive streak with this linear part of the yolk-blastopore of Elasmobranchs; and he says ('Comparative Embryology,' vol. ii, 1st ed., ch. xi, p. 240), "That it (primitive streak) is in later stages not continued to the edge of the blastoderm, as in Elasmobranchii, is due to its being a rudimentary organ."

The modification which I would propose to suggest in the comparison is as follows. The primitive streak of the Amniota is, as is well known, partly involved in the tail fold, and tucked under on to the ventral surface of the embryo. It thus becomes divided into a dorsal part, at the front end of which is the neurenteric canal or its rudiment, and a ventral part. The dorsal part is in birds for some time placed in a dilated posterior part of the still open medullary groove called the sinus rhomboidalis. This part I would compare to the dorsal part of the blastopore shown in the same position and relations in my figs. 3 and 4. The ventral part, on the other hand, I would compare to the part of the blastopore which in Elasmobranchs runs along the ventral side of the tail

to the yolk-stalk (my woodcut, *c d*); while the linear part
of the yolk-blastopore in Elasmobranchii (my woodcut, *e f*) is
unrepresented or rudimentary in Aves and Amniota gene-
rally—is, in fact, the rudimentary part referred to by Balfour
in the above quotation from the ' Comparative Embryology.'

The comparison has the advantage of bringing together the
growing points of the embryos in the two cases. In
Amniota the primitive streak is the growing point where the
cells are proliferated, out of which the greater part of the
embryo is formed. In Elasmobranchii the tail swellings
which form the sides of the dorsal and ventral parts of the
embryonic blastopore (my woodcut, *a b c d*) are the points
where the active growth takes place, as a result of which the
hinder part of the embryo is formed. Indeed, the prominence
of the tail swelling is due to the mass of mesoderm-cells pro-
duced by this proliferation at the edge of this part of the
blastopore.

The proliferation of mesoderm takes place in a rudimentary
fashion in Elasmobranchii, at all points of the circumference
of the blastoderm; which circumference, gradually creeping
over the yolk and enclosing it, constitutes the lips of the
widely open blastopore; but the proliferation is very feeble
except at the notched embryonic rim, the growth of which
forms, as above described, the tail end of the embryo.

It is interesting to notice the different manner in which the
tails of Elasmobranchii and Amniota are formed. There is in
the former no tail fold as in the latter, but simply a bilateral
bending round of the posterior tongue-like projection formed
by the growth backwards of the notched part of the embry-
onic rim.

The above account of the Elasmobranch blastopore is not
given for the first time, although when I did my work—now
some years ago—I was unaware that a correct account of the
process had been published by Schwarz in 1889 (' Zeit. f. wiss.
Zool.,' Bd. xlviii).

Kastschenko, in the previous year, published an excellent
paper on Selachian development in the ' Anatomischer An-

zeiger,' vol. iii, p. 445, in which he calls attention to the fact that Kowalevsky, in a paper published in Russian in about 1870, was the first to describe it correctly. That Kowalevsky's description, if correct, as maintained by Kastschenko, should have been overlooked, is of course attributable to the fact of its being written in Russian, and not reproduced in any of the more commonly known European languages. It seems a great pity that an observer of the eminence of Kowalevsky should thus secrete his work and render it unavailable to science.

Kastschenko's account of the matter is as follows :

" The closure of the medullary tube presents in the dog-fishes interesting peculiarities, which were first discovered and correctly described by A. Kowalevsky. . . . . The medullary folds are continuous at their hinder ends with the caudal lobes, and by means of the latter with the general edge of the blastoderm. Each caudal lobe presents a marked knee-shaped bend, the point of which is directed backwards.. The lateral limbs of the paired caudal lobes approach one another on the ventral side of the embryo ; and when the medullary folds fuse on the dorsal surface the adjacent caudal lobes also fuse. By the fusion of the former the medullary tube is formed, and by the fusion of the latter the neurenteric canal and the hind gut. The hind gut, therefore, is the immediate continuation of the medullary tube, and the neurenteric canal must be regarded as nothing else than a portion of the blastopore. Further forwards the hind gut remains for some time open ventralwards, but eventually this opening also fuses, the anus appearing considerably later in the same place."

This account, however, as will be gathered from my description, does not give the whole gist of the matter. It fails to notice the slit-like form of the dorsal part of the blastopore which perforates the floor of the medullary canal, and the author does not appear to understand, or at any rate fails to draw attention to the fact that the ventral opening leading into the hind gut is part of the blastopore, and is continuous with the slit-like non-embryonic part of the blastopore running along the yolk. The only point in which it supplements

Balfour's description is in the account given of the formation and of the at first open condition of the hind gut.

I quite agree with Kastschenko's remarks on the view that the embryo is formed by the fusion of two separate halves. It must, however, be admitted that the embryo is formed by a bilateral growth; that there are two growing points—one in each caudal lobe, which contributes to its development. With regard to the growth of the blastoderm, I agree essentially with Balfour, but I differ from him as to the growth of the embryo. His views are expressed in the following passage ('Comp. Emb.,' 1st ed., ch. iii, p. 35; Mem. Ed., vol. iii, p. 43):—"This rim [the embryonic rim] is a very important structure, since it represents the dorsal portion of the lip of the blastopore of Amphioxus. The space between it and the yolk represents the commencing mesenteron, of which the hypoblast on the under side of the lip is the dorsal wall. The ventral wall of the mesenteron is at first formed solely of yolk, held together by a protoplasmic network with numerous nuclei. The cavity under the lip becomes rapidly larger, owing to the continuous conversion of lower layer cells into columnar hypoblast along an axial line passing from the middle of the embryonic rim towards the centre of the blastoderm." The italics are mine, and are used to bring out the point in which my view is divergent from Balfour's. He regards the embryonic rim, at its first appearance, as marking the hind end of the future embryo, which is formed by a differentiation forwards of the blastoderm, as already established. I, on the other hand, regard the same point as marking the extreme front end of the future animal, and consider that the notched embryonic rim grows over the yolk uniformly with the rest of the blastoderm edge. It certainly does so extend itself, at any rate until the stage of my fig. 1, and of fig. 2 also, allowing for the shoot back of the caudal tongue. And it appears to me that this view—which is, to a certain extent, in accordance with the view of Roux on the growth of the Amphibian embryo ('Anat. Anzeiger,' vol. iii, p. 705)—must be looked upon as being nearer the

truth than Balfour's; for if Balfour's view is correct, the embryonic rim being stationary in growth backwards—all the differentiation being forward—ought, from the first, to be placed in a bay of the edge of the blastoderm.

According to my view, then, the blastoderm grows uniformly over the yolk at all points of its circumference. Indeed, its edge is everywhere raised into a marked ridge, which is continuous with the embryonic rim. The difference between the growth at the embryonic rim and elsewhere consists in the fact that, as the former extends over the yolk, a trail of columnar epithelial cells is left separated from the yolk by a space, whereas elsewhere the raised edge of the blastoderm simply slides over the yolk, leaving, as far as one can see, little (possibly a few mesoderm-cells) or no trail.

Further, it is clear, from what I have said above, that the notch of the embryonic rim represents the anterior end of the blastopore, and that on the view of embryonic growth above stated the blastopore does at one time or another perforate the whole length of the medullary plate. Posteriorly it does actually form for a short time a slit through the medullary plate, but anteriorly it keeps closing up as the embryonic rim grows backwards, so that it is never present in this region as more than a notch.

It will be maintained by some that this view of the growth of the embryo, and of the relation of the blastopore to the medullary plate, is incompatible with the objection to the concrescence theory above formulated. To this the reply would be that the body of the Elasmobranch embryo is no more formed by the fusion of two lateral halves than is the body of the Peripatus embryo, in which nearly the whole of the ventral surface is at one time traversed by the long blastopore.

The phenomenon we are in both these cases dealing with is the closure of the blastopore; and to talk about concrescence and fusion of two halves is merely obscuring the real question, and seeking to explain a process of growth by a phrase which has no satisfactory meaning.

Before leaving this part of my subject I may point out that

while the anus is formed within the area of the blastopore, and is in some Vertebrates actually a persistent part of the blasto-pore, in no Vertebrate has the mouth been traced into connec-tion with the blastopore. The fact that no such connection has been established is not surprising when one remembers how early the anterior part of the blastopore closes in Elasmo-branchs and Amphibia, and must not be taken as proving that the blastopore never extended in front of the present medullary plate on to the ventral surface of the head. I shall return to this question in the part of this paper which deals with the Vertebrate head.

It will be seen from the above account that the behaviour of the blastopore of Elasmobranchs—in its relation to the anus, neurenteric canal, and growing point—resembles very closely that of the frog as described in the admirable paper by Assheton and Robinson in vol. xxxii of the ' Quarterly Journal of Microscopical Science.'

2. On the Formation of the Mouth and Gill-clefts in Elasmobranchs.

I have had a number of drawings made of the head of embryos of Scyllium canicula to illustrate certain points in the formation of the mouth and clefts. Some of the points have been known before, and some are, I believe, recorded for the first time.

The mouth makes its first appearance in Stage I as a row of dots lying in the middle line between the two mandibular arches (fig. 5), and connected by a kind of shallow groove in the ectoderm, along which the ectoderm and endoderm are fused. These pores soon become connected (fig. 6) to form a long slit, which extends from the ventral point of junction of the mandibular arches forward along the depression between the latter as far as the pocket of ectoderm which is destined to give rise to the pituitary body. The first rudiment of the mouth actually extends into the rudiment of the pitui-tary body. At the front end of the buccal slit the fore-gut, the notochord, the ectoderm, and the mesoderm are all con-

tinuous with each other.   The mouth soon widens and shortens (figs. 8, 10, 12) until it attains its adult form.

The mandibular arch is at first directed almost from before backwards (figs. 5, 6, 7), and its anterior end is under the mid-brain.

The hyoid arch is also directed very much backwards, though not so much as the mandibular; and its anterior (dorsal) end is well in front of the auditory sac (fig. 7).

The branchial arches are also directed backwards, but the inclination is less in the posterior arches than in the anterior (fig. 9).

The question now arises, what is the meaning of this backward direction of the visceral arches?   The only answer that I can suggest to this question is that the same cause which has produced the flexure of the brain, and of the front end of the notochord, has affected the arches.   If this is so the cranial flexure should really be called cephalic flexure, for it affects not merely the brain, but all the organs of the head.

To account for this flexure we must either suppose that there has been a great forward extension of the dorsal anterior end of the head, which would carry the dorsal ends of the arches forward, and, if the anterior end of the notochord and the infundibulum, i. e. the anterior end of the cranial axis, remained fixed at the front end of the mouth, would also cause the flexure of the brain and anterior part of the notochord; or that there has been a great shrinking of the ventral parts of the head just behind the mouth.   If either of these views is correct, it necessarily follows that the mouth was originally a nearly vertically directed slit looking straight forward.   It may even have extended on to the dorsal surface.

The early slit-like form of the mouth is very remarkable, and may be regarded as being in favour of the view that the mouth is derived from the anterior part of the slit-like blastopore, though I admit that this does dot constitute a very powerful argument.

The extension forward of the first rudiment of the mouth into the pituitary pocket is also very remarkable.

In Scyllium and Raja the hyobranchial cleft is formed before the spiracular cleft.

It is interesting to notice in this series of heads the manner in which the at first straight mandibular arch is bent upon itself at the point which will become the point of articulation of the upper and lower jaws. The part anterior to the angle develops a forward projection and forms the upper jaw—the part behind is bent ventralwards and outwards and forms the lower jaw. The widening and shortening of the mouth seems largely due to this bending of the mandibular arch (cf. series of figures of heads in ventral and side view).

The view that the mouth is derived from the anterior end of the blastopore was originally put forward in my paper on " The Origin of Metameric Segmentation " (' Quart. Journ. Micr. Sci.,' 1884). Considering the early stage at which the anterior end of the blastopore closes in Vertebrates, and the relatively late appearance of the mouth, one would not expect to find any direct embryological evidence in support of this view. For the argument and indirect evidence in favour of it I refer the reader to pp. 73 et seq. of my paper above mentioned. To that evidence I now add the long slit-like form of the primitive Elasmobranch mouth.

3. Segmentation of the Mesoderm and Development of Nerves.

V. Wyhe[1] describes the cranial mesoderm in Scyllium as segmenting from behind forwards, and he says that in Stage I —and not before—the whole of the cephalic mesoderm is broken up into somites, and that all these somites contain a cavity except the first.

Kastschenko[2] says that the first somite is formed at what appears to be the junction of the head and trunk, and that the segmentation of the mesoderm extends backwards and forwards from this point. Anteriorly it becomes more and more

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[1] ' Ueber die Mesodermsegmente u. d. Entwick. d. Nerven d. Selachier-koppes,' Amsterdam, 1882.

[2] ' Anat. Anzeiger,' vol. iii, p. 462.

indistinct as the front end of the embryo is approached, so that the anterior part of the cephalic mesoderm is at no stage of development broken up into somites. This unsegmented part of the cephalic mesoderm, which corresponds, according to Kastschenko, to several somites, is comprised in the second somite of Wyhe. The first somite of Wyhe occupies, in Kastschenko's opinion, a special position. Kastschenko's observations were made on the genera Scyllium and Pristiurus, but he does not state precisely the ages of the embryos to which his observations refer, nor distinguish between the genera in describing his observations. As the different genera of Elasmobranchs differ, as I hope to show, very remarkably in the condition of the mesoderm and during these early stages, this latter point is one of considerable importance.

It is perfectly obvious to anyone who examines Elasmobranch development that the work of these two observers has been exceptionally thoroughly and carefully done; and if the results and views which I have arrived at differ from theirs, I would wish my work to be considered alongside of theirs, not as contradicting, but as supplementing it, by the future workers who succeed in obtaining a fuller and more accurate knowledge of the development of the different genera of this interesting group.

Balfour (' Elasmobranch Fishes,' Mem. Ed., p. 302), in describing Pristiurus, says that " coincidently with the appearance of a differentiation into a somatic and splanchnic layer the mesoblast plates become partially split by a series of transverse lines into protovertebræ." This statement I can entirely confirm for Pristiurus and Scyllium; its importance has not been fully appreciated or understood. What it means is this, that the body-cavity at the very first sign of its appearance (differentiation of mesoderm into somatic and splanchnic layers) is segmented.

Balfour goes on to say, " In the head, so far as I have yet been able to observe, the mesoblastic plates do not at this stage (D) become divided into protovertebræ." The term head

here must be regarded as meaning the anterior end of the body, for it is not possible in these young embryos to distinguish the head from the trunk. I am, however, in entire agreement with the statement that there is a stage in which there is a considerable tract of mesoderm in front of the first formed somite, which is entirely unsegmented, and with no signs of differentiation into somatic and splanchnic layers. But in Pristiurus this stage is of very short duration, for, according to Balfour, even in Stage D there is a cavity in the anterior part of the mesoderm. I can entirely confirm Balfour as to the presence of this cavity at this early age in Pristiurus; but it is not, as he seems to imply, ever continuons with the general body-cavity. It is, indeed, a somite—the second or mandibular somite of v. Wyhe,—and its appearance is followed by the breaking up of the mesoderm between it and the first so-called trunk somite into successive and contiguous but indistinct somites. I am not able to say in what order these somites are formed, whether from behind forwards, as Kastschenko maintains, or in the reverse direction. All I can say on this subject is that in Pristiurus the mandibular somite is formed before those behind it, and that in Scyllium I have an embryo a little older than Stage F, but younger considerably than Stage G, in which the whole of the mesoderm in front of the first so-called trunk somite is broken up into somites successively traceable in a series of transverse sections. The first of these somites (the second of Wyhe) is the most distinct and, I expect, the first formed, as in Pristiurus.

This early segmentation of the anterior part of the mesoderm into somites almost exactly like those in the hinder part of the body is a morphological point of great interest. It is very transitory in the genera mentioned, and disappears before any trace of the pharyngeal pouches are formed, except in the case of the mandibular somite, and possibly also of the one next it. In Stage I, where, according to v. Wyhe, the segmentation of the anterior part of the mesoderm is complete, I cannot find in either Scyllium or Pristiurus or Raja any of the somites described by him as the fourth, fifth, and sixth;

moreover the posterior limits of the third cannot be made out in Stage I.

Dohrn,[1] however, in his fifteenth study describes a complete mesodermal segmentation as occurring in Torpedo mar-morata at a stage in which the mandibular and hyo-branchial pouches could be made out. The embryos in question were considerably younger than the embryos in which v. Wyhe first observed the segmentation of the cranial meso-derm, and Dohrn ascribes them to Stage F; but the above pouches being present, he was able to compare his cephalic myotomes with those of Wyhe. He makes out ten myotomes in front of the hyoid pouch, arranged as follows:

4 myotomes in the place of Wyhe's first.
3   „   „   „   second or mandibular.
3   „   „   „   hyoid.
2 or 3 „   „   „   fourth.

He admits that they are very transitory structures, and that they have lost their distinctness (by fusion with one another) in Stage G, i. e. before the stage at which v. Wyhe first saw them. Having a very practical acquaintance with the great variation of the mesoderm in embryos of different genera of Elasmobranchs I do not venture to impugn the accuracy of Dohrn's observations on a genus which I have not examined; but knowing the extreme difficulty of satisfactorily observing these rudimentary cranial somites, even when they are un-doubtedly present, I cannot help feeling that it is desirable that Dohrn's statements should receive some confirmation. This confirmation is, to a certain extent, supplied by Herr Killian's[2] recently published work on Torpedo ocellata. I say "to a certain extent," because Killian's list of somites does differ slightly from that of Dohrn. I think that it is possible, and I trust that Dr. Dohrn (and Herr Killian) will forgive me for making the suggestion, that he has been misled by deceptive appearances afforded by the somites at the time of their disappearance. I know very well that in looking

[1] 'Mittheil. a. d. Zool. Station zu Neapel,' Bd. ix.
[2] 'Anat. Anzeiger,' Ergänzungsheft, 1891.

through any one series of sections it is very easy to make out what appears to be a great number of somites, but on carefully comparing the two sides of the embryo, and on estimating the intervals which the somites occupy, it is in my experience always found (after Stage F in the head region) that, with the exception of the first three head segments and the three posterior segments, these supposed somites are in embryos, in which the rudiments of the spiracle and hyoid cleft are apparent, quite irregular, and are either simply spaces in the mesoderm or remains of broken-down somites. This result comes out still more forcibly if one attempts to confirm one's observations on one embryo by similar observations on another embryo of the same size.

But even if Dohrn is right in his enumeration of the anterior somites, it is clear that Torpedo differs much from Scyllium, Raja, and Pristiurus, whether my account or Wyhe's be taken as correct. For in Torpedo there are four somites where in the other genera there is most unquestionably one, e. g. the somite of Wyhe.[1]

It would appear, then, that if the number of primitive cranial somites in any given region of the head does really differ in closely allied genera in the manner indicated by the divergent observations of Wyhe, Dohrn, Killian, and myself, the supposed indications of segmentation which are found in the adult, and are constant throughout the Vertebrata, can have very little value as real tests of the primitive metameric segmentation—of the segmentation which obviously persists in the trunk region, and which begins with the segmentation of the mesoderm, and is moulded upon it in the manner characteristic of all metamerically segmented animals.

We may, I think, even go further, and say that the adult arrangements of nerves and branchial arches, &c., characteristic of the Vertebrate head, must have arisen subsequently to

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[1] I leave out of consideration the supposed somite anterior to the premandibular somite (first of Wyhe), which has been described by some observers in Acanthias, Torpedo, &c. I have seen traces of it in Scyllium, but it is in that genus merely a diverticulum of the premandibular somite.

the disappearance of the primitive segmentation. This position will be still further strengthened if my contention turns out to be correct, viz. that in embryonic development the meso-dermal cranial segments do largely become indistinguishable before the adult landmarks have appeared.

If my arguments and facts are sound, it follows that any attempt to elucidate the structure of the adult head from the point of view of its being composed of a series of segments comparable to those of the trunk is foredoomed to failure; and the result of the whole inquiry shows up most thoroughly the weakness of the position of those who hold embryological research to be of small importance in comparison with the study of adult structure.

To a student of the multitudinous changes of structure which an organism passes through in the course of its existence it seems strange even now, and in the future will ever seem stranger to the philosophical morphologist, that one condition of structure only, and that the most complex and inexplicable, should have been regarded by anyone as holding the key to the solution of even a simple anatomical problem.

To sum up the matter, v. Wyhe holds that there are nine cranial segments which can be traced into the adult. Dohrn holds that there is a much greater number of cranial somites, some of which can be traced into the adult, and some of which disappear. I agree with Dohrn in asserting that the anterior mesoderm is completely segmented in Stage F, but maintain, in opposition to him, that it is not possible to say how these segments are related to adult structures, because they have for the most part vanished before any of the adult landmarks have appeared.

The premandibular somite of Balfour (the first somite of Wyhe).—There can be no doubt this is not, as Balfour sup-posed, separated off from the mandibular.

Kastschenko says that it develops from what he calls the prechordal portion of the gut, which becomes solid when the medullary plate is formed, and then subsequently again acquires a cavity. I find myself unable to accept this account

of the origin of the first somite. It is true that at the time
of the formation of the medullary plate the notochord stops
some little distance short of the front end of the body, and
there is a portion of the gut in front of it; but this is only a
temporary state of affairs, and is due to the fact that the front
end of the notochord, which is developed from behind for-
wards, is not yet formed: moreover the solid mass of endo-
derm referred to by Kastschenko is present at the front end of
the gut even at this stage. When the notochord has acquired
its furthest anterior extension in Scyllium, just before
Stage G, it terminates in a solid mass of cells, which is con-
tinuous also with the front end of the gut. The notochord
has hitherto during the whole of its growth been continuous
in front with the endoderm, and its condition at the period
referred to is merely a persistence of that continuity. Wyhe's
account of the anterior end of the notochord appears to me to
be quite correct.

When the notochord has acquired its utmost anterior
extension there is no portion of the gut in front of it, but
merely this solid mass of cells, with which both it and the gut,
and afterwards the ectoderm of the buccal slit and pituitary
body, are continuous, and which underlies the very front end
of the medullary tube. If this mass of cells be regarded as
partly consisting of the anterior end of the notochord still un-
differentiated, it may be said that the notochord reaches in
Scyllium, at any rate, to the very front end of the neural
tube; in other words, that Scyllium at this stage is truly
cephalochordate in the sense that Amphioxus is cephalo-
chordate.

The solid mass of cells in which the notochord and gut
terminate becomes in Scyllium and Pristiurus very early,
before Stage G, connected with the ventral ectoderm. Wyhe,
who connects this fusion with the formation of the mouth,
puts it down as taking place later in Stage H; but I can posi-
tively assert that in Scyllium and Pristiurus it is present
before Stage G—before any trace of the cranial flexure has
appeared.

There can be no question that the first or preoral somite develops in connection with this solid mass of cells, but whether entirely from it, as Wyhe appears to maintain, or only partly from it, is difficult to say.   In Scyllium there are very clear indications that a part of the tissue from which the somite develops is derived from a paired ingrowth from the ectoderm.   In Stage G the cell mass is continued forwards on each side in continuity with the ectoderm, and these paired tracts present the appearance of ingrowths.

The mass of cells of which I am speaking presents very remarkable differences in its relation to adjacent organs in the different genera that I have examined.   In Scyllium and Pristiurus it is continuous with the ventral ectoderm throughout its whole extent from the earliest stage at which I have seen it, i. e. Stage F, or the earliest stage at which the ventral ectoderm is folded in.

In Scyllium it is for the most part not continuous with the medullary ectoderm, unless there is such a continuity, of which I am not certain, at its very front end.   In Pristiurus and Raja it is markedly continuous with the medullary ectoderm throughout its entire extent, while in Raja the dorsal lateral outgrowths, which are soon formed from it, are also continuous with the medullary ectoderm.   Further, Raja differs from the other two genera in that this cell-mass is not continuous with the ventral ectoderm at all (excepting through the endoderm and buccal slits).

As Wyhe has correctly stated, the first or premandibular somite of Balfour is formed by the hollowing out of this mass of cells and its lateral prolongations, and Kastschenko seems to be justified in placing it in a different category from the other somites.   It differs from the other somites in two respects: (1) in its connection at origin with the ectoderm, either of the body-wall or of the neural tube (Raja, Pristiurus) ; (2) in its continuity with its fellow across the middle line.

Before leaving this cell mass which gives rise to the first somite, and which eventually breaks off from the various

organs with which it is at first continuous, i. e. notochord, ectoderm, and gut, I should like to point out a resemblance in its early condition to the primitive streak of the Amniota. Like the primitive streak, it is a densely packed mass of nuclei in continuity with all the layers and organs of the body. The ectoderm, endoderm, notochord, and mesoderm, all are continuous with it; and as the primitive streak is the growing point for the hind end of the embryo, so it appears to contribute in a similar manner to the front end.

The Anterior Somites in Raja.—In Raja the segmentation of the anterior mesoderm and the prominence of the first two somites are not nearly so conspicuous as in the other genera. The condition of the anterior mesoderm after its separation from the endoderm is quite different from that in Scyllium and Pristiurus. It does not assume the condition of an " epithelium " arranged round the cavities or the incipient cavities of somites. On the contrary, it at once assumes the form of " embryonic connective tissue," i. e. of a mass of stellate cells all connected together by their processes. In other words, it at once takes on the form which is only secondarily attained by the same mesoderm of the two other genera after passing through the epithelial condition. This difference in the early structure of the cephalic mesoderm of Raja and Scyllium is another proof, if such were needed, that the distinction between " mesenchyme " and epithelial mesoderm to which the Hertwigs have so prominently called attention has not the importance which they attribute to it. The cavities of the first two somites make their appearance in this stellate mesoderm at about Stages G, H. But they are at first inconspicuous, having the appearance of blood-vessels, and are without the conspicuous epithelial lining. In fact, the cells lining them have at first simply the characters of the reticulate mesoderm tissue, of which, indeed, they are merely a part.

CONTINUITY OF CELLS AND LAYERS.

The continuity between the different layers and organs of the embryo to which I first called attention in Peripatus is found in all Vertebrate embryos that I have examined. In fact, there is a network of pale protoplasmic fibres extending inwards from the nucleated protoplasm of the various surfaces. When this network has nuclei at the nodes, we get the reticulated tissue, or embryonic mesoderm, or mesenchyme. In Scyllium it is at first sparse and without nuclei. In Raja, on the other hand, it is very richly developed, and rich in nuclei. In Raja, in other words, the protoplasmic connections passing between the various organs and layers are very conspicuous and well marked. In Scyllium this tissue is at first without nuclei, as I have said. But soon it acquires nuclei and becomes denser. Where do the nuclei come from ? In my opinion they are derived partly from the epithelial walls of the somites, partly from the anterior mass of mesoderm in which the notochord, gut, &c., ends, and partly from the growing tissue of the caudal swellings, and perhaps also from the neural crest.

We now pass on to speak of the neural crest in those Elasmobranchs which I have studied.

The nerve crest was first discovered by Balfour in the trunk region of Elasmobranch embryos. Marshall[1] has observed it in the chick, and describes it as occurring in the anterior part of the spinal cord region and extending continuously forward into the fore-brain.

Van Wyhe and Kastschenko also both describe the nerve crest in the Elasmobranch embryos they examined as reaching from the region of the fore-brain continuously backwards. The cranial nerves and the posterior roots of the spinal nerves grow out from the nerve crest, and the nerve crest persists itself in part as the longitudinal commissure. Both Balfour and Marshall state that this longitudinal commissure extends

[1] 'Quart. Journ. Micr. Sci.,' vol. xviii.

back continuously from the root of the glossopharyngeal to the spinal cord, connecting together the posterior spinal roots and the roots of the vagus and glossopharyngeal. It is not, however, developed in front of the glossopharyngeal, the nerve crest atrophying between the ninth and seventh, and between the seventh and fifth nerves.

My observations agree with this account except in one point, and that relates to the nerve crest. In Scyllium and Pristiurus the nerve crest is not a continuous structure, as Wyhe and Kastschenko assert (Balfour and Marshall have no observations on the cranial part of the nerve crest in Elasmobranchs). It is in three separate pieces. The first of these is found in the anterior part of the brain; the fifth nerve and presumably the ophthalmicus profundus grow out from it. The second is found a little further back, and gives origin to the seventh and eighth nerves. The third piece occurs a little further back, and reaches from the hind brain continuously back the whole length of the spinal cord. The ninth and tenth cranial nerves and the posterior roots of all the spinal nerves grow out from it. It is this latter part of the nerve crest which gives rise to the longitudinal commissure of Balfour.

There are three views as to the origin of the peripheral nerves.

1. According to Hensen's[1] view, the rudiments of the nerve-fibres are present from the beginning as persistent remains of the primitive connections between the incompletely separated cells of the segmented ovum.

2. Balfour[2] regarded them as cellular outgrowths from the central nervous system extending to the periphery. The original continuity between the central and peripheral organs, which must have existed, has, it was supposed, been lost in ontogeny by rupture, and reacquired by means of these outgrowths.

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[1] 'Virchow's Archiv,' vol. xxxi, 1864.

[2] 'Development of Elasmobranch Fishes,' Mem. Ed., p. 384, vol. i.

3. The view of His,[1] which was previously held by Bidder. According to this view the nerve-fibres are the elongated processes of cells. The anterior roots are derived from non-cellular outgrowths of the spinal cord, consisting of the elongated processes of the nerve-cells of the central organ. The fibres of the posterior roots, on the other hand, are the elongated processes of the ganglion-cells of the ganglion on the posterior roots. Processes of these cells grow out to the periphery and inwards to the centre.

Balfour expressed on a priori grounds a strong preference for the view of Hensen, but rejected it on the ground that there was no evidence for the connection which it demanded. Now, however, we know that in many types the segmentation of the ovum does not bring about a complete separation of the cells of the ovum.[2]

There is no such separation in Peripatus; and in many Arthropoda—if not in all—it is known not to take place. It does not take place in Elasmobranchs, as I can certify from my own observations; but for a summary of the facts and a discussion of the whole question I must refer the reader to my monograph already quoted.[3]

If the segmentation of the ovum does not bring about a complete separation of the cells of the germ, as it was formerly supposed to do, then the connections required by Hensen's theory exist.

Turning to the special case before us of the Vertebrata, I have in the present paper dwelt upon the fact (see above, p. 581) that the cells of the young embryo (subsequent to cleavage) are connected by delicate processes, and that these processes are often extremely fine, and unite together into networks below the epithelial arrangement of the protoplasm which is characteristic of the surfaces. This network is sometimes of a very

[1] 'Anatomischer Anzeiger,' vol. iii, p. 500.

[2] See Self, "Monograph on the Development of Peripatus capensis," in 'Studies from the Morphological Laboratory of the University of Cambridge,' 1889, pp. 47—50, and pp. 130, 131.

[3] Loc. cit., pp. 99—106.

loose mesh, and its fibres are always delicate; and it is no doubt often torn and destroyed by the preserving processes to which the embryo has to be subjected. But delicate as it is, there can be no doubt of its existence in Vertebrate embryos; and there can be no reasonable doubt that it is derived from the processes and strands left between the cells as a result of the incomplete cleavage of the ovum. There can be no doubt, I say, that the network exists; but that the peripheral nerve-fibres and the central nerve-fibres are derived from it has not yet been shown. That is the point which now needs investigation, and I hope myself to treat of it in a future paper.

Meanwhile I may say that there is in my opinion evidence to show that the whole of the nervous connections (by nerve-fibres and otherwise), both in the central organ and at the periphery, are developments of this pre-existing network, which connects together at all times the whole of the cells derived from the fertilised ovum.

I do not dispute for one moment the description given by Dohrn[1] of the structure of particular stages in the development of a nerve-fibre; but in saying that it consists of a row of ecto-derm-cells laid on end to end he is, I think, going beyond his facts, being led to such an interpretation of the appearances not so much by observation of previous stages as by a process of reasoning based upon the cell theory of structure, which theory implies that the animal body at one stage of its onto-geny consisted of cells which are separate from one another and only secondarily fuse to form the adult tissues and com-binations.

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[1] 'Studien z. Urg. d. Wirbelthierkörpers,' No. 17.

EXPLANATION OF PLATE XXXV,

Illustrating Mr. Sedgwick's "Notes on Elasmobranch Development."

Fig. 1.—Embryo of Scyllium canicula, $2\frac{1}{2}$ mm. in length. The hinder end of the embryo is notched. The medullary groove is just beginning. The tail swellings of Balfour are well marked.

Fig. 2.—Embryo of Raja ? sp., 4 mm. in length. The medullary groove is closed except at the hind end. The notched embryonic part of the edge of the blastoderm has grown faster than the rest, and come to project over the surface of the yolk. The sides of this projection are already slightly bent ventralwards. They will eventually meet and form the ventral part of the caudal region of the body.

Fig. 3.—Raja ? sp. Embryo of Stage E or F, $4\frac{1}{2}$—5 mm. in length. The medullary canal is still open, but the medullary folds are almost touching except behind, where the medullary canal widens out in a wide medullary groove, in the floor of which is placed the dorsal part of the blastopore. The blastopore is slit-like, but dilated in front; posteriorly it is continued round the hind end of the body into the ventral portion.

Fig. 4.—Raja ? sp. Stage E or F, 5—$5\frac{1}{2}$ mm. in length, a little older than Fig. 3. Medullary canal closed except behind, where it widens out and encloses the blastopore. The blastopore is slit-like, but the hinder end of the dorsal portion is faintly marked.

Figs. 3 and 4 are somewhat diagrammatic, but they show correctly the relations of the medullary groove and dorsal part of the blastopore. I hope to publish figures of the sections through them shortly.

Fig. 5.—Ventral view of head of Scyllium canicula between Stage I and K. Total length 7—8 mm. The two first pharyngeal clefts are open. The mouth rudiment is present as a longitudinal groove in the ectoderm of the buccal depression, which is fused with the endoderm. At intervals there are perforations along this groove. The groove reaches into the rudiment of the pituitary body. The mandibular arch is present as a backwardly directed longitudinal ridge, and bounds the buccal depression externally.

Fig. 6.—Ventral view of head of Scyllium canicula a little older than the preceding. The buccal groove has become a longitudinal slit.

Fig. 7.—Side view of head of Scyllium canicula a little younger than Stage K. Total length about 9 mm. I could not distinguish any trace of

the limbs. I do not think the fourth slit is open. The posterior end of the mandibular arch is slightly bent ventralwards.

Fig. 8.—Ventral view of head of same embryo drawn to a slightly smaller scale. The anterior part of the buccal slit has become much wider.

Fig. 9.—Side view of head of Scyllium canicula about Balfour's Stage K. Total length about 11—12 mm. External gills have appeared on the first and second branchial arches. The ventral bend of the hind end of the mandibular arch is more marked.

Fig. 10.—Ventral view of the same head drawn to a slightly smaller scale. The future angle of the jaw can be distinguished, the mouth being widest at that point. The posterior slit-like part of the mouth is still present.

Fig. 11.—Side view of head of Scyllium canicula about Balfour's Stage L. Total length about 16 mm. The external gills have increased in number, and are present on the mandibular arch. The angle of the jaw where the lower part of the mandibular arch bends ventralwards is very marked.

Fig. 12.—Ventral view of same head drawn to a smaller scale. The mouth has much widened, and the posterior slit-like part has almost entirely disappeared. The anterior part of the mandibular arch has a process towards the middle line. The hinder end of the body has been tilted upwards so as to bring the fronto-nasal process into view.

On the Paired Nephridia of Prosobranchs, the Homologies of the only remaining Nephridium of most Prosobranchs, and the Relations of the Nephridia to the Gonad and Genital Duct.

By

Dr. R. v. Erlanger.

With Plates XXXVI and XXXVII.

Introductory and Historical Part.

By the study of the development of Prosobranchs and fresh-water Pulmonates, especially of Paludina vivipara (8), Bythinia tentaculata (9), and Planorbis corneus (10), I was led to adopt Professor Ray Lankester's (19) view on the homology of the only remaining kidney or nephridium of most Prosobranchs. I found that the kidney of Paludina and Bythinia, which in the adult lies to the left of the anus, was, before the torsion takes place, situated to the right of the anus, and consequently must be homologous to the actual left kidney of Fissurella, Patella, Haliotis, Trochus, &c. Whilst in so-called leiotropic species[1] the actual kidney is the left one, it is just the contrary in dexiotropic species, such as Planorbis, where the actual right kidney is, before the torsion, situated to the left of the rectum. I also found in Paludina a rudiment of the actual right kidney lying before the torsion to the left of the rectum, and observed that its duct was converted into that of the genital gland. Comparing

[1] Leiotropic would correspond to the German term "rechtsge-wunden," as used by conchologists ; dexiotropic to "linksgewunden."

this fact with the statement that the right kidney of Zygo-
branchs and Cyclobranchs serves as a duct for the sexual
products, I was led to the conclusion that the actual right
kidney disappears or becomes highly modified, while its duct is
converted into that of the genital gland.

Reading the works on the comparative anatomy of Gastro-
pods, I was struck by the contradictory statements about the
reno-pericardiac duct in Zygobranchs, Cyclobranchs, and
Prosobranchs. Ray Lankester, in his first paper on Patella
(18), stated that he found a communication between the actual
left kidney of Patella and the pericardium. In a second
paper he only describes a right reno-pericardiac duct in the
same species. V. Jhering (16) could not find any communi-
cation between either kidney and the pericardium in Fissurella
and Patella, at least, as he says, with any certainty, while he
was able to find it in Haliotis. Bontan (2) failed to see the
left kidney in Fissurella, and could not find a reno-peri-
cardiac canal in the right kidney. B. Haller (13) describes a
right reno-pericardiac duct in Fissurella. Wegmann does
not mention any communication for either kidney and the
pericardium in Patella (28), although he found a left reno-
pericardiac duct in Haliotis (27), where the right kidney has
no opening into the pericardium. Cunningham (7), at Ray
Lankester's suggestion, re-examined Patella, and found a
reno-pericardiac duct for both kidneys. The last anatomist
who studied the kidneys of a great number of Prosobranchs, R.
Perrier (23), describes a right reno-pericardiac duct in
Fissurella and Patella, but could not find a left one in
either of these two species. On the other hand, he found only
a left pericardiac duct in Haliotis, Trochus, and Turbo.

The conclusion to be drawn from this historical résumé is,
I think, that the subject was well worth a new investigation.
I accordingly have studied five different species of Fissurella,
Emarginula, Puncturella (closely related to Rimula),
one species of Patella, a Tectura, one species of Haliotis,
one of Trochus, and a Turbo. My results, as the sequel
will show, are somewhat surprising as regards Fissurella and

Patella, whilst they only confirm prior investigations as regards Haliotis, Trochus, and Turbo.

I further hope to offer a plausible explanation for the contradictions between my predecessors and myself, and must add that to attain this end I expended more time and trouble than it cost me to arrive at the conclusions which I intend to describe in this paper.

Methods employed in the Present Investigations.

In order to fix the tissues as rapidly and as perfectly as possible I made use of the following fluids, viz. Kleinenberg's fluid (picro-sulphuric) with a few drops of osmic acid, and a mixture of sublimate and glacial acetic.[1] The specimens of Patella and Fissurella were dropped living into these fluids, left there for about a quarter of an hour, then thoroughly washed with water, and afterwards hardened in spirit from 40 per cent. up to 90. I tried Flemming's fluid (chromosmic acetic), but was obliged to discard it, as it made the tissues too brittle. When the specimens have been in spirit some time they can be easily taken out of the shell by pulling them gently with a pair of forceps. They then come out perfectly uninjured.

For Trochus I had to use a different method, as the shell and operculum make the animal impervious to the fixing liquid. The shell in the species which I investigated is so thick and hard that it cannot be removed while the animal is living without injuring it severely. I therefore put the Trochuses into sea water and 1 per cent. absolute alcohol (according to Signor Lo Bianco's method) in order to draw the anterior half of the body out of the shell. After a day or two the animals are paralysed without being killed. It is then possible to pull the anterior half of the body out of the shell and to plunge them into the fixing liquid, which can then easily penetrate into the shell and the mantle cavity. The specimens were then hardened in spirit for a few days. It is then

[1] Sublimate 5 per cent. in sea-water 3 parts, and 1 part of glacial acetic acid.

possible to crack the shell with a hammer and to remove it with a pair of forceps without injuring the specimen in any way.

The rest of my material was not obtained fresh, but had been preserved in spirit. The preservation was satisfactory in nearly all cases.

The stain used was principally alum carmine, which is very valuable for molluscs, and can be used to stain in bulk. In many cases I used hæmatoxylin or methyl green as a plasma stain. They easily penetrate when only a part of a specimen has been prepared for sections.

In most cases I sectionised only the pericardium and the mantle cavity, after having removed the foot and other organs with a razor or a pair of scissors. In this way a great deal of time and trouble may be saved, and the stains penetrate more easily. It is of course necessary to have hardened the specimen thoroughly beforehand. By the same method it is easy to dissect out the pericardium, mantle cavity, and kidneys. Such a preparation can then be mounted whole in balsam, and is most useful for the topography of the different organs.

The sections were cut with Yung's microtome; the embedding was done in chloroform and paraffin. Besides dissections and sections I also made use of injections, at the special request of Professor Ray Lankester. I myself am strongly prejudiced against this method, as it is very likely to mislead. I used soluble Berlin blue, and injected by blowing the solution through a fine glass pipette with the mouth. Applied in this way the injections method confirmed the results obtained by dissection and sections in every case. When, on the contrary, I used a syringe and a strong pressure, the coloured fluid often broke through the walls of the pericardium and kidney in places which I consider as loci minoris resistentiæ, and which shall be described in the sequel.

Before beginning my correspondence with Professor Ray Lankester on the points at issue I had always injected from the pericardium, as morphology and physiology show that the liquid which fills the pericardium is expelled by the kidney,

and consequently in a centrifugal direction.   By request of Professor Ray Lankester, I made some more injections from the kidneys, with the same results, which I have already described.   Personally I do not think the latter method of injection a good one.   I consider it even less reliable than the first, as a great many animals possess a valve between the pericardium and the reno-pericardiac duct, in order to prevent the excreted liquid from flowing back into the pericardium.

The shells of most of the species of Prosobranchs studied by me in the course of these investigations were determined by Professor Boettger, in Frankfort-on-the-Main, and Dr. Kobelt, in Schwanheim, near Frankfort-on-the-Main.   I beg both these gentlemen to accept my thanks for having so readily fulfilled my request.

DESCRIPTIVE PART.

I propose to begin with Haliotis and Trochus, where a reno-pericardiac canal (left) really exists.   I shall afterwards describe the other forms in which no such a structure exists. I adopt this order so as to show that I have not overlooked the communication between pericardium and kidney, which is really quite easily to be found in Haliotis, Trochus, and Turbo, even by dissection alone, whilst in Fissurella and Patella no trace of such a structure could be found.

1. Haliotis tuberculosa.

This species has been studied by v. Jhering (16), Wegmann (27), Haller (13), and Perrier (23).   I am happy to be able to confirm Jhering's, Wegmann's, and Perrier's statements.   Jhering's figures are certainly a little schematic, as Haller remarks; but whilst Haller's figures are very good and exact, his interpretation is quite incorrect.   He considers the duct of the right kidney exclusively as the genital duct, and describes a communication between the right kidney and the left (Haller's papillary sack) which really does not exist at all.

Fig. 1, a view of the dorsal surface of Hal. after removal of the shell shows the pericardium (Pc.) in situ at the hinder

end of the mucous gland (*muc.*), and the mantle cavity (*M.*). To the left lies the left kidney (*Nl.*) which is considerably smaller than the right one (*Nr.*), of which a part can be seen on the right side of the pericardium. All these organs are viewed by transparency through the mantle. In order to display the external openings of the kidneys, it is necessary to lay open the mantle cavity and to turn back the rectum, which is dorsally attached to the mucous gland. A dissection of this kind is figured in fig. 2, where both nipples are to be seen (*Xl.* and *Xr.*) at the base of either ctenidium (*Bl.* and *Br.*), and to either side of the rectum (*R*). The right ctenidium (*Br.*) is about a fifth smaller than the left. This fact has already been mentioned elsewhere, but I desire to lay special stress on it, as it will be made use of in the comparative part of this paper.

The right kidney is a large gland, the shape and relations of which have been accurately described by Wegmann (27), while Perrier (23) has ably figured its histological structure. Having no intention of entering into histological detail I simply refer the reader to Perrier's memoir on this subject.

The left kidney has the shape, and in large specimens the size of a hazel-nut; the numerous papillæ which it contains give it externally a velvety appearance (Wegmann).

When the pericardium has been dissected out, opened transversely from behind and the heart (ventricle and both auricles) removed, the opening of the left reno-pericardiac duct into the pericardium can be easily seen (fig. 3, *Y*); it is situated to the left of the rectum, and lies between this and the left auricle. This is shown in a frontal section (fig. 9). The reno-pericardiac duct is, as Perrier has already stated, a canal of no inconsiderable length, and its presence can be readily detected in frontal sections, while, on account of its direction, it is somewhat more difficult to trace in transverse sections. There is no proper genital duct in Haliotis, and the gonad communicates with the right kidney by a slit-like opening, which, according to Perrier (23), has a special valve closed in all times except in that of sexual maturity.

2. Trochus turbinatus, Born.

The topography of the pericardium and kidneys is practically the same in Trochus as in Haliotis; Turbo is so nearly allied to Trochidæ that I think it unnecessary to give a full description of this form.   My friend Mr. Macbride and myself have dissected two large specimens of Turbo, and have found that it differs but very slightly from Trochus, and these differences are so small that they may be neglected entirely with regard to the points which form the subject of this memoir.

Haller has made the same erroneous statements about Turbo as about Haliotis, which have already been corrected by Perrier, with whose observations my own agree perfectly.

When the mantle cavity has been opened by a longitudinal section parallel to the attached side of the only remaining left ctenidium (*Bl.*, fig. 4), and viewed from the ventral surface, the rectum (*R.*) is seen in the shape of a long tube opening into the mantle cavity (*M.*) by the anus (*A.*), which is situated at about the fifth of the total length of the gill from its proximal end.   To the left (in reality the right) of the rectum the duct of the right kidney is seen as a long tube opening into the mantle cavity by an orifice (*Xr.*).   To the right (in reality the left) another wider tube is to be seen, which does not run parallel to the rectum as the duct of the left kidney.   This tube or sac is the left kidney, which also opens into the mantle cavity by a button-hole-like orifice (*Xl.*).   The left kidney or papillary sac extends back to the pericardium (*Pc.*), which has been opened in order to show the two auricles of the heart. The axis of this organ is oblique to the longitudinal main axis of the body—a fact which is already apparent in Haliotis, but to a lesser degree.

In a series of transverse sections beginning at the anus, or, better, at the point at which the rectum issues from the pericardium anteriorly, and extending back to the point at which the rectum issues from the pericardium posteriorly, a large sinus (*bas.*, fig. 13) can be discerned lying ventrally to the

rectum (*R*.), and above the mantle cavity (*M*.). This sinus is morphologically equivalent to the basibranchial sinus existing in Haliotis and in Fissurella-like forms, where we shall meet with it again. Perrier (**23**) and Bernard (**1**) have shown that the blood coming from the right kidney, the head, and the epipodium is collected here, and hence is sent to the ctenidium or ctenidia.

The vein (*v*.) which in Trochus runs into the afferent vein of the ctenidium is seen to the left of the rectum, dorsally from the left kidney (*Nl*.) or papillary sac. This organ has the closest resemblance to the corresponding one of Haliotis, and shows a great number of the characteristic papillæ.

The right kidney (*Nr*.) is to be seen on the same section to the right of the basibranchial sinus. The section only shows the part which acts as a reservoir for the urinary secretion, and has been called urine chamber (Urinkammer) by B. Haller (**13**).

Projecting into the ventral wall of the papillary sac can be seen the transverse section of a duct which lies between the mantle cavity and the papillary sac or left kidney. In fig. 3 (*Y*.) it is seen to open into the left kidney; two or three sections further back this communication is entirely obliterated (*C*, fig. 8). Proceeding still further backwards, the duct in question can again be seen to open into a space lying ventrally to the basibranchial sinus (fig. 12), and which, as another section still further back clearly shows, is a ventral and anterior prolongation of the pericardium (*Pc*.). In fig. 12 two distinct portions of this cavity may be seen (*Pc*. and *Pc*.), the one already described and opening into the reno-pericardiac duct (*y*.), the other separated from the first by the basibranchial sinus (*bas*.) lying immediately below the rectum, which is already enveloped by the ventricle (*V*.). Fig. 7 shows that further backwards the two portions unite (*Pc*.), and that now the basibranchial sinus (*bas*.) is divided by the pericardium into two separate halves.

By comparing the transverse series with horizontal ones in which the reno-pericardiac duct can be readily found, and with

dissections, it can be clearly seen that the portion of the pericardium leading into the reno-pericardiac duct is a longish prolongation extending ventrally and anteriorly to the heart. Its position corresponds to that of the reno-pericardiac duct already described in Haliotis.

The right kidney, the properly secreting part of which is to be seen in fig. 12 (*Nr.*) below the urine chamber, does not communicate either with the pericardium or with the left kidney or papillary sac. There is a very short true genital duct in Trochus and Turbo. The gonad which lies quite close to the right kidney discharges its products into the mantle cavity, as v. Jhering has already stated.

3. Fissurella, Emarginula, Puncturella.[1]

These species, differing exceedingly slightly in the general appearance, position, and histological texture of both kidneys, can be all described at a time.

I examined five different species of Fissurella, viz. the four different species which are to be found in the Gulf of Naples, that is to say, Fissurella costaria, Bast. (=mediterranea =europæa), Fiss. græca, Linné, Fiss. nubecula, L., and Fiss. gibberula, Lamarck. I also dissected a Chilian species, which I believe to be the Fiss. maxima.

I think that a few hints concerning the determination of the four species found at Naples may prove useful, as I found considerable difficulty in determining them with the diagnoses given by conchologists.

Fiss. costaria, also called reticulata, is the largest species, and can attain a length of 4 to 5 cm.; the shell, which anteriorly is narrower than posteriorly, is provided with about twenty well-marked ribs diverging from the fissure; between these ribs there are several others less well defined. Fiss. græca is generally about half the size of the species which I have just described, the shell is just as broad in front as behind, the ribs are all about the same size, and formed by a succession of scale-like processes of the shell, which make it

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[1] Pelseneer (**22**) has found the left kidney in Scutum (Parmophorus).

much rougher than that of F. costaria. Fiss. gibberula is mostly about a third smaller than F. græca, and can be easily recognised, as the part of the shell situated behind the fissure is higher than that situated before the slit. The shell has, therefore, a hunchbacked shape, whence the Latin name. The shell of F. gibberula is nearly quite smooth.

If the shell of the three species I have just described be placed with the aperture downwards, the slit upwards, on a table, it can be readily noticed that the shell only touches the table at its anterior and posterior end. If, on the contrary, the same experiment is made with the shell of F. nubecula it will be seen that the rim of the shell touches the table in every point of its circumference. F. nubecula is the smallest of the Neapolitan species, being generally smaller than F. gibberula; occasionally, however, very large specimens get nearly as big as those of F. græca. The shell of F. nubecula is much flatter than those of the three other species.

I could state a great many other minor points by which the four species I have just described might be distinguished, but I think that those I have mentioned will suffice, as they afford a good and convenient criterion. The Emarginula I examined also belongs to the fauna of the Gulf of Naples, but is difficult to obtain. Dr. Kobelt, in Schwanheim, near Frankfort-on-the-Main, who had the kindness to determine a part of my material, labelled it Emarginula Huzardi, Pay. This species is rather scarce here, and I worked on specimens which had been preserved in strong spirit by Dr. Schiemenz, who kindly placed several of them at my disposal. The preservation was quite satisfactory.

The Puncturella, which like Fiss. maxima is a Chilian form, was collected by Chierchia in November, 1882, and was less satisfactorily preserved, the gills and epithelium of the mantle cavity being somewhat macerated. Fortunately the preservation of the kidneys was good.

B. Haller (13) having given a very fine figure of the shape and position of the right kidney of Fissurella costaria I thought it superfluous to give another. When the shell has

been removed the right kidney is seen surrounding the peri-
cardium on the right side.   It sends a prolongation to the
right, which extends further forwards than the pericardium
itself, and another one to the left, so that the pericardium is
entirely surrounded by the right renal organ, which extends
far back nearly to the posterior end of the animal.   In order
to see the left kidney it is necessary to remove the mantle
funnel which projects through the slit of the shell (fig. 22, *Fi.*),
with the underlying sinuses of the funnel, also to open the
pericardium dorsally and to lift up the left auricle.   It is
better to remove the heart entirely by cutting the rectum
anteriorly and posteriorly to the ventricle, the two auricles at
the origin of the branchial efferent veins, and the aorta (*ao.*),
which issues from the ventricle posteriorly, ventrally, and on
the left.

Such a dissection is to be seen in fig. 5.   The bottom of
the pericardial cavity is displayed (*Pc.*) ; on the right a part of
the right kidney (*Nr.*) is seen by transparency through the
ventral wall of the pericardium.   *R.* and *R.* are the two cut
ends of the rectum on its way through the pericardium ; *ao.* is
a section of the aorta, which has just left the heart and
pierces the ventral wall of the pericardium.   The left kidney
(*Nl.*) is exceedingly small compared with the huge right renal
organ.   It lies embedded in the anterior wall of the peri-
cardium, between this and the basibranchial sinus. It generally
has the shape of a sac (fig. 19) provided with a bent duct
leading into the terminal papilla.   Sometimes, however, as in
fig. 5, it is slightly ramified.

In order to see the anterior openings of both kidneys and
their nipples or papillæ it is best to make a transverse section
of the mantle cavity just in front of the anus.   This I did on a
well-hardened specimen, first making a horizontal section with
a sharp razor, passing just below the bottom of the mantle
cavity, then a transverse one just in front of the anus.

Fig. 6 shows a preparation obtained by this method.   The
mantle cavity is seen to be divided into four portions or nooks
by the branchial supports (*br.*) on either side.   One may,

therefore, distinguish an upper and a lower nook of the mantle cavity on either side (*Msr.*, *Msl.*, *Mir.*, *Mil.*). The anus lies at the end of a well-defined papilla which occupies the centre of the cross which divides the mantle cavity in the way which has just been described. On the ventral side of the anus, just above the floor of the mantle cavity (*Ma.*), a projecting ridge parallel to the mantle cavity may be seen. This ridge is formed by the basibranchial sinus (*bas.*). The ridge itself bears the two papillæ on which the external apertures of the two kidneys lie. The papilla of the left kidney (*Pr.*) is larger, has a slit-like opening (*Xr.*), and is situated anteriorly on the right edge of the basibranchial ridge. The papilla of the left kidney (*Pl.*) is much smaller, and lies further back in the left inferior nook of the mantle cavity. Its aperture is nearly round, and in most cases very difficult to find in dissections. In the preparation just described, the ctenidia which are cut transversely display perfectly the double feathered condition characteristic for the forms with two equally developed gills. The relations of the ctenidia to the auricles (*aur.*), the ventricle, the nipples of the anus, and the kidneys are shown in fig. 10, which is drawn from Fiss. maxima, in which the pericardium and mantle cavity have been opened ventrally.

At the base of the papilla of the right kidney (*Pr.*), between this and the pericardium, that is to say, in the hinder wall of the basibranchial sinus, two openings can be seen. The one lying towards the middle line, distinguished by a brownish colour, is the beginning of the left kidney running into its papilla (*Xr.*); while the other one, lying outside of the first, is the genital duct (*Gd.*). Both kidney and genital duct have been cut through transversely in the course of the dissection. The same thing magnified ten times is shown in fig. 18. A bristle has been introduced into the genital duct, and is seen to issue out of the sectionised right kidney—a proof that the genital duct opens into the papilla of the right kidney. The left kidney can be easily detected in Fiss. reticulata, maxima, gibberula, and græca; it is a remarkable fact

that it has entirely atrophied in Fiss. nubecula, where even the papilla has disappeared.

Fig. 23 shows a transverse section through the pericardium and both kidneys of Fiss. græca. Dorsally and in the middle line the cavity of the pericardium (*Pc.*) can be easily seen. In it lies the ventricle (*V.*) surrounding the rectum (*R.*), to the right of which an artery is seen in transverse section. The section is not absolutely transverse, so that only one auricle, the left one (*aul.*), is to be seen. Fig. 10 had shown that both auricles are of the same size, and situated symmetrically on either side of the ventricle and the longitudinal body axis. On the ventral side of the pericardium lies the basibranchial sinus (*bas.*); to its right lies the beginning of the right kidney (*Nr.*), with the commencement of the genital duct (*Gd.*) situated to the right of the latter. Fig. 26, a part of a transverse section through Fissurella nubecula, shows the genital duct (*Gd.*), containing young ova, opening into the beginning of the right kidney (*Nr.*), which also contains some ova. This shows that the sexual products are really discharged through the right nephridium. To the left of the basibranchial sinus (*bas.*) is the left nephridium (*Nl.*). The figure shows the tremendous difference in the size of the nephridia. The section of the left is the largest one in the whole series, and still it is surprisingly small, compared to the right kidney, which extends from one side to the other of the section, and insinuates itself between the different viscera (*Nr., Nr.*). Fig. 21 is a transverse section through the papilla of the right kidney in Fiss. gibberula, showing the external opening of the right kidney (*Xr.*), situated between the genital duct (*Gd.*) and the beginning of the right kidney cavity (*Nr.*).

Von Jhering, Haller, and Perrier are unanimous in saying that the left kidney does not open into the pericardium. Whilst Jhering and Boutan failed to detect a reno-pericardiac duct in the right kidney, Haller and Perrier have described a right reno-pericardiac canal.

I have carefully studied a great number of transverse and frontal series through Emarginula and Puncturella, and

the four Neapolitan species of Fissurella, and have abso-
lutely satisfied myself that no such thing exists. The spe-
cimens of Fissurella had been most carefully preserved and
stained, the sections extended without a break through the
whole length of the pericardium. Fig. 16 shows the point
in which the right kidney runs into the nipple immediately on
the ventral and right side of the pericardium. In this section,
as in all others, the brown epithelium of the right kidney is
seen to extend over the whole inner surface of the nephridium,
without any interruption whatever.

Perrier, in his answer to a letter which I wrote to him on
the subject, stated that he had made an injection into the peri-
cardium of Fiss. costaria, and that the coloured fluid had
penetrated into the right kidney. This can be easily accounted
for. The beginning of the right kidney lies immediately below
the pericardium, and its cavity is only separated from that of
the pericardium by the epithelium of the kidney, the exceed-
ingly thin tunica propria of this gland, and the delicate wall
of the pericardium. When an injection is forced into the peri-
cardium under a high pressure—with a syringe, for instance—
the liquid bursts through the wall of the pericardium and
kidney at the point just mentioned. I have myself made the
experiment. When using the method described in this paper
such a thing never happened; when, on the contrary, I used
a syringe, I very often found the injection in one or the other
kidney, sometimes in both, as they both come into the closest
proximity with the wall of the pericardium. This place in
both kidneys probably corresponds to the spot in which the
nephridium was constricted off from the pericardium or cœlom,
and is very likely homologous to an abortive funnel in both
cases.

I have been quite unable to find any trace of a reno-peri-
cardiac canal like the one figured by Perrier (23) in pl. i,
fig. 3, of his paper. The above-named gentleman, at my
special request, has very courteously promised to look over
his preparations, giving special attention to this point, but I
have not yet received information as to the result.

I can also account for Bela Haller's statements on the point at issue. The funnel he describes is nothing but the section of the aorta (compare his pl. i, fig. 1, with my own fig. 3, and Boutan's [2] pl. xxiii, fig. 6). The renal end of his reno-pericardiac duct is nothing else than the genital duct (compare my fig. 18 with his fig. 2, pl. i). The same anatomist describes an entirely imaginary genital duct, which is only the inferior right mantle nook shown in my fig. 6. Had Haller carefully studied unbroken series of sections (he figures sections, but has probably not been able to cut complete series) he would certainly have avoided these mistakes.

Perrier certainly directed his attention almost entirely to the left kidney, rather neglecting the point whether a right reno-pericardiac duct really existed. The canal which he figures would be quite satisfactory were the opening into the kidney to be seen, which is not the case. He was evidently misled by Haller and by misunderstanding v. Jhering, who says that he could not satisfy himself as to the presence of a right reno-pericardiac duct. The latter information was obtained from Perrier's answer to my letter.

Emarginula Houzardi.—The anatomy of this species, as seen after the removal of the shell through the very thin mouth, is illustrated in fig. 11. It closely resembles that of Fissurella. The right kidney, however, seems to be somewhat smaller than in Fissurella.

Fig. 27 is a perfectly transverse section through the pericardium and both kidneys of Emarginula. The external opening of the left kidney (Xl) is very plainly seen in the section.

Puncturella spec.?—The same remarks I made about Emarginula can be applied to Puncturella.

Fig. 15 shows a transverse section through the pericardium and both kidneys of this species, which is certainly more primitive than Fissurella and Emarginula, as the visceral hump (fig. 22, $H.$) is much better developed here.

4. Patella cœrulea and Tectura spec.?

Special attention was given to Patella, as my results are absolutely contradictory to those of Ray Lankester (**18, 19, 20**) and Cunningham (**6**). Patella cœrulea is the only species of Patella found in the Gulf of Naples, and is the same which Cunningham has studied. I therefore do not doubt that the study of Patella vulgata would lead to the very same conclusions.

Perrier (**23**) has evidently neglected the right reno-pericardiac duct in Patella, as he neither describes nor figures it.

The shape and position of both renal organs are displayed in fig. 14, drawn from a hardened specimen after removal of the shell, hood, and dorsal pigment. The pericardium (*Pe.*) lies to the left and at the hinder end of the mantle cavity (*M.*). Its position and shape have been accurately described by Ray Lankester (loc. cit.) and Wegmann (**28**), who has written a short but valuable monograph of Patella vulgata, unfortunately paying but little attention to the relations of the kidneys and the pericardium.

The little or left kidney (*Nl.*) lies immediately to the left of the pericardium, between it and the right kidney (*Nr.*), which covers very nearly the whole of the visceral mass. The papillæ of the kidneys lie on either side of the anus (*A.*) (*Xr.* and *Xl.*), and may be easily found when the hood or anterior mantle-flap has been removed.

Sections (transverse and horizontal) show that the kidneys are situated on either side of the rectum (*R.*) (fig. 25 *a*) (*Nr.* and *Nl.*). Their general histological appearance closely resembles that of Fissurella. It is a well-known fact that the rectum does not perforate the ventricle and pericardium in Patella, so that the rectum, the left kidney, and the anterior part of the right kidney are situated entirely to the left of the pericardium. The blood-sinuses of both kidneys are, as Perrier (**23**) has shown, in relation with the auricle of the heart, while in Fissurella, Haliotis, and Trochus this statement can only be applied to the left kidney.

As regards the presence or absence of reno-pericardiac ducts in Patella I have come to the same conclusions as for Fissurella, Emarginula, and Puncturella, and must also add Tectura to the list of Prosobranchs having no communication between the kidneys and the pericardium.

Injections made according to my method, which I owe to Professor Mayer's kindness, led me to the same conclusions. When a higher pressure is used the injection may penetrate into either kidney, but generally it is easier to drive it into the left. The places in which this happens are always the same, and can be readily found by dissections and sections without using injections.

Fig. 25 a shows that towards the latter end of the mantle cavity (M.) the left kidney comes very near the wall of the pericardium. In this section there is still a thickish muscular partition between the left kidney (Nl.) and the cavity of the pericardium. This is the continuation of the partition between the mantle cavity and the pericardium. Still further back (fig. 25 b) the mantle cavity (M.) recedes more and more from the pericardium (Pc.), and the muscular partition entirely disappears in a spot (†) which very probably corresponds to an abortive funnel. In this place the cavity of the left kidney is only separated from that of the pericardium (Pc.) by the secreting epithelium of the kidney, the tunica propria of the same organ, and the flat epithelium of the pericardium. This accordingly is the likeliest spot for an injection to break through either from the pericardium into the left kidney or vice versâ. Even when using an immersion I was unable to find the smallest opening in this place when examining series of a specimen which had not been injected. I wish to remark here that Mr. Cunningham states that he has been able to trace the reno-pericardiac ducts in sections through an uninjected specimen.

I myself have already pointed out that to my mind the method of injection is of very small value in such cases, and very likely to mislead the investigator. I remind the reader that many French anatomists, who seem to be particularly

partial to injections, have, in molluscs, described a very complicated system of arteries, capillaries, and veins which were simply the artificial results of the injections.

A corresponding spot also exists in the right kidney, at the end of the so-called subanal tract of the right kidney (fig. 20). In this place, in which both Lankester and Cunningham have stated they found the reno-pericardiac duct, the right kidney also comes right up to the pericardium. It remains, however, separated from its cavity by a thin layer of muscular tissue. This circumstance explains why it is easier to get the injection from the pericardium into the left kidney than into the right renal pouch, and also accounts for the fact that Ray Lankester (18) first found the left kidney of Patella opening into the pericardium.

Tectura, as has already been mentioned, closely resembles Patella. There, nevertheless, are a few points on which I desire to direct the reader's attention. As in Puncturella compared to Fissurella and Emarginula, the visceral hump is far better developed in Tectura (fig. 17, H.) than in Patella. Both in Tectura and Puncturella the difference in the size of both kidneys is smaller; both renal pouches are simple in their shape, that is to say, less ramified.

Fig. 24, a frontal section through the pericardium (Pe.) and both kidneys of Tectura, is given to show that the left kidney (Nl.), which is still smaller than the right (Nr.), lies entirely to the front of the rectum (R.), and in the same longitudinal line as the right kidney. This fact will be made use of in the comparative part of this paper.

Conclusions and Comparative Part.

The results of this investigation may be summed up as follows :

Trochus, Turbo, and Haliotis possess a left reno-pericardiac duct only.

Fissurella, Emarginula, Puncturella, Patella, and Tectura possess no reno-pericardiac duct whatever.

The genital products always pass through the right renal organ, either by bursting of the gonad through the walls of the right kidney, as in Patella and Trochus,[1] or being admitted through a kind of valve (Haliotis), or transported to the right renal papilla by a special genital duct (Fissurella).

The inference to be drawn from these statements is that, according to the view first expressed by Ray Lankester (19), the only remaining nephridium of most Prosobranchs corresponds to the actual left kidney of forms possessing two renal organs. In my paper on the development of Paludina (8) I have stated that this view was supported by embryological evidence, the actual nephridium of Paludina being situated before the torsion to the right of the anus, while the rudiment of the actual right kidney lies to the left of the rectum before the torsion takes place. I have further shown that, as Sarasin[2] had already stated, the actual nephridium in Bythinia tentaculata was, before the torsion, situated to the right of the anus, as must be the case in all leiotropic forms. In Planorbis, on the contrary, which is a dexiotropic species of fresh-water Pulmonates, the actual right nephridium is, before the torsion, situated to the left of the rectum (10).

These facts, compared with the fact that the genital gland in the above-mentioned species of Zygobranchs and Cyclobranchs, or, according to the new French terminology (3), Diotocards and Heterocards, opens into the right kidney, and that in Paludina the duct of the rudimentary right kidney becomes the genital duct, show clearly—

1. That the only remaining kidney in most Prosobranchs is the actual left one.

2. That the actual right kidney has disappeared or become

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[1] In Turbo and probably also in Trochus, the genital gland has a separate opening into the mantle cavity (v. Jhering (16), and "Sur les relations naturelles des Cochlides et des Ichnopodes," in ' Bulletin scientifique de la France et de la Belgique,' xxiii, 1 Partie, 1891, pp. 148—257, pl. iv—vi).

[2] Sarasin, P., " Entwickelungsgeschichte der Bythinia tentaculata," in ' Arb. z. Inst. Wurzburg,' Bd. vi, 1882, pp. 1—68, Taf. i—vii.

transformed, and that a part of it corresponding to the duct forms a part of the genital apparatus.

The next question is, what has become of the glandular part of the actual right kidney? R. Perrier (**23**), in his extensive memoir on the anatomy of the kidneys of Prosobranchs, came to the conclusion that the only remaining kidney of most Prosobranchs is homologous with the actual right kidney of those forms which are provided with two renal organs. He inferred this from the fact that the actual right kidney of birenal forms is always the larger one, and always shows the characteristic brown renal secretory epithelium. I have already given my reasons against this inference, and will add that it seems more rational to admit that all the organs of the actual right side (left before the torsion) have disappeared in higher Prosobranchs. This opinion is supported by the facts that in Haliotis the right ctenidium is smaller than the left, and that in Turbo and Trochus the only remaining ctenidium and auricle are the actual left. R. Perrier says that the actual left kidney, which is fast disappearing in Fissurella, much reduced in Patella, and highly modified in Haliotis, Trochidæ, and Turbo, where it is called papillary sac, has transformed itself into the so-called nephridial gland, a distinct part of the only remaining kidney of most Monotocards.

According to Perrier the transition between Diotocards and Monotocards is formed by the Patellidæ or Heterocards. The left kidney has become located between the pericardium and the right kidney. The sinuses of both kidneys are in communication with those of the auricle. Supposing, says Perrier, that the thin wall between the left and the right kidney in Patella disappeared, the left kidney would become part of the right kidney; thus apparently there would be only one kidney remaining, and the left one would exactly correspond to the nephridial gland. This hypothesis is realised by Ampullaria,[1] which, according to Bouvier (**4**), shows two kidneys, of which the actual right one (according to Bouvier) has the same shape and position as (*ex. gr.*) the only

[1] The species examined was leiotropic.

remaining kidney of Paludina, and opens into the mantle cavity; the other one (left according to Bouvier) has no external opening, but communicates with the other renal organ. Whilst the first renal organ is formed of lamellæ, the roof of the second one is coated with a vascular web, which constitutes the renal tissue.

The vascular irrigation of the first kidney is the same as in the renal organ of Lamellibranchs, as the venous blood which has passed through it goes to the gills before reaching the heart, whilst the venous blood of the second flows directly into the auricle.

" The two renal organs of Ampullaria are, therefore (says Bouvier), placed exactly in the same conditions as those of Haliotis, according to Wegmann's description, the second kidney performing the same part in the circulation as the only kidney of Monotocard Prosobranchs."

Bouvier, therefore, argues that the first kidney is homologous with the right kidney, the second with the left kidney of Diotocards.

I myself hold the opposite view on the same subject. Bouvier's most valuable argument is derived from the circulation in the renal organs. Perrier (23), however, has shown that, as we proceed higher in the series of Prosobranchs, the circulation in the kidney undergoes very considerable changes. The quantity of blood passing through the right kidney before going to the gills decreases more and more as we proceed from Haliotis to Trochus and Turbo. In Monotocards the only remaining kidney has developed an entirely new and different system of blood-irrigation, and its circulation has grown quite independent of the general one. His argument, therefore, turns out to be much less important than it seemed at first.

The position of the kidneys, as far as I can make out by examining the figure given by Bouvier of a dissected Ampullaria, would rather tend to prove that the large vascular sac lies more to the right, the smaller lamellar organ to the left of the rectum and the body axis. The size and shape of both

kidneys further supports my own view. The lamellar organ closely resembles the kidney in the embryo of Bythinia, as described by myself (9), while the size of the left kidney and the simplicity of its structure strongly remind one of the right renal organ in Diotocards and Heterocards.

I must now beg the reader to refer to my fig. 24 of Tectura species which shows that the left kidney (the small one) lies quite anteriorly to the large right one, a fact which might explain the position and homologies of the renal organs of Ampullaria, further supporting my own view on the subject.

Bouvier, whose abilities as an anatomist I value most highly, has only examined specimens of Ampullaria preserved in spirit, and exclusively made use of the method of dissection. I must venture, therefore, on technical grounds, to question some of his statements. It seems highly improbable to me that the lamellar renal organ should have no communication with the pericardium, whilst the kidney of Bythinia, which is closely similar to it in shape and texture, clearly shows a well-defined reno-pericardiac duct. This point, and the communication of the two renal organs, ought certainly to be reinvestigated in well-preserved specimens by the method of sections. Bouvier has shown and stated that the organisation of Ampullaria closely resembles that of Paludina. Ampullaria (says Bouvier) is a large Paludina, which by adaptation to a new mode of life has acquired a lung. The results obtained by the study of the development of Paludina may therefore safely be applied to Ampullaria, all the more as the species described by Bouvier is leiotropic like Paludina. I consequently am quite convinced that the lamellar kidney of Ampullaria is homologous with the actual left kidney of Paludina, the vascular sac in Ampullaria to the rudimentary right kidney in Paludina.

Before taking leave of the kidneys of Prosobranchs, I must yet mention the curious organ in Dolium described by P. Schiemenz (26) under the name of anal kidney (Afterniere). This organ consists of two brown glandular

lob'ules situated on either side of the anus: they have been
described by Haller (13). On their surface can be descried
numerous canals, having the appearance of blood-vessels.
These canals, however, are by no means blood-vessels. They
unite from both sides and run into a large sinus which enve-
lops the rectum. The sinus opens to the right of the anus
by a large lip-like orifice. This opening must not be mis-
taken for the female genital opening, which lies to the left of
it. The anal kidney does not communicate with the peri-
cardium, while the actual left kidney is plainly seen to possess
a reno-pericardiac duct.[1]

In this instance also it would be highly desirable to study
the development of the left kidney, and of the anal kidney
of Dolium, as under the present state of our knowledge
it is doubtful, although probable, that the so-called anal
kidney really represents the modified right kidney of
Dolium.

To return to R. Perrier's hypothesis, this author is ready
to admit that his ideas on the homology of the only re-
maining kidney of Monotocards can easily be reconciled
with my own. He was led to believe that one kidney had
disappeared in these forms. The only circumstance which
induced him to suppose that the remaining kidney was the
actual right nephridium was the predominance assumed by
the right kidney in Diotocards, an argument derived from
analogy, and "peu probant," as Perrier himself wrote to me.
It would be necessary to ascertain whether the nephridial
gland really represents one or the other kidney. I hope to be
able to solve this problem in the course of time, having
already collected materials for the study of the development
of Cassidaria, which Perrier chose as a type for his descrip-
tion of the nephridial gland. If according to Perrier the
nephridial gland represents the actual left nephridium, we
should be led to conclude that in Paludina and Bythinia,
where no such gland exists, the actual right kidney has

[1] Dr. Schiemenz had the kindness to show me the organ just described in
several specimens of Dolium preserved in spirits.

disappeared, and the remaining left is homologous with the nephridial gland of other Monotocards.

To the best of my knowledge, it is more probable that in most Monotocards the actual right kidney has disappeared, and that possibly it has been transformed into Perrier's nephridial gland.

A. Lang, in a short pamphlet purposing to explain the asymmetry of Gastropods by mechanical processes, has also arrived at the conclusion that in Monotocards the whole actual right complex of originally paired organs has disappeared. This pamphlet is evidently the result of Lang's study of literature previous to the publication of the part of his text-book of comparative anatomy dealing with molluscs. Whilst most of the ideas expressed in Lang's paper are certainly not new, his mechanical explanation of the asymmetry is certainly very ingenious. Bütschli, the last zoologist who dealt fully with the same question, had not attempted a mechanical explanation of this difficult problem. Unfortunately several facts are a serious impediment to Lang's theory. For instance, it is difficult to understand why Trochus and Turbo, the shells of which are just as highly coiled as that of the common snail, should have retained two auricles and two kidneys if the pressure brought to bear by the shell on the actual right side of the body is held to have caused the disappearance of the corresponding set of organs. Our present knowledge of the development of Fissurella and Patella, which we owe to Boutan (2) and Patten,[1] is another serious objection, as it is well known that the shell of both these species is originally nautiloid.

Another point which seems not to have met with any attention from Professor Lang is that up to the present it must have seemed highly probable that Perrier's view on the homologies of the only remaining kidney in Monotocards, viz. that it represented the actual right one of Diotocards, must be correct. Lang neither mentions Perrier's exhaustive

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[1] Patten, "The Embryology of Patella," in 'Arb. Zool. Inst. Wien,' Bd. vi, 1885, pp. 149—174, Taf. xiv—xviii.

treatise nor my own paper on the development of Paludina, in which I was the first to maintain the opposite view.

Of course, I am quite prepared to admit that many facts and statements must needs escape the attention of the author of a text-book comprising the whole of the animal kingdom, especially when the writer had not previously himself worked for some time upon the group he describes.

I now propose to compare the results afforded by the study of Prosobranchs with those obtained by other workers from the remaining groups of Gasteropods and molluscs in general.

The Heteropods are now almost generally considered as modified Prosobranchs. As was to be expected accordingly, the nephridium is situated to the left of the anus and rectum.

We now come to the so-called Euthyneura. I have already stated that the development of Planorbis (10) confirmed my view of the homologies of the only remaining nephridium, which is also supported by the evidence collected from several other papers by different investigators dealing with the same question[1] with regard to Pulmonates. Of all Opisthobranchs, the Tectibranchiata are certainly the least modified forms and the most nearly related to Prosobranchs. The anatomy and a part of the embryology of Aplysia has been recently studied by my friend Signor Mazzarelli. According to him the external opening of the renal organ in the adult lies ventrally and to the left of the anus and rectum. The kidney in the larva or veliger stage most probably corresponds to an organ which has been described as an eye by Lacaze-Duthiers. The position of the organ, which in the larva lies to the left of the rectum and anus (after the torsion has taken place in a leiotropic direction), shows that it must be the actual left kidney. The development shows that the mode of formation of this organ closely agrees with that of the kidney of Paludina, Bythinia, and Planorbis. Before the torsion it lies to the right of the rectum. Its glandular part has a mesodermic origin; the

[1] I must refer the reader to the list of embryological papers given in my memoir on 'Paludina' (8).

duct is formed by a short invagination of the ectoderm. The external opening is clearly seen in the veliger stage to the left and a little below the anus. According to Signor Mazzarelli there is even evidence to prove that a similar but rudimentary renal organ was, before the torsion, situated to the left of the rectum.

In Pulmonata and Tectibranchiata the torsion (leaving out of account the torsion of the visceral hump, which is an independent process [Bütschli] from that of the general torsion) is already less marked than in Prosobranchia. The original twisted condition is still more reduced in Pteropoda and Nudibranchiata, where a kind of untwisting has very probably taken place. This explains why the actual left kidney (the right one before the torsion) apparently does not occupy the same position as in Gasteropods, though in reality its position is homologous, provided we suppose that a secondary untwisting has taken place to a greater or lesser degree.

In all other groups of Mollusca, that is to say, in Placophora, in Scaphopoda, in Lamellibranchiata, and Cephalopoda, the renal organ is paired.

I have not yet mentioned the Aplacophora or Neomenia, as Pruvot (25) has recently thrown considerable doubt on Hubrecht's (15) views on the morphology of the uro-poetic apparatus in this interesting group. Pruvot considers the ducts leading from the pericardium (Hubrecht) as nothing but oviducts, the pericardium itself as a mere egg-pouch. The unpaired glandular part, considered by Hubrecht as the secreting part of the nephridia, is called a shell-gland by the French anatomist. Pruvot describes as an excretory gland a new organ which he calls the cloacal ridge (bourrelet cloacal).

I cannot attempt to explain away Pruvot's statements concerning the secreting part of the kidney, not having studied myself the anatomy of Neomenia. However, I think that this can be easily done for the pericardium and ducts of the nephridia.

Pruvot, after having in a preliminary account of his investi-

gations denied that the organ hitherto described as heart really represented the blood-propelling organ, has in the full account of his researches admitted that the heart really exists, but in a very rudimentary form.   Nevertheless he refuses to admit that the pouch, in the roof of which the heart forms a gutter-like invagination, really represents the pericardium.   His chief objection is that the epithelial lining of this pouch produces the ova and the spermatozoa.   The conception that the cavity of the pericardium in molluscs represents the reduced cœlom, and that the cavity of the gonad originally formed a part of this cœlom, is evidently unknown to Mr. Pruvot.   The fact that the epithelial lining of the pericardial cavity represents the gonad or genital epithelium is the best proof that the Neomenia show a very primitive condition in this point, reminding one strongly of the condition to be met with in a great number of Annelids.   I therefore further conclude that Pruvot's oviducts are really the nephridial ducts which possibly have lost their connection with the glandular part of the renal organ.

Having discussed Pruvot's rather isolated conception of the uro-poetic organs of Neomenia, I think it advisable to compare the results which I have obtained by the study of Prosobranchs with those of other anatomists on the corresponding organs of the remaining molluscs.

In an account of the development of Paludina I have shown that the gonad arises as an evagination of the pericardium or cœlom, which gradually becomes constricted off and acquires a special duct leading into the mantle cavity. The glandular portion of both renal organs has a similar mode of formation, and the original lumen of the evagination leading into the pericardium becomes the reno-pericardiac funnel. The cavity of the gonad and those of both nephridia are, therefore, parts of the cœlom.

In Paludina the actual right kidney has disappeared, and its duct, formed by an introflection of the ectoderm, becomes the efferent duct of the gonad. The actual left kidney persists, and remains in communication with the pericardium.

The history of the development will explain the facts we have met with in the anatomy of the forms dealt with in this paper. In Fissurella and allied forms, as well as in Patella and Tectura, both renal organs have lost their communication with the part of the cœlom represented by the pericardium. The shape and size of the right kidney, and the way it extends between all viscera, pervading the whole body of Fissu̇rella and Patella, are explained when we consider it as a part of the cœlom. The fact that the nephridia in Fissurellidæ and Patellidæ have lost their communication with the pericardium is certainly surprising, especially as no other such cases are known in molluscs. In other groups, however, we meet with parallel cases—so, for instance, in Hirudinea, in which most species have lost the opening of the nephridia into the cœlom. Besides, it must be remembered that both Fissurella and Patella, which by reversion to the primitive external symmetry have regained or preserved to a great extent the original internal symmetry, are in reality highly modified forms. This is abundantly shown by the story of the development of the shell (loc. cit.).

I will now attempt to give an idea of the condition of the uro-poetic apparatus in the hypothetical ancestral form of molluscs, and to show how the present condition in actual living groups can be easily deduced therefrom (see diagram). It is now a view accepted nearly by all morphologists, that the ancestral form of molluscs was of bilaterally symmetrical build, and that the anus (*A*.) and mantle (*M*.) cavity were situated at the aboral end of the animal. Spengel,[1] Bütschli (5), Ray Lankester (21), Lang (17), and others have built up their theories on the asymmetry and torsion on this hypothesis. The existence of the bilateral symmetry further implies that the ancestral form possessed paired ctenidia (*Br*.), osphradia, and nephridia (*N*.). The nephridia were tubes with a medium glandular portion having a communication (*Y*.) with the pericardium (*Pc*.), and opening at the basis

[1] Spengel, J. W., " Die Geruchsorgane und das Nervensystem der Mollusken," 'Zeitschr. f. wiss. Zool.,' 1881, xxxv, pp. 333—384, Taf. xvii—xix.

of either ctenidium at the end of a papilla (*P.*). The rectum (*R.*) passed through the pericardium (*Pc.*), but ventrally from the heart, without perforating the ventricle (*V*), opening by the
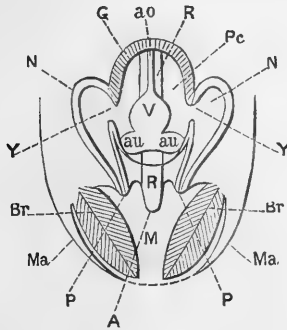
Diagram of the reno-pericardial system of the ancestral form of molluscs. *ao.* Aorta. *au.* Auricles. *Ma.* Mantle. The other letters are explained in the text.

anus (*A.*) in the middle line, at the bottom of the mantle cavity (*M*). Most probably the entire anterior half of the epithelial lining of the pericardium, that is to say, the portion anterior to the region occupied by the heart, represented the gonad (*G.*). The sexual products dropped into the cavity of the pericardium passed through the funnel into the nephridium, and passed out of the nephridial duct into the mantle cavity. This condition would be nearly realised by the actual Neomeniæ.

Very soon the portion of the pericardium, the lining of which represented the epithelium of the gonad, became constricted off the pericardium (compare the development of Paludina). This is the case in all other groups of Mollusca except Neomeniæ.

According to Pruvot, the gonad in Aplacophora is formed by two symmetrical portions only separated by a ridge. The gonad is unpaired in Placophora, Cephalopods, and Gasteropods. These certainly being less modified forms than Lamellibranchiata and Scaphopoda, where the gonad is paired, it is reasonable to infer that the original

condition was an unpaired gonad. This having become
separated off from the pericardium was accordingly obliged to
form a new communication with the outer world. In Zygo-
branchs, Tectibranchs, and in Scaphopods the gonad
enters into communication with the actual right nephridium
by bursting open its wall, or by opening into it by a special
duct, probably evolved from a part of the gonad itself. In
higher Prosobranchs the genital duct is probably the duct of
the actual right aborted kidney (Paludina). In the archaic
forms of Lamellibranchiata, which according to Pelsener (22)
are modified Prosobranchs, such as Nucula, the genital
products are expelled into the initial part of the nephridia,
that is to say, alongside of the reno-pericardiac funnel,
while as we proceed higher, independent genital ducts
are formed. In Chitons we find an independent pair of
genital ducts, as in Cephalopods. It is as yet not known
whether these ducts are in any way related to those of the
nephridia.

According to Grobben (12), Sepia, probably the most
archaic form, shows a large cœlom, communicating with the
nephridia by two openings or funnels. The cavity of the
cœlom is coated with an epithelium, and encloses in its
anterior portion the heart with its vessels, the branchial
hearts and the pericardial gland, formed by differentiated
peritoneal epithelium. The hinder portion of the cœlom,
divided off incompletely from the anterior one by a septum,
encloses the gonad, which probably belonged originally to the
epithelium of the peritoneum, and the stomach.

All the evidence which I have collected in the comparative
part of this paper tends to show that the molluscs are true
cœlomate animals, and that the condition of the genital and
renal organs is closely similar to that of primitive Annelids.

Before concluding this paper I wish to remark that I am
fully prepared to have the truth of my statements on Fissurella
and Patella questioned. I have given myself the greatest
trouble to find both reno-pericardiac ducts. Being unable to
see the left one, the existence of which I was most anxious to

prove for reasons which the reader of this paper will easily understand, I began to look for the right one. I thought that, having once found the right one, its shape and position would enable me to see the left one. Meeting with the same negative results, I then examined Haliotis and Trochus, when I immediately found the left reno-pericardial duct only, but this with the greatest ease. I then carefully re-examined my preparations of Fissurella and Patella, and came to the results which I have fully described in this paper. I next considered it my duty to explain the contradictory statements of my predecessors, and hope to have done this successfully. Nevertheless I will be most happy to confess that I have been wrong in this matter, provided I am shown an uninterrupted series of sections through an uninjected specimen displaying one or both reno-pericardiac ducts in Fissurella or Patella. A valid proof of the existence of both reno-pericardiac ducts in both these species would exactly suit my theory on the homologies of the kidney in Monotocards.

In my paper on Bythinia I have fully expressed the opinion I hold on the value of the method of sections. I have stated that in my humble opinion a thorough study of the entire animal or embryo by other methods must always precede that of sections. However, I maintain that sections alone can, under the present state of anatomical technique, give us an exhaustive knowledge of complicated topographical questions and of delicate anatomical points. The method of sections is most valuable when the existence of a communication between an organ and another organ or the outer world is to be proved. In this case sections are the only true test. My figures show that when a reno-pericardiac opening really exists it has the shape of a well-defined canal, generally of no inconsiderable length, as in Haliotis and Trochus. I have seen the same structure in Paludina, Bythinia and Aplysia, in Signor Mazzarelli's series of sections. For these reasons I think it is fair that I should require to be shown similar ducts in Fissurella and Patella before I give up my own view on the points I have discussed in this paper.

It is my pleasant duty to thank the Office of Public In-
struction of Baden for the table which it generously placed
at my disposal, hereby enabling me to make further researches
on the comparative anatomy and embryology of molluscs.
The excellent organisation which the Zoological Station of
Naples owes to its director, Professor A. Dohrn, always kept
me supplied with an abundance of material. I must specially
thank Sr. Lo Bianco for the trouble he took in procuring for
me the numerous specimens which I required for my researches.
I am further indebted to Professors Eisig and P. Mayer for
valuable technical hints, and to my friend Mr. MacBride, who
kindly undertook to read the manuscript of the first paper
which I have published in the English language.

Naples Zoological Station;
        April 9th.

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Additional Note.—I have lately examined Trochus and Turbo, in order to test v. Jhering's and B. Haller's statements about the existence of a proper genital duct (loc. cit.).

V. Jhering's figure is absolutely schematic, and is not accompanied by a detailed description. As he failed to recognise the existence of the left kidney, it is highly probable that he mistook one or the other renal duct for the genital duct.

Haller simply mistook the right renal duct for a proper genital duct, having stated that the right kidney opened into the left one.

I was unable to find any proper genital duct in Trochus turbinatus and Turbo rugosus, although I used both the method of dissection and that of unbroken series of sections.

On the other hand, the appearance, position, and the vicinity of the genital gland to the glandular portion of the right kidney and the beginning of the right renal duct are the same as in Haliotis. I am therefore led to the conclusion that the sexual products are expelled by the right kidney.

Should this conclusion be correct (an absolute proof can only be given by finding ova or sperma in the right renal duct), all primitive Prosobranchs would be devoid of a proper genital duct, the right renal duct serving also as a genital

duct.   In higher developed forms the glandular portion of the right kidney has disappeared, whilst its duct forms a part of the proper genital duct.

For the present I can only say that, contrary to v. Jhering's and B. Haller's assertions, there is no proper genital duct in Turbo and Trochus.

Naples ; June 1, 1892.

EXPLANATION OF PLATES XXXVI and XXXVII,

Illustrating Dr. R. v. Erlanger's paper " On the Paired Nephridia of Prosobranchs."

List of Reference Letters.

A. Anus.   *Ap.* Anal papilla.   *Au.* Auricles of the heart.   *aur.* Right ; *aul.* Left auricle.   *ao.* Aorta.   *B.* Gill (ctenidium).   *Br.* Right ; *Bl.* Left ctenidium.   *bas.* Basibranchial sinus.   *bs.* Branchial rod or support.   *C.* Reno-pericardiac canal.   *E.* Eye.   *F.* Foot.   *Fi.* Mantle-fissure.   *G.* Gonad genital gland.   *Gd.* Genital duct.   *H.* Visceral hump.   *Ha.* Heart.   *I.* Intestine-gut.   *L.* Liver.   *M.* Mantle cavity.   *Ma.* Mantle.   *Mb.* Bottom of the mantle cavity.   *Msr.* Right superior portion ; *Msl.* Left superior portion; *Mir.* Right inferior portion ; *Mil.* Left inferior portion of the mantle cavity.   *muc.* Mucous gland.   *mu.* Muscle.   *N.* Nephridium.   *Nr.* Right; *Nl.* Left nephridium-papillary sac.   *P.* Papilla of the nephridium.   *Pr.* Right ; *Pl.* Left papilla.   *Pc.* Pericardium.   *R.* Rectum.   *S.* Stomach.   *si.* Sinus.   *V.* Ventricle of the heart.   *ve.* Vein.   *vt.* vena transversa.   *vbe.* Branchial efferent vein.   *X.* External opening of the nephridium.   *Xr.* External opening of the right ; *Xl.* External opening of the left nephridium.   *Y.* Opening of the nephridium into the pericardium.   *y.* Opening of the reno-pericardiac canal into the pericardium.

Fig. 1.—Dorsal view of Haliotis tub. after removal of the shell. $\frac{1}{1}$.

Fig. 2.—Dorsal view of Haliotis tub. The mantle cavity has been opened, the rectum and the mucous gland thrown back, so as to display the external openings of the kidneys. $\frac{1}{1}$.

Fig. 3.—Pericardium of Haliotis tub. opened; heart removed.   The

front wall of the pericardium is seen from behind, and shows the left kidney by transparency, and the inner opening of the reno-pericardiac duct. $\frac{5}{1}$.

FIG. 4.—Mantle cavity of Trochus turb., opened and extended. Ventral view of the gill, pericardium, nephridial ducts, and rectum. $\frac{2 \cdot 5}{1}$.

FIG. 5.—Pericardium of Fissurella cost., opened dorsally; heart cut out. Both kidneys seen in situ by transparency. $\frac{6}{1}$.

FIG. 6.—Mantle cavity of Fissurella cost., in transverse section, displaying the shape of the mantle cavity, the position of the gills, anus, nephridial papillæ, and basibranchial sinus.

FIG. 7.—Transverse section through the pericardium and both kidneys of Trochus turb., showing the reno-pericardiac canal. $\frac{32}{1}$.

FIG. 8.—Transverse section through the basibranchial sinus and both kidneys of Trochus turb., showing the reno-pericardiac canal. $\frac{32}{1}$.

FIG. 9.—Horizontal section through the pericardium and both kidneys of Haliotis tub. $\frac{32}{1}$.

FIG. 10.—Ventral view of the mantle cavity and pericardium of Fissurella max. The pericardium is opened ventrally; the heart is displayed. $\frac{1}{1}$.

FIG. 11. Dorsal view of Emarginula Huzardi, after removal of the shell. Anatomy seen by transparency through the mantle. $\frac{1}{1}$.

FIG. 12.—Transverse section through the basibranchial sinus of Trochus turb., showing the reno-pericardiac canal opening into the pericardium. $\frac{32}{1}$.

FIG. 13.—Transverse section through the basibranchial sinus of Trochus turb., showing the reno-pericardiac canal opening into the left kidney. $\frac{32}{1}$.

FIG. 14.—Dorsal view of Patella coer, after removal of the shell, pigment, and hood; displaying the relations of the pericardium and both kidneys, the papillæ of the anus and both kidneys. $\frac{1}{1}$.

FIG. 15.—Transverse section through the pericardium and both kidneys of Puncturella spec.? $\frac{32}{1}$.

FIG. 16.—Transverse section through the pericardium of Fissurella gibb., showing the right kidney opening into its papilla. $\frac{32}{1}$.

FIG. 17.—Tectura spec.? viewed from the left side after removal of the shell, showing the position of the pericardium. $\frac{1}{1}$.

FIG. 18.—Papilla and genital duct of Fissurella cost., cut transversely and viewed from behind; a bristle has been introduced into the genital duct, and comes out through the duct of the right kidney. $\frac{10}{1}$.

FIG. 19.—Left kidney of Fissurella cost., dissected out. $\frac{12}{1}$.

FIG. 20.—Transverse section through the pericardium and subanal tract of the right kidney in Patella coer. $\frac{70}{1}$.

FIG. 21.—Transverse section through the right renal papilla and genital duct of Fissurella gibb. $\frac{100}{1}$.

Fig. 22.—Puncturella spec.? viewed from the left side after removal of the shell. $\frac{1}{1}$.

Fig. 23.—Transverse section through the pericardium and both kidneys of Fissurella græca. $\frac{32}{1}$.

Fig. 24.—Horizontal section through the pericardium and both kidneys of Tectura spec.? $\frac{32}{1}$.

Fig. 25 a.—Transverse section through the pericardium and both kidneys of Patella coer. $\frac{70}{1}$.

Fig. 25 b.—Transverse section through the pericardium and the left kidney of Patella coer. $\frac{100}{1}$.

Fig. 26.—Transverse section through the right kidney of Fissurella nubecula, showing the genital duct opening into the right kidney, which also contains eggs.

Fig. 27.—Transverse section through the pericardium and both kidneys of Emarginula Huzardi. $\frac{32}{1}$.

Lenses and eye-pieces used with Abbé's camera lucida.—Zeiss, oc. 1 (achr.), obj. a³ (achr.) = $\frac{32}{1}$. Zeiss, oc. 1 (achr.), obj. 16 mm. focal distance (apochrom.) = $\frac{70}{1}$. Zeiss, 2 (apochrom.), obj. 16 mm. focal distance (apochrom.) = $\frac{100}{1}$.

The above-mentioned eye-pieces and lenses were used for the drawing of the figures of microscopic sections; the whole series of the apochromatic objectives, 8 mm., 4 mm., and 2 mm. immersion, were used during the course of this investigation, with apochr. eye-pieces 6 and 8. The drawings of dissected specimens were done with Nachet's dissecting stand and a camera lucida.

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