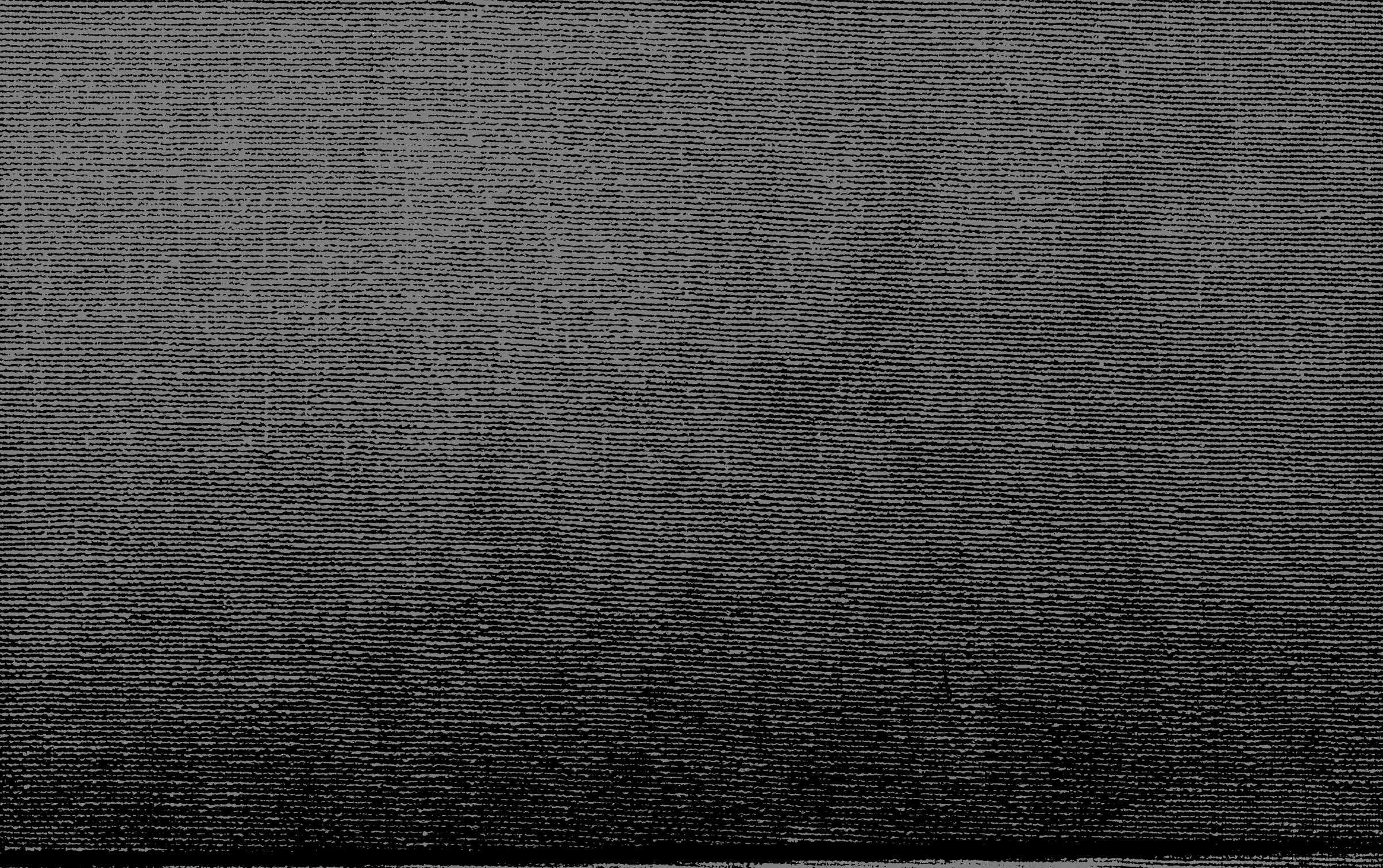

QUARTERLY JOURNAL

OF

MICROSCOPICAL SCIENCE.

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VOLUME 40.—NEW SERIES.

With Lithographic Plates and Engravings on Wood.

LONDON:
J. & A. CHURCHILL, 7, GREAT MARLBOROUGH STREET.
1898.

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The Constitution and Development of the Society of Termites: Observations on their Habits; with Appendices on the Parasitic Protozoa of Termitidæ, and on the Embiidæ.

(Concluded from Vol. 39, p. 315.)

By

Professor B. Grassi in collaboration with Dr. A. Sandias.[1]

With Plates 16—20 in Vol. 39.

The Mode of Development of the Castes in Termes and Calotermes.

I. Development of the Substitute or Complementary Royal Forms.

A. Experiments and Observations on Calotermes under Natural Conditions.

1. The royal pairs of about eighty nests were removed at different times from January to July.[2] After the following October each nest was found to contain a substitute pair, i. e.

[1] [By a misunderstanding, for which the translator is not accountable, the paragraphs containing the observations of Dr. Sandias referred to on p. 253 were enclosed in Part I of this paper between square brackets. All such paragraphs occurring in the text of that part should be credited to him; in the following pages they are distinguished by being placed between asterisks.

On the other hand, all such footnotes of both parts, and such portions of the text of the present instalment, as are contained in square brackets have been added by the translator, some notes not contained in the original having been supplied for this translation by Professor Grassi.—W. F. H. B.]

[2] All these nests were left with a more or less numerous population of soldiers, larvæ of different ages, and nymphs.

forms of a golden-yellow colour, with the compound eyes almost always pigmented, with or without wing-buds and with the abdomen dilated, particularly in the female, which as a rule did not possess the genital appendices. In the majority of examples the wing-rudiments were absent or very short, but exceptionally they approximated in length to those of a nymph. Sometimes one member of the royal pair did, and the other did not possess wing-buds.

The nests not infrequently contained eggs, but never a true royal pair.

2. Either the king or queen alone was removed in the spring from about a dozen nests. After October each nest contained a yellow, i. e. substitute king, associated with a black queen if the king had been taken away; if the queen, a black king with a yellow queen. Therefore the true royal form which had been removed was still wanting.

Corollary.—After loss of the royal forms the orphaned nest furnishes a pair of substitute forms; if one only is missing, it is replaced by a substitute of the same sex, and in such cases a black example mates with a yellow.

Individuals capable of reaching sexual maturity are selected for development into substitute forms, but the most advanced, the nymphs, are not preferred.

3. Many nests orphaned in July, August, and September provided themselves with a substitute royal pair, notwithstanding that perfect examples, that is, black and with fully formed wings, were abundant in those months.

Corollary.—Orphaned nests do not have recourse to perfect insects in order to provide themselves with a king or queen. There is no justification for the belief that nests orphaned at the swarm-period adopt a pair of perfect insects, because thickly populated nests are never found naturally to contain a king and queen of small size.

4. Certain nests orphaned in March and April and reopened in June and July contained a limited number only (six or eight to twenty) of royal substitutes, which were still sexually immature.

5. Five orphaned nests were re-examined after not less than two years' interval. They possessed substitute pairs of the usual type, except that the yellow colour was deeper, and the abdomen, especially of the queens, more distended. Several of these royal forms had no sign of wings.

Others were opened after three or four years, and contained substitute forms precisely similar, save for a more marked enlargement of the abdomen, just as in true kings and queens of the same age.

The progeny in all the above-mentioned nests consisted as usual of soldiers, nymphs, &c.

Corollaries.—There is no subsequent growth of the wings in the royal substitutes.

Their progeny is identical with that of the perfect royal forms.

6. Two nests orphaned a couple of years previously contained no royal pair, but merely some five or six examples in process of becoming substitutes. Neither eggs nor new-born larvæ were present, but the rest of the brood was normal. This was observed in April.

These nests had clearly been orphaned a second time after having once possessed a substitute royal pair.

7. The substitute pairs were removed from several nests which were furnished with them; the colonies provided others.

8. It is not uncommon in nature to find substitute pairs in trees which have been felled or broken by a gale or other accident. In such cases the true royal pair was evidently left behind in the severed portion, and the nests were consequently left orphaned.

Corollary.—Loss of the royal couple is a frequent natural phenomenon.

B. Experiments and Observations on Termes under Natural Conditions.

1. I have never found a single nest containing a true royal pair, though on one occasion such a pair was discovered under natural conditions, but without offspring. By keeping twenty or thirty winged examples of both sexes in a glass jar

filled with rotten wood, I have often got a pair to lay eggs, and once I obtained larvæ in various stages of growth.

Lespès claims often to have found true royal pairs in France. But serious doubt has been thrown on this statement, to which I shall refer in the section on Historical Accounts.[1]

2. In Sicily, Termes multiplies by means of complementary or substitute sexual forms.

3. If two colonies of Termes, both containing complementary queens, are taken from different trees at some distance away and intermingled, they are found to fraternise without the least difficulty.

This has been demonstrated for Termites collected respectively at places six and eight kilometres apart.

It is certain that the insects commingled in the last two experiments belonged to absolutely distinct colonies, and it must be inferred that the Termites of different nests fraternise. And therefore, in spite of their mutual recognition, the application to them of those criteria, which are so effective in deciding whether bees belong to the same hive or not, becomes excessively difficult. One can satisfy oneself that friendship takes place equally whether complemental queens are present in one or in both of the united populations; and this proves that such queens live peaceably together without signs of jealousy even when derived from separate nests.

4. Termites readily migrate from tree to tree, and carry

[1] [Perris, who was a very accurate observer, records ('Ann. Soc. Ent. Fr.' [5], vi, 1876, p. 201) the discovery in the Landes on June 10th of two pairs of Termes lucifugus buried in the ground at the stump of a pine, and each accompanied by a few (four) eggs. Pérez ('Comptes Rendus,' cxix, 1894, pp. 804—806) also performed the experiment of furnishing winged T. lucifugus with an artificial habitat. The couples were put into a jar containing earth and a large block of decayed wood. They buried themselves in the earth, and selected domiciles under it in the cracks of the wood. Pairs established on April 29th were found to be flourishing on July 4th and August 30th, and the abdomen was gradually becoming distended. They had penetrated more deeply into the wood by the latter date, and were found with difficulty. On October 15th six sexual forms were found in the same cavity together with two young workers and an egg. They avoided the light, and no further growth of the abdomen had taken place.]

their eggs and young with them. Consequently many trees which contain a brood are devoid of complementary queens.

5. A trunk containing a numerous population but no queens is cut down and removed a certain distance away. The colony, finding itself orphaned, at once sets to work to rear substitute royal forms. The same thing happens when the complementary queens are killed; and if a few only are destroyed, a few are formed.

All these experiments were begun from November to February, and I did not search for the substitute queens till ten months later. In a single case I began the experiment at the end of March, and looked for substitutes in June; instead of the usual kind I found partially infuscate forms with torn wings, such as I have previously described as originating from imagos, which are still white, and as yet incapable of flight.

I have met with similar examples on various other occasions, when my knowledge of Termitidæ was still too imperfect to allow me to complete the observations then made.[1]

Corollaries.—Termite societies inhabiting trees devoid of complementary or substitute forms, either developed or in process of development, are certainly in connection with colonies in other trees which contain such forms. So far is this true that, if the former are removed to a distance from the latter, they at once set themselves to rear complementary or substitute examples. The insects are therefore fully aware when they have royal forms at hand, and regulate themselves accordingly. Complementary queens are only found in nature in one out of every fifteen or twenty trees infested with Termites; and it must be recollected in connection with this fact that the inhabitants of a trunk destitute of such queens bestir themselves to form them whenever the means of communication with the parent nest containing them are interrupted by distance or other inconvenienccs, or whenever they think fit to do so.

It will consequently be seen that there actually exists a

[1] Since this work was written I have been able to establish four other cases altogether similar to the one described above.

certain number of Termite nests, each of which extends into several trees; and it is impossible in a particular case to decide without great difficulty whether any given examples represent an entire colony, and whether the inmates of a tree destitute of parental forms belong to one community rather than to another.

c. Anatomical Observations on the Generative Organs and their Connection with the Alimentary Canal.

Though I have already said a good deal about the substitute and complementary king and queen, it becomes necessary to give certain details about their generative organs, which my investigations prove beyond doubt to be precisely identical with those of the perfect insect.

I begin with those of Calotermes. The substitute queen possesses a bundle of six ovarian tubes in each ovary (Pl. 19, fig. 1), which increase in length concomitantly with her growth in bulk, and eventually become over a centimetre long (Pl. 19, fig. 2), whereas those of recently developed substitute queens measure scarcely half a centimetre. As a rule but one tube of each ovary contains a nearly ripe ovum, which is always larger than the most advanced ova of the remaining tubes. The ovarian tubes may occasionally be bifurcated at the lesser (anterior) extremity. Vitellogenous cells are absent, but an ovarian follicle exists. The receptaculum seminis is elongated, and never contains a large quantity of semen. The accessory colleterial glands (Pl. 19, fig. 3) are also long; the oviducts are short. The genital appendices, as we said before, are absent, save in very rare cases, in which they differ from those of the male by their less proximity to each other.

All these characteristics, with the exception of the occasional presence of the appendices, are met with alike in the true queen.

The number of follicles which compose each testis of the king is difficult to determine, but is about nine. However, it may confidently be stated not to differ in the true and sub-

stitute kings respectively. Like the ovaries, the testes increase with the growth in bulk of their possessor, and are therefore notably smaller in a small than in a large king (Pl. 19, figs. 4, 5). The vesiculæ seminales and genital appendices are present, but there is no intromittent organ. In all these respects the true and substitute kings are identical.

Both kinds are found to contain ripe spermatozoa, though always few in number, in the vasa deferentia. There is a special liquid which rarely exhibits spermatozoa in the structures which, by analogy with other insects, I call the vesiculæ seminales; and spermatozoa are always absent in both the vasa deferentia and vesiculæ of examples which still possess fully formed wings and of young substitute kings.

No writer has hitherto observed the spermatozoa, on account of their unusual character and small size; in describing them I shall limit myself to those features which can be made out without special preparation, as I have not investigated them minutely.

In order to make sure of their complete maturity, spermatozoa were taken for examination from the spermatheca of the queen (Pl. 19, fig. 12). They are relatively short, varying from 12 to 20 μ in length, non-motile, flattened, and therefore differing in shape according as they are viewed from the face or the side. When seen from the face they are elongate and subquadrangular, like a grain of rye, or better, a seed of zinnia; sideways they appear more or less regularly linear. One extremity usually presents a characteristic thickening, and there are certain cilia-like appendages which are better visible when the spermatozoon is seen sideways. There is no difference between the spermatozoa of the true and those of the substitute king.

I now pass to Termes lucifugus. In the queen the ovarian tubes are so numerous as to be counted with difficulty, but there are about thirty-six on each side; though they do not exceed half a centimetre in length in the largest individual, their shortness in comparison with those of Calotermes is more than compensated for by their number (Pl. 18, figs. 1—4). They

appear at first sight to be less numerous in young substitute and in perfect queens; but this is deceptive, because many are so far arrested in growth as to be with difficulty made out. The remainder of the female generative organs (Pl. 18, fig. 10) present no marked differences from those of Calotermes.[1]

In Termes alike the number of testicular follicles is difficult to count, but is about eight (Pl. 18, figs. 5—8). Neither in this nor in any other respect is there any difference between the sexual organs of the substitute and of the perfect king.[2]

Spermatozoa are present in Termes lucifugus in the spermatheca (in small quantity, from 150 to 1000 in round numbers) and vasa deferentia, just as in Calotermes. For examination they were collected by preference from the spermatheca (Pl. 18, fig. 9). Their form is round, about 2 to 4 μ in diameter. The immature spermatozoa in the testis possess a tail, which is lost as they ripen. They are also non-motile.

The degree of development of the generative organs in examples in process of becoming substitute kings or queens varies concomitantly with the stadium in which they are. In Calotermes, in which the phenomena can be better followed, I find that the development of the gonads proceeds very slowly in the royal substitutes, and that the pigmentation of the compound eyes is almost always the earliest diagnostic character. The female genital appendices are usually shed at the next succeeding ecdysis.

I have spoken of the substitute forms in course of growth, but which have not yet moulted, as larvæ of such forms; they

[1] In Termes numerous unicellular glands, each furnished with a cuticular duct, are crowded under the columnar epithelium of the spermatheca and open into its lumen (Pl. 19, fig. 6). I have not examined Calotermes for them. The accessory colleterial glands are two in number, one usually long and full of secretion, the other shorter and narrower, its secretion being generally very scanty.

These glands have a common aperture in connection with the ninth true sternite, whilst that of the vagina, into which the spermatheca opens, is in the eighth true sternite. These observations apply to Termes only.

[2] The intromittent organ is wanting, and the male genital opening lies in the ninth true sternite.

are, as a rule, distinguished by the pigmented eyes and the presence of genital appendices in both sexes.

The gonads are sexually differentiated, and the vasa deferentia and oviducts are present at the time of birth, but there is no trace of the external genitalia, which do not become evident until the larvæ are divisible by the greater or less size of their head.

The soldiers and workers are of both sexes, as Lespès discovered for Termes, and Müller confirmed for the soldiers of Calotermes. Observations made by means of longitudinal and transverse sections allow of my giving some additional particulars. The gonads are evident in every soldier and worker, and persist in a nearly uniform stage of development, regardless of age; and all the component parts of the generative apparatus of one or the other sex are invariably present, though of very small size. Both the external and internal genital organs are less reduced in the Calotermite soldier than in the soldier and worker of Termes, and are better developed in these forms of both species than in the larvæ at the time when the head is beginning to vary in size.

The greater part of the abdominal cavity is occupied by the gonads and the alimentary canal (the hinder part of the foregut, the chylific ventricle, and intestine). The intestine presents a cæcal ampulla, which is sometimes greatly dilated, but is much less so at other times, and may even be contracted. In the first case it occupies a large part of the abdomen, and must undoubtedly exercise an indirect pressure on the genital glands, except during the earliest stages of life. The amplitude of the cæcum stands indisputably in an inverse ratio to that of the degree of development of the gonads, and its greater or less size is correlated with the presence or absence of certain parasitic Protozoa. These are fully described in an appendix. They are here mentioned with reference only to the singular fact of their absence in substitute or complementary royal forms, whereas they are present, at least in small numbers, in the perfect insects.

The ampulla also contains bacteria (Spirilla and Leptothrix), which I have not specially studied, for they are not absent in the complementary or substitute forms, and occupy but little space. But it is crammed, so to speak, with Protozoa, either Cercomonadidæ (Monocercomonas, Dinenympha), Pyrsonymphidæ, or Lophomonadidæ. Termites free from Protozoa are generally distinguishable by the naked eye, on account of the immaculate abdomen; in those which possess them the abdomen presents a more or less distinct yellow patch, due to the cæcum being visible through its transparent walls. This patch derives its colour from the vegetable aliment of the Termites, with which a large number of the parasitic Protozoa are filled (see the Appendix); it is indistinguishable when the food is colourless, and a Termite may then be supposed to be free from Protozoa, whilst it is really harbouring an enormous number.

Protozoa are absent in the new-born larvæ, and make their appearance in Calotermes concomitantly with the appearance of the four secondary Malpighian tubules, and with the acquisition of a twelfth antennal joint, the third and fourth being glabrous. These examples, described in the preceding chapter, vary from 2·5 to 3 mm. in length, and may possess either a large or a small head. If the former is the case their ampulla is comparatively dilated, and contains both Monocercomonads and Lophomonads; whereas those with a small head contain Monocercomonads only, the Lophomonads first appearing in a later stadium.

Monocercomonads, Dinemympha, and Pyrsonymphidæ begin to appear in Termes lucifugus when there are twelve all pilose antennal joints and eight Malpighian tubules, four large and four small, and the body averages a little more than 2·25 mm. in length; and they are sometimes present and sometimes absent in such specimens. More precisely, they are generally absent in small-headed and present in large-headed individuals; but they are constantly to be found when the body length exceeds 2·5 mm.

The Lophomonadidæ do not appear, at least as a rule, until

the body exceeds 2·5 mm. in length, and the eight Malpighian tubules have become similar.

In general, Protozoa may be said to make their earliest appearance in the large-headed forms both of Termes and Calotermes, at a time when the Malpighian tubules are evidently divisible into two groups, large and small.

If we disregard the absence of Protozoa in the very young larvæ, they may be said constantly to be present in the winter, when the insect development is arrested, in all members of the community except the complementary or substitute forms, and to be then very abundant in the soldiers and workers, somewhat less so in the larvæ and nymphs, and scarce in the true kings and queens. I should add that they are found, usually in small numbers, in every winged example which leaves the nest.

In summer the Protozoa are somewhat differently distributed on account of the frequent ecdyses of the Termites, before each of which they die off, to be subsequently re-acquired.

That Protozoa are absent in the complementary or substitute forms is a fact that has been verified in hundreds of cases. But it is apparently contradicted by the following observations:

1. A certain number of Protozoa were found in queens of Termes lucifugus, evidently of great age, as their shrunken ovarian tubes and empty spermatheca showed. That these examples had certainly borne eggs was demonstrated, firstly, by the presence of a granular yellowish substance, never found in examples which have not oviposited, and situated at the mouth of the ovarian tubes in the oviducts; and secondly, by the similarity in condition of the upper and lower ovarian tubes, the former being always much the smaller in virgin examples.

2. Whenever Protozoa were present in any numbers in a substitute king or queen of Calotermes, the host was found to be depauperised (e. g. by infestation with acari), and exhibited an imperfect development of the testes or ovaries.

3. Numerous Protozoa were found in a few substitute kings and queens of Calotermes which had been kept some days in a glass jar containing a little wood, together with many other examples of the same species taken from different nests. But during this time the royal specimens had been taken out and put back several times, and may be regarded as having been disorganised.

4. Lastly, a very few kings were found to contain a moderate number of Protozoa in midwinter,—that is to say, at a time very far removed from the period of copulation.

Allowing all due weight to these exceptions, we may still say that they are far from disproving, but rather confirm the rule, which may be thus formulated:—When the genital organs are mature in individuals of which the wings are not fully developed, Protozoa are absent, and the dilated cæcum is therefore of minimal size. The absence of Protozoa does not, however, connote the incapacity of the insects in question to possess them.

But here a grave question presents itself. Is the absence of Protozoa a cause of the ulterior development of the genital organs, or simply a consequence? Observations for the purpose of solving this question were undertaken on complemental or substitute forms in process of development (larvæ or immature examples). In these Protozoa were usually, but not invariably, absent. The significance of this last fact is not easily estimated, but after careful consideration I feel justified in offering the following explanation:—Examples in process of becoming complementary or substitute sexual forms do not habitually possess Protozoa; they may from time to time become infected with them, but they rapidly get rid of them independently of the moults. Possibly the development of the genitalia is arrested dnring the infection.

It follows from all this that in complemental and substitute forms there is an accelerated or anticipated maturation of the genital organs which appears to be in intimate relation with the loss of the parasitic Protozoa from the cæcum. Such a loss in growing

specimens, in which the generative glands are still very immature, or before they are in a condition to undergo compression by the cæcal ampulla, suggests that the absence of Protozoa may not be unconnected with the maturation of the glands.

The experiments and observations described below were undertaken by me in order to determine better the importance of these parasites.

D. Experiments and Observations on Nests kept in Glass Tubes.

(*a*) Calotermes.

1. Numerous small nests were made in glass tubes, each containing from fifteen to forty examples of different ages, but no adults and no royal pair.[1] After a few days from two to six incipient substitute forms, characterised by the pigmented eyes, were found in each tube; they were usually obtainable in about a week if the tubes were carried in the waistcoat pocket, and in summer they often appeared as soon as the fourth day. In fact, after as short a time as thirty or forty hours one could tell which individuals were capable of acquiring the ocular pigment.

The abdomen of these specimens presents a characteristic appearance, being hyaline and destitute of the tinge of colour caused by the food (wood). They evidently possess no Protozoa. But those which assume this appearance do not always acquire the ocular pigment,—that is, they do not always become royal substitute larvæ. The eyes become (invariably?) pigmented without an antecedent ecdysis.

The formation of such incipient royal substitutes never takes place in any tube containing a royal pair. But if the king and queen are removed for twenty-four or forty-eight hours, or are injured, a few substitute forms appear at times.

[1] The wood in such nests should not be white, or the presence or absence of Protozoa cannot be readily distinguished by examination of the specimens with the naked eye or a simple lens.

These facts have been confirmed on very many occasions.

2. Furthermore, a nest was made up of three large larvæ only; after a fortnight one of these exhibited pigmented eyes, and was therefore in process of becoming a substitute form.

3. The formation of substitutes may take place at any time of the year, and is never delayed for more than a fortnight if the tube is carried in the waistcoat pocket.

4. If a nest containing growing examples is furnished with adults ready to fly, or with the wings shed in the act of capture, the formation of royal substitutes takes place as usual, while the perfect insects bore through the cork and escape in search of fresh quarters. This confirms the observation that Calotermes does not make use of perfect insects capable of flight as substitute forms; and the reason of this is certainly to be found in that prepotent instinct towards quitting the nest which arises in all Termitidæ as soon as they become fully coloured imagos.

5. If a nest is formed only of examples with not more than twelve antennal joints, substitute forms are not developed within the usual period. But if a few small-headed larvæ, with from fourteen to sixteen antennal joints, are included, they are promptly transformed into larvæ of royal substitutes.

6. A colony of about two hundred Calotermites is collected and separated into some fifteen nests, the royal pair being purposely killed. Every nest will furnish two, three, or four substitute forms.

Another colony of equal size is collected and put into a single glass jar after destruction of the royal pair; it will form from four to six substitutes.

The same number of Calotermites will therefore produce a very large or a very small quantity of substitute forms according to circumstances.

These simple experiments at once suggest that the ordinary members of the colony are capable of development into these forms; and further justification for this view is found in the fact that specimens in different stages of development (without,

or with more or less evident rudiments of wings) may be found in process of elevation to this dignity.

7. In a few tubes (2, 3, or 4 per cent.) no substitute forms are developed, in spite of the presence of individuals apparently capable of undergoing that destiny.

I have established the fact that there is no development of royal forms during May and June in tube-nests which contain only nymphs.

And here I ask the reader to admire the talents of these little animals!

What is the use of furnishing royal substitutes when all the inhabitants of the colony will soon become fully winged, and will disperse to found new nests? An occasional nymph may be sacrificed to become a substitute form. But that takes place only at a time remote from the swarm-period, or when there are younger examples present.

A few nests, indeed, form no substitutes, and that without any justifiable motive. I am unable to decipher their intentions, but the fact remains that these republics, imbued, as they seem to be, with Malthusian doctrines, find their parallel in certain beehives which cease to concern themselves with the possession of a queen.

8. In nests formed of individuals presumably free from Protozoa (that is immediately before, during, or after an ecdysis) a certain number of incipient substitute forms (at first larval, and later immature forms) appeared without any marked delay. They preserved themselves free from Protozoa for some time at least, but the other occupants became infected simultaneously with the appearance of these incipient substitutes.

9. Even if all the inmates of the nest contain Protozoa, some of the usual substitute forms are produced without material delay.

10. In the eighth case the appearance of Protozoa was due either to their unsuspected presence in some individual which infected the others, or to the accidental inclusion in the nest of fresh dejecta of examples containing Protozoa (the nests having been made with wood previously inhabited by Calotermites).

The experiment was therefore repeated several times with increased care in the selection of specimens, and with the employment of wood which had been occupied by Termes (many parasitic Protozoa of which are not conspecific with those of Calotermes).

In this way it was possible successfully to establish several nests which remained free from Protozoa for over a month. Nevertheless only a few examples from among all those of suitable age became substitute forms.

It is thereby demonstrated that the mere absence of Protozoa is insufficient by itself to stimulate the maturation of the genital organs. But it is certain that the first indications which point to the development of a substitute form date from their disappearance. However, this disappearance also takes place in examples approaching an ecdysis, and by the use of tube-nests it can be made out with certainty that the incipient substitute royal forms (larvæ) undergo a moult a few days after the eyes begin to darken. The ecdysis may therefore be responsible by itself for the disappearance (death) of the Protozoa, which would then be indicated as a secondary phenomenon.

11. Nests of examples free from Protozoa are difficult to keep alive. This raises the question whether the cause of death is due to the absence of the parasites, or simply that the inmates are in process of moulting, or are just entering or quitting a moult. Recently moulted examples have an especially tender cuticle, and undoubtedly require a greater amount of moisture in the air and food; they are therefore more delicate, and this alone may sufficiently explain the difficulty in question. But I shall return to this point later.

12. Examples in process of becoming royal substitutes reached maturity in the tubes in very few cases (after three or four months; the tubes in question were packed in a box and taken to North Italy and Germany). As a rule, the nest dies away before they are fully developed.

So considerable a delay in maturation appears only natural if it is recollected that examples are selected for the throne at a time when their generative organs are very backward in development.

Forms destined to become substitutes may be said generally to become re-infected with Protozoa at the end of a week or ten days; they lose them in about a month at the succeeding moult, but may subsequently re-acquire them. Too much importance should not be attached to these facts, because they are exhibited when the nests are mostly in bad condition.

From the foregoing observations I infer that the transformation of ordinary into substitute forms must be dependent on a change, either quantitative or qualitative, in the character of the ordinary diet. This is a necessary sequence from what can be made out in the tube-nests, in which, in fact, we have seen that a small quantity of decayed wood suffices not merely to keep the Calotermites alive, but to enable them to rear up substitute forms.

I shall therefore record a series of observations on the aliment of Calotermes, which have been carried out by means of tube-nests.

Their food and drink consist of—

1. Wood.
2. The matter excreted or disgorged by other Calotermites.
3. The exuviæ of each other.
4. The corpses of other Calotermites.
5. Their own saliva or that of others.
6. Water.

*Calotermites triturate dead or dry wood (and cork) with their mandibles, and devour the fine meal which is thus obtained. This meal is not wholly used for food, but, as we have mentioned more than once, is employed partly for building, and is also partly regurgitated (vide infra). Wood is therefore the basis of the alimentation of Calotermes.

The insects are consequently nourished on a material very

deficient in nitrogen, and their slowness of growth must be correlated with this fact. They must certainly digest cellulose or lignin, or both of these ternary substances.

The mandibles of the soldiers are incapable of gnawing up wood. This explains why these forms die after a short time if kept in isolation—a fact I have repeatedly established by forming nests entirely of soldiers (vide infra). Nevertheless, they eat wood which has been triturated by other members of the colony.

The dejecta play an important part in the nutrition of Calotermes, and may be said to form the ordinary diet. Lignin (by the phloroglucin reaction) and cellulose are found to be present in the fæces, which appear under the microscope as a very fine detritus, in which traces of fibres or spiral vessels are to be made out with difficulty. They appear to the naked eye as cylindrical scybala, usually a little over half a millimetre, but sometimes as much as a millimetre in length. Their colour varies in accordance with that of the wood in which the Calotermites are living, and is commonly dirty white if derived from cactus, red-brown or brown if from almond, &c. The scybalum is often brown with one extremity reddish, and is usually quite brown when stale. If carefully examined with a lens it is seen to be prismatic rather than cylindrical, owing to the impressions it receives from the rectal plicæ.

Calotermites ingest this substance either fresh or old, and may use it for building, either directly or after ingestion and subsequent regurgitation. By prolonged observation of the living animal, or by suitable examination of the contents of the alimentary canal, it is easy to satisfy oneself that but a very small proportion of what is ingested is regurgitated.

The dejecta are greatly preferred at the time of elimination, probably because, as is obvious, they are then less dry (Calotermes does not habitually drink, as I shall mention later).

In order that they may be obtained in this condition, their elimination is provoked by means of the antennæ and maxil-

lary, possibly also the labial palpi (I have not been able definitely to establish the latter point).

When a Calotermite wishes to feed, he accosts one of his fellows and caresses the abdomen with the antennæ and palpi. If the one thus accosted is prepared to eliminate, he at once extrudes the scybalum from the anal aperture. The other then removes it, chiefly by aid of the maxillary palpi, and usually in two operations separated by a short interval, drawing it at first half, and then completely out. He then rapidly seizes it with the mandibles, suspending his caresses for this purpose, and when he has possessed himself of it, nibbles at and ingests it little by little.

If the insect accosted is unable to furnish what is required, he is at once abandoned and another pursued, and in this way several examples are solicited in turn until one produces the substance desired. But a single scybalum is rarely sufficient, and consequently the one which has devoured it immediately sets off to procure another.

A scybalum, of which the extrusion has been caused by an individual even of royal stock, is sometimes seized and quickly devoured by another.

If the example solicited is prepared to eliminate, it stands still, otherwise it runs away; and the thing becomes more curious when the dejecta are either not quite ready for extrusion, or when but a small amount is ready. The one solicited runs away and the other pursues; and this most probably is the explanation of the so-called amorous passages seen in many termites and described in the preceding chapter.

The operation of caressing the apex of the abdomen to procure elimination of the dejecta is performed alike by the soldiers, but with some difficulty, on account of the large size of their mandibles. The soldier achieves his purpose by placing himself so that his head forms nearly a right angle with the body-axis of the example caressed, and the buccal apparatus situated on the under side of the head comes into contact with the anal aperture of his fellow, who in turn is held steady by the soldier's mandibles resting on the upper

side of the posterior extremity of the abdomen. Palpation is accomplished to some extent by the mandibles, but principally by the fore-legs, as is sometimes observed in other members of the colony.[1] The soldier ingests slowly and with difficulty.

Other individuals do not fail to perceive what is taking place, and make attempts at robbery, which are sometimes successful.

After provoking the elimination of the dejecta, a large example sometimes retires to allow a little one to partake; and the latter has been occasionally noticed to have previously twitched the legs, and especially the tibiæ of the larger one, perhaps as a warning that it was hungry.

It should be added that much fæcal matter is spontaneously deposited in the middle of the nest, and, if not ingested, is then carried away by its eliminators, who employ it in forming barricades, or store it up in uninhabited parts of the nest.

When an individual has finished eating, another approaches and licks over the front of the head and antennæ with rapid movements of the palpi, evidently to clean them; not uncommonly two clean each other alternately. Then one may stop for a moment while the other continues the process, and passes to the occiput and then to the legs, going from their base to their apex. The recipient of these attentions stands still for some time, and then rapidly takes to flight, probably because the other has bitten one of its tarsi on reaching it. This cleansing operation is usually resorted to after a meal, but is sometimes manifested without that preliminary.

If the mouth of certain examples is attentively observed, it will often be seen to exhibit a microscopic reddish-brown pellet, which gradually increases in bulk till it forms a rounded mass of about a millimetre in diameter. This pellet is obviously composed of regurgitated food, and is sometimes used for building; at other times another example approaches, seizes and devours it.

The excreted or disgorged substances are occasionally

[1] Excretion may also be stimulated by gently stroking and compressing the abdomen with a feather.

yellowish and semi-fluid, and the exact method of nutrition is then more difficult to ascertain.

Though ecdysis is a very frequent phenomenon in Calotermes, it is difficult to find exuviæ in the nest. This, coupled with the fact that the alimentary canal sometimes contains portions of cast skins, suggested to me that they are habitually devoured. Attentive observation shows that this is really though not invariably the case. Sometimes the assistants at a moult eat the exuviæ bit by bit as they are shed; and at other times an example seizes the skin directly it is cast, and carries it away for a greater or less distance to eat part or the whole. The proctodæal cuticle may or may not be shed with the rest of the exuviæ. If it is not, it may be passed either spontaneously, or as a result of the palpation and pressure employed to produce defæcation by another insect, which then draws out and devours, instead of the dejecta, a white substance, which is the cuticular lining in question.*

Besides the practice of eating the cast skins, I have mentioned that at times a Calotermite may apparently finish the operation of licking another by a bite. The carnivorous propensities of these insects do not stop there. Any example not in normal condition (e. g. which is shrivelled, or which shows signs of being ill by remaining long motionless for some undecipherable reason, or which is unable completely to moult the skin) is eaten before death by its companions. It is not uncommon to see a soldier decapitate a living nymph, or to find an example with the abdomen eaten and the legs still in motion. At times a victim attempts to retreat, but is then usually decapitated at a stroke by a soldier. But this mode of execution is not always resorted to, and the antennæ may be bitten off first, &c.

*I may add in passing that so great is the ferocity of the soldiers that one may be occasionally seen furiously to assault five or six other individuals and bite off heads and abdomens, &c. The object of these massacres, which take place only when the nest is thrown into disorder, is not quite clear.

Probably the soldier imagines that his companions are the cause of the disturbance.

The king of a queenless nest was observed to be dead, but a few hours later the body could not be found—a clear proof that it had been devoured. This king had shown signs of illness a few days previously, and the population had provided a certain number of substitute larvæ.

On one occasion nine examples, of which one was a soldier, were detected at night in the act of devouring a royal substitute in process of moulting. The wretched animal was still alive, and writhed all over its body to free itself from the torture. The nine assassins were probably annoyed at the light to which they were exposed, for they at once stopped eating, and jointly carried off the victim by means of their trophi to a darker part of the nest. Meanwhile many others crowded up, evidently to partake in this feast of royal flesh.

Occasionally one is seen to lick the leg of another for some time, and then suddenly to bite off the tarsus.

The tubes often contain nymphs with imperfect wings which have been nibbled off by their companions; and it is remarkable that the right fore-wing is usually selected in those destined to become substitute forms. This has been already referred to, as has the constant mutilation of the antennæ in the royal pairs. The preceding observations suggest as an explanation of the latter curious phenomenon that the antennæ have been gnawed off. Possibly the royal examples bite off each other's antennæ, for they are found to be already mutilated in solitary royal pairs, that is without offspring.

An individual which has been some time dead is no longer eaten.

The saliva next claims attention: it issues in connection with the labium as a colourless and distinctly alkaline liquid, which contains no structures discoverable with the microscope. This liquid collects on the labium as a small drop, which may be employed either as a cement in building or as food for others. These may either possess themselves of the drop and then retreat a little way to swallow it gradually, or they may

receive it from the one which secretes it and clearly provides it for them as an article of diet.

The assimilation of a drop requires a certain number of acts of deglutition, which may be counted, usually four or five.

Examples may sometimes be seen to perform several such acts in order to swallow their own saliva as it trickles from the labium.

Termitidæ do not habitually drink; but Calotermites (imagos, soldiers, &c.) which are moribund from drought may be seen to apply the mouth to wood saturated with water and suck the latter up, keeping the mandibles still and moving the rest of the mouth parts. When the water is drained from one spot they move to another.

Other parched examples (soldiers kept some time in a dry place) have been observed to have recourse to a drop of water placed in a watch-glass, in which they were found. When the watch-glass was examined from below, the soldiers were seen to move both the mandibles and maxillary palpi when drinking, holding the legs as erect as possible, and the abdomen turned up so as not to wet the body.

We may disregard these exceptional cases to consider how and when the different forms are nourished.

Wood-meal is eaten by all except the very young, which first begin to do so when the apex of the mandibles and maxillæ becomes coloured. This statement applies equally to the dejecta, vomit, exuviæ, and dead bodies, except that the young appear to feed on the two former substances earlier than on wood.

I shall specially mention the following phenomena as having been observed: the soldiers are sometimes supplied with vomit standing mouth to mouth; they also regurgitate it; the royal forms feed alternately on each other's dejecta; large examples may solicit from small.

Saliva is given or yielded in abundance to larvæ which are too young to eat wood, and to those in course of becoming royal substitutes, and a certain quantity is also given to the other small-headed forms.

It is only with much patience that such phenomena can be determined, but it can be done with complete certainty. It has been especially noticed that an incipient royal substitute was approached after a recent ecdysis by different individuals which administered saliva to it; and it should be added that after the last moult the nymphs make repeated acts of deglutition to swallow the saliva secreted by their own glands.

Moreover saliva is seen to trickle from the labium of, and to be swallowed by incipient royal substitutes which have just finished moulting, if they are not supplied with it by their companions. The secretion may stop for a few minutes and then recommence. The forms which secrete saliva for others are large larvæ or nymphs.*

Individuals fed with saliva exhibit a great transparency of the abdomen, an indication that they are in progress of becoming royal substitutes. They contain no Protozoa, or contain them dead, and their death or disappearance is most probably a direct result of the action of the saliva.

It is a moot point whether the maturation of the generative organs is due solely to the saliva or to the absence of Protozoa as well; but the latter, as my previous remarks show, is not by itself a sufficient cause.

I have frequently asked myself whether the Protozoa have not an important digestive function, since the comminuted wood passes almost entirely through their bodies. It is probable, but not proved.

*Termitidæ can go without food for several days; the soldiers especially eat much less than other forms, and can endure more than eight days' abstinence. Exercise, therefore, costs the colony little.

As is only natural, when the fæces have repeatedly passed and repassed through the bodies of the insects, they are no longer sufficient to support life. For this reason a nest of nothing but soldiers dies, because they are incapable of gnawing up fresh wood. But the addition of a single large larva to a colony of ten or a dozen soldiers is enough to keep them alive.*

(*b*) Termes lucifugus.

Experiments on this species are much more difficult, and can only be carried out in large glass receptacles, which do not permit of the necessary observations. Substitute forms were easily obtainable in greater or less numbers in the few cases in which the Termites survived in abundance and in good health for some months. On this point I have nothing to add to what is recorded in other parts of the present work.

The complementary queens are objects of tender care not only on the part of the workers, but of the larvæ, and are much more assiduously cleaned than the substitute queens of Calotermes. Five or six companions will stand round one of these queens at the same time, one cleaning her legs, another the antennæ, a third the abdomen, &c.

Their nutrition exhibits the same features as those related for Calotermes.

Conclusion.

The facts recorded justify the conclusion that the saliva of both Termes and Calotermes exercises a marvellous influence on individuals in process of becoming perfect insects; it effects their transformation into substitute or complementary royal forms. This is substantially a most remarkable phenomenon of neoteinia. But whereas neoteinia, or the sexual maturity of forms retaining larval characteristics, is dependent in Amphibia on the environment, in Termitidæ it is essentially subordinate to the nutrition.

II. Development of the Soldiers and Workers.

Termitidæ possess three special castes—workers, soldiers, and neoteinic forms (according to the expression used in the preceding paragraph). The latter are not only arrested in

development, but may present characters peculiar to themselves, and differing from those of the perfect insects (e. g. the long outstanding abdominal setæ and black maculation in Termes). Special and still more pronounced characteristics are exhibited by the other larvæ of arrested growth, that is the castes of soldiers and workers. The proof just given that one, the neoteinic caste, is dependent on nutrition, is enough to suggest on a priori grounds that this may be equally true of the others.

Strictly a priori the soldiers may be regarded as further differentiated workers, and indeed at the beginning of their development they exhibit but a single known characteristic, the enlargement of the head as in the worker. The soldier therefore begins by possessing the characters of the worker. In other words, we may say that an individual destined originally to become a perfect insect is capable of undergoing a paranomalous development, of acquiring certain characteristics which, by absolutely destroying its fertility, no longer allow of its becoming a perfect insect. If the differentiation of the head is arrested when certain structural modifications have been acquired (simple increase in its size, a special form of pronotum, &c.), and the individual is limited further to uniform growth in stature, except for the augmentation in the number of antennal joints, we have a worker. But if, instead of this limitation, the mandibles and labrum become elongated while the maxillæ and labium do not alter, then we have a soldier. In short, worker and soldier follow a common path for some distance; at a given point one, the worker, continues along it, while the other, the soldier, diverges from it. And with this are connected the facts that young soldiers and workers are indistinguishable, and that certain Termitidæ (Calotermes) possess soldiers, and others (Anoplotermes) workers only.

But these inductions all require the control and support of direct observations; and mine indeed are not quite complete, as I have been unable to keep Termes alive for a sufficient time.

Nevertheless a few crucial tests have given results entirely in accordance with the above induction. These are:—

1. Transformation into a soldier larva, and subsequently into a soldier, can take place at very various ages.

As in Calotermes, the soldier may originate from examples with from twelve to seventeen antennal joints, and therefore of very different sizes and with or without rudiments of wings (see § 2). Newly formed soldiers are therefore to be found of different dimensions (small, medium, and large), and with a variable number of antennal joints.

The small soldier is an inhabitant of newly established nests which experience the want of soldiers, and therefore anticipate their development.

2. Various observers have found soldiers with more or less well-marked wing-rudiments in the nests of exotic Termitidæ, and have regarded them as an inexplicable abnormality. My belief, on the contrary, is that their development is the result of unusual or extraordinary nutrition, and is in harmony with my previous statements. If it be correct, it should be possible to put Termitidæ under such conditions as would compel them to produce similar monstrosities. That such a thing is really possible is shown by the following experiments.

*In winter small Calotermite nests of nymphs alone, and therefore free from soldiers, are established in tubes containing triturated wood, and are kept in a warm place, in the pocket or near a stove.[1] After a time they are found to contain not merely a certain number of nymphs in process of becoming royal substitutes, but others as well, which are being transformed into soldiers, and are distinguished especially by an elongation of the mandibles and labrum as well as by the greater size of the head. The wings are simultaneously reabsorbed until barely a vestige remains. These nymph-soldiers, as they may be termed, become perfect soldiers after an ecdysis. It cannot be positively stated that all succeed in reaching the

[1] It should be added that the development of Termitidæ kept in a warm place proceeds even during the winter, the queens prematurely laying eggs, which hatch precociously, &c.

goal, because they remain unchanged in some nests for months together. The pigmentation of the eyes found in some of these nymph-soldiers is curious but intelligible.

Similar experiments were made on natural nests in trees by removal of a large number of soldiers, and after some time a few nymph-soldiers were found in them.

I was led by this to suspect that such forms may at times be normally found in intact nests, and by paying particular attention to the origin of the large soldiers I actually found that normal nests in which the soldiers originate from larvæ with wing-buds, or nymphs, are not rare; this phenomenon has escaped the most accurate investigators, including Fritz Müller, on account of the reduction of the wing-rudiments, which perhaps disappear completely in the case of larvæ.*

The following fact shows that these phenomena hold good equally for Termes.

In a small nest of Termes lucifugus kept in a glass jar I obtained a nymph-soldier which had almost exactly the thorax of a nymph of the second form, and the head of a soldier. At the time of formation the nest contained a certain number of perfect insects, many workers, a few soldiers, and some undifferentiated larvæ. This nymph-soldier was found in it six months later, together with a number of workers, an ordinary soldier, and a nymph of the second form. Death had evidently claimed many victims during the six months that the nest lasted.

I have met with similar nymph-soldiers on other occasions. All these observations are sufficient to show that the casts of workers and soldiers denote merely a paranomalous development of individuals capable of becoming perfect insects.

3. Further evidence is afforded by the fact that the newly born larvæ are relatively alike inter se,—relatively only, because they are not all identical in bulk. This was observed by taking Calotermites in the act of hatching (they emerge at one pole of the egg) and comparing them after preservation by a uniform method.

*It is hardly practicable to discover Termes in the act of quitting the egg; consequently a large number of examples must be collected, subdivided according to their different stadia, and then compared.

*4. The newly hatched larvæ are fed on saliva, to which, after some time, is added a clear yellowish vomit, probably largely diluted with saliva; later still they begin to adopt other articles of diet.

Examples of which the head is beginning to enlarge must receive but a very small amount of saliva, as is shown by the woody or fæcal colour of the intestinal contents. But those in which the head remains narrow continue for a longer time to receive pure or nearly pure saliva, which is afterwards always administered in some quantity.

5. If a large number of small and narrow-headed larvæ are added to small tube-nests, some without and others containing soldiers, part of those in the former nests are generally found to develop a large head, which is not normally the case in the latter.

It is clear, therefore, that the colony short of soldiers tends to furnish itself with them, as it does with royal forms.

But proof is required that the small large-headed larvæ really become soldiers. A calculation of the proportionate numbers of the castes in colonies of Calotermites shows that of the soldiers to be very small—at most one in every fifteen or twenty in a very populous nest, or one to every five or six others in a very small colony. Now, in the aforesaid soldierless nests one large-headed larva appears to every five or six inmates, and in natural nests the small large-headed larvæ are very few in proportion to those with a small head.

All this goes far to furnish the necessary proof. And the matter becomes a certainty if we study the nests of Termes, in which the young, with already differentiated heads, show a large percentage of large-headed forms, in agreement with the fact that a considerable number of workers is developed in addition to the soldiers.

Evidently, then, the large-headed larvæ are des-

tined to become workers and soldiers, or the latter alone in Calotermes, in which workers do not exist.

Although details cannot be given, it may be concluded that the development of the soldiers and workers is consequent on the less quantity of saliva which they receive; and with this is associated the earlier appearance of Protozoa, and their constant presence in great abundance.

The method by which the soldier is further differentiated from the worker is a more obscure matter to determine. Is the larger amount of nutriment which the latter receives the cause of such a phenomenon? In any case it is certain that the essential factor must be one of nutrition.

Numerous experiments have shown that the soldiers cannot become royal substitutes, and further that they themselves are incapable of transforming larvæ or suitable nymphs into substitute forms.

General Considerations and Retrospective Note on Thysanura.[1]

1. The soldiers, workers, neoteinic forms (complementary or substitute kings and queens), and the true perfect insects are all derived from similar ova; in other words, every ovum must be regarded as inherently capable of giving rise to any one of these four classes of forms. It follows that the differences between them connote something absolutely unrelated to the distinctions of sex; and the existence of Termite castes has therefore no bearing on the theory which postulates that every somatic cell is derived partly from the mother and partly from the father.

The following points are also of importance:

(1) The neoteinia of Termitidæ is no isolated phenomenon, but is paralleled in many other insects, including the Orthoptera (sensu lato).

Sexually mature forms, some with well-developed, others with short wings, and that independently of sex, are met with

[1] [Grassi, 'Atti Acc. Lincei' (4), iv (1887), pp. 543—606, pls. i—v; with bibliography of the author's antecedent memoirs on Thysanura.]

in certain species of grasshoppers. Similar phenomena occur in Psocidæ, Perlidæ, &c.

(2) The Embiidæ, which, as will be seen later (Appendix II), have been regarded as near allies of the Termitidæ, though they are nothing of the sort except in so far as they belong to the same order, the Orthoptera (s. lat.), exhibit a sexual dimorphism comparable with that subsisting between the soldiers and other Termite forms, namely, a much greater development of the mandibles in one sex than the other.

As the marvellous effects produced by differences in nutrition remain always indisputable, this would lead us to believe that the intimate structure of Termitidæ may form, so to speak, a nidus specially adapted for their manifestation.[1]

2. The great influence exercised on the genitalia by the salivary secretion is a fact which finds its analogue in bees. And inasmuch as these insects are very remote from Termitidæ, and are of independent phylogeny, it must be inferred that we are in presence of a marvellous phenomenon of convergence.

3. If the aliment can exercise a profound influence on the genital organs in forms so remote from each other as Termitidæ and bees, we may fairly suppose it to produce a direct effect on these organs in many other animals. And this seems to me to form a serious argument in favour of the possibility of inheriting acquired characteristics.

No light is thrown on the mode of development of the

[1] [In other words, the intimate structure of the protoplasm in other species of Orthoptera already exhibits a tendency towards the production of better developed mandibles in certain individuals (males). We may therefore suppose that at one time the ancestors of Termitidæ exhibited a much greater development of the mandibles in one sex than the other. Similar suppositions, and others which readily present themselves to one's mind facilitate, in my opinion, the understanding as to how this character may present itself in the soldiers without the invocation of direct inheritance. (See the introduction to my memoir on "The Anatomy of the Thysanura.") G. B. Grassi, December, 1896.]

workers, soldiers, and neoteinic forms, either by the comparison instituted by Darwin or by the existence of worker bees capable of oviposition.[1]

I regard it as possible that the characteristics of these three classes of forms may be suddenly manifested, because, as I have previously suggested, the latent tendencies may be pre-existent in the as yet undifferentiated Termite larvæ.

4. Since the development of workers, soldiers, and neoteinic forms is arrested, as many of their characters show, and since the metamorphosis of Termitidæ is incomplete, the development of castes in this family is entirely different from that which occurs in bees, ants, &c.

Further, the fact remains that the workers, soldiers, and neoteinic forms on the one part and the perfect insects on the other part are not separated by that enormous difference of instincts which is manifested between the queen and worker bees. On the contrary, the instincts of the former group of forms are possessed generally by the other members of the Termite colony.

5. In the desire to estimate the importance which attaches to the facts disclosed of my investigations on Termitidæ, I propose to begin by the consideration of a preliminary question. In earlier geological periods the Termitidæ were represented in Europe by numerous species, which are now reduced to two, and confined to S. Europe, whilst a large number still exist in the tropical and subtropical zones.

This and other facts already discussed indicate that the different European distribution of Termitidæ at other periods was probably dependent on the warmer climate which then prevailed over this part of the world.

These considerations, together with the fact that our termite

[1] [Of late I have changed my mind, having had occasion to meet with a nymph-soldier (vide p. 14) of Termes lucifugus with well-developed ovarian tubes, and return to the supposition that the phenomena of inheritance in the sterile casts of Termitidæ (workers and soldiers) can be interpreted as I have pointed out in the introduction for the bees, i. e. by the exceptional existence of workers and soldiers capable of oviposition.—G. B. Grassi, December, 1896.]

queens are very far from attaining the colossal dimensions so well known in exotic species, suggest that our European species may be in process of degeneration.

Such a belief would rest on the behaviour of the royal forms in the Termes society; but is this foundation really valid? At first sight Termes lucifugus certainly appears to be very different from the species hitherto described, but an examination of the many brief and incomplete accounts we possess allows me to assert that all the Termitidæ of other countries really fall into the two primary types I have observed in Calotermes and Termes. These are—

(1) A colony presided over by a king and queen, which have possessed and shed fully developed wings. When orphaned it is headed by a pair of royal substitute or neoteinic forms.

(2) A colony at the head of which are numerous neoteinic queens, the kings, also neoteinic, being present for short periods only. The colony is not founded by the royal forms which govern it; or, more exactly, the neoteinic forms have been raised by a detached portion of a pre-existing colony, which thus founds a new and independent society.

Hagen discovered, but could attach no meaning to, a certain number of examples in other species, e.g. Termes morio, Latr., which must be interpreted as neoteinic royal forms.[1] Fritz Müller states[2] that Hagen wrote to him that the Asiatic and African queens are all true imagos with wing-stumps, but that all those from Brazil and America generally are in the nymph garb; but this is contradicted by Müller, who found hundreds of true queens in Brazil, as well as those in nymph form.

I am myself acquainted with nymph-like forms of several tropical species; I therefore conclude that all Termitidæ are reducible to the two main types above mentioned, and see no

[1] [A better example of a neoteinic form is given by Hagen in Termes debilis, Heer, 'Linn. Ent.,' xii, p. 207, pl. i, fig. 26.]

[2] ['Jen. Zeitschr.,' 1873, p. 457, note 3.]

further reason for regarding the European species as degenerate.

6. This question settled, another arises as to which of the two European forms must be considered the more primitive. Evidently Calotermes, for the following reasons:

(1) The ovary is composed of seven ovarian tubes only, and is therefore in a relatively archiac condition (see my studies on Thysanura);

(2) The soldiers are still furnished with eyes;

(3) The colony is headed by individuals which have possessed wings suitable for flight;

(4) The art of building is little developed in all species of the genus;

(5) The queens of this and other species are relatively small, and therefore resemble the females of other insects;

(6) There are no workers.

This aggregate of facts leads one to say positively that Calotermes is the more primitive, while none of them justifies the supposition that it is a degraded form.

7. What interpretation is to be placed on the singular fact that all the winged forms of Termes are inexorably lost? Does this loss take place everywhere, and have not numerous cases occurred in France in which they have been found to establish new nests, as I have shown for Calotermes? These are questions which admit of no definite answer, because the observations made in France by Lespès are not entirely reliable, as Hagen has pointed out.

Fritz Müller, whilst denying that the winged forms can possibly found new colonies, and supposing, on the contrary, that they enter orphaned nests, furnishes the clue to the problem by a most original comparison.[1]

He compares the winged forms to perfect flowers, and the substitute nymphs, as he regards them, to cleistogamic flowers. The object of the winged forms, like that of the perfect flowers, would be to render interbreeding difficult; and that of the substitute nymphs, like the cleistogamic flowers, would be to

[1] ['Jen. Zeitschr.,' 1873, Beitr. iii, pp. 451—463.]

furnish a means of escape from the considerable danger to which the perfect insects are necessarily exposed.

In other words, according to Müller, Termites furnish royal substitutes only if orphaned at a time when there are no winged forms. Their procedure is really different, but the comparison between them and plants possessing both perfect and cleistogamic flowers still holds good. Calotermes may be compared with a hypothetical plant, in which seed is produced by perfect flowers, and not by cleistogamic flowers unless the others fall; Termes (in Sicily) to a plant in which the perfect flowers do not produce seed, but the cleistogamic flowers seed abundantly.

The separate swarming of the sexes, discovered by myself, fully confirms this conclusion.

Lastly, the phenomena exhibited by Termes indicate an advance on those found in Calotermes.

8. It will be seen from the second appendix to the present work that there is no direct evidence of relationship between Termitidæ and Embiidæ if we disregard the wings, which, according to Westwood,[1] Hagen,[2] and Redtenbacher,[3] are somewhat alike. But Redtenbacher himself, the most competent authority of the three, concludes that the Embiidæ are separated by the wing-structure as an entirely special group, which exhibits relationship with the Termitidæ and Blattidæ, as well as with the Perlidæ.[4] Thus there cannot be said to be any close connection between the Termitidæ and Embiidæ even in wing-structure, while the number of the Malpighian tubules, the arrangement of the testes and ovaries, the spermatozoa and alimentary canal, all present differences of the highest importance.

The Embiidæ, it is true, lead a sort of gregarious life, but they do so under conditions very different from those found in Termes, since they show no sign of division into castes.

[1] ['Trans. Linn. Soc.,' xvii (1837), pp. 369—373, pl. ii.]

[2] ['Canad. Ent.,' 1885.]

[3] [Ann. K. K. Nat. Mus. Wien,' i, pp. 153—232, pls. ix—xx.]

[4] [A similar conclusion is arrived at by Brongniart, 'Insectes Fossiles,' p. 215.]

9. If the Termite colony were headed only by royal forms, such as have been described, without sign of wings, the phenomenon would occasion no surprise, but all valid proof would be wanting that the Termitidæ themselves had ever possessed wings.

This consideration may throw some light on the origin of the Thysanura. My researches on these insects showed that their characters are partly primitive, and partly connect them intimately with the Orthoptera (s. str.). Such a connection seemed, however, to be contra-indicated by the hypothesis, which I accepted, that the Thysanura have never possessed wings, structures of which I have been unable to find the least indication.

But I am compelled to reject this hypothesis, owing to what I have seen take place in Termitidæ under my eyes, and to admit that the Thysanura may originally have possessed wings, which they have subsequently and entirely lost. They must be descended from primitive forms in which the first abdominal segment remained independent, with its sternite complete and not fused with the metasternum as in Termitidæ; and not a few winged Orthoptera (s. lat.) are known to exist which have the first abdominal sternite separate as in Thysanura.

And this leads me to regard the division of Insecta into Apterygogenea and Pterygogenea as unfounded, whatever Brauer may assert.[1]

In other words, I admit that all existing species of insects may once have possessed wings.

Historical Accounts and Developments.

I leave to the writer of a complete monograph on the Termitidæ the task of elucidating point by point the contributions to a knowledge of them which Dr. Sandias and I have made and set forth, but I cannot here pass in silence over some preceding accounts of special significance.

[1] 'Zool. Anz.,' 1888, pp. 598—600.

In a preliminary note I have recorded what has been made out about the neoteinic forms from the works of those who have previously studied the subject, and especially from the paper by Fritz Müller, and I repeat my remarks textually.

"To the genius of Fritz Müller belongs the credit of having conceived the novel, brilliant, and most plausible hypothesis of the royal supplemental pairs in the Termite kingdom, an hypothesis supported partly by the observations of other writers, and partly by his own.[1]

"In Termes lucifugus, a South European species, Lespès discovered two kinds of nymphs, which he called respectively the nymph of the first and the nymph of the second form. The former was more active and slender, and possessed long and broad wing-rudiments, which completely covered the base of the abdomen; it became a perfect insect and left the nest from about the 15th to the 20th of May. The nymph of the second form was much rarer, its abdomen was larger and heavier, and the wing-rudiments were short, narrow, and situated at the sides of the thorax. When first found in February the latter nymphs were equal to the others in length (6—7 mm.), but subsequently exceeded them (8—10 mm.), simply through enlargement of the abdomen, especially in the female. The abdominal tergites were then insufficient to cover the sides of the body, and were distinctly separated from each other by soft membranous interspaces. In fact, a dilation of the abdomen had taken place which corresponded with a much greater development of the testes and ovaries than that existing in the nymphs of the first form. The nymphs of the second form remained unaltered till July, when they turned brown, but they gradually became very much rarer. Although Lespès did not continue his observations beyond that month, he supposed that these nymphs were metamorphosed in August into winged males and females, and swarmed like those of the first form. From the latter he derived the small kings and queens occasionally found in the nests; from those of the second form he derived the large kings and queens. This

[1] 'Jen. Zeitschr.,' 1873, Beitr. iii.

supposition was based on the lesser development of the gonads in the small king and queen, just as in the nymph of the first form, and on their much greater development, as in the nymph of the second form, in the large king and queen, or as he terms them simply, the king and queen. But these degrees of development may be explained, as Müller remarks, simply by differences of age and of the seasons when the observations were made.

"Moreover Hagen and Müller observe that the royal forms possess wing-stumps, and this presupposes a degree of development of the wings which appears unattainable by the nymph of the second form, of which the wing-buds are still very short in July. To the writers quoted must be added Bobe-Moreau,[1] who studied a species, presumably Termes lucifugus, in South Europe, but failed to observe the second swarming conjectured by Lespès to take place.

"According to Müller, the nymph of the second form remains wingless and never abandons the nest, in which he believes it to become sexually mature under certain circumstances. He adds that fertile examples with the appearance of a nymph have been described as the queen in different species, not only in Termes lucifugus (Joly),[2] but in T. flavipes, T. arenarius, and Calotermes flavicollis (?). Müller does not believe that the swarming of Termitidæ can lead to the foundation of new nests; whilst he does not positively (geradezu) deny the possibility of this happening in Calotermes, he absolutely excludes it for all species of Termes, Eutermes, and Anoplotermes, studied by himself. The effect of swarming in his opinion is simply to provide royal pairs for unoccupied thrones, and the colony must avoid the enormous amount of labour and the serious consumption of its members entailed by swarming, and be certain of possessing a king and queen by the retention in the nest of a royal pair produced therein. Now this pair is the offspring of the same parents, inasmuch

[1] ['Mémoire sur les Termites observés à Rochefort, &c.,' 1843.]

[2] ['Mém. Ac. Sci. Toulouse' (3), v (1849), pp. 1—37, pls. i—iii.]

as a nest usually contains but a single royal couple, and their interbreeding must therefore vitiate the stock. Swarming brings about the intercourse of examples from different nests, and diminishes the evils of interbreeding by leading to the pairing of non-consanguineous royal forms. This, therefore, is its proper function.

"In the realisation of this advantage it may too easily happen that the orphaned population is unsuccessful in providing a fresh royal couple for the throne. In this case the royal substitute pairs, or sexually mature nymphs of the second form, step in and thus ensure the safety of the colony. Their slow development is correlated with this function, and their reduction in number during July may possibly indicate that they are killed off when no longer required, and that the colony does not keep alive the large number originally provided.

"This hypothesis was supported by the following observation made by Müller himself in Brazil. He found in the solid core of a Eutermes nest no less than thirty-one substitute queens, which were seen to lay eggs, together with a single true king, possessing wing-stumps; a true queen was wanting. These supplemental queens bore a general resemblance to workers, but were twice as large; their wing-buds were mostly very short (about half the length of the segment from which they took origin), but were markedly longer in a very few examples. The antennæ were fourteen-jointed as in the workers (those of the soldiers having thirteen, of the perfect insects fifteen joints). Their head might be taken for that of a worker, except for the presence of small pigmented compound eyes.

"These are the whole of the particulars given in Fritz Müller's paper. Hagen, quoted by Müller, takes a different view, and believes that all African and Asiatic queens originate from perfect insects, and those in America directly from nymphs.

"Latterly von Jehring (in Brazil) has published two notes on alternation of generations in Termitidæ.[1] He regards the substitute queens, found on a single occasion by Müller, and

[1] 'Ent. Nachr.,' xiii (1887), pp. 1—4, 179—182.

never by himself, as abnormal forms (oviparous workers), like the soldiers with rudimentary wings described by Hagen, and as of no importance in the termite economy. He considers that the nymphs of the second form found in Termes lucifugus are to be explained either as the result of seasonal dimorphism, or as belonging to a species inquiline with the one to which the nymphs of the first form belongs (he claims to have observed the latter phenomenon in many American species !).

"Müller[1] has declared von Jehring's criticism to be insufficient, though without advancing any fresh facts, and they do not appear to me to deserve further consideration."

From these quotations it will be seen that Hagen and Fritz Müller have endeavoured to correct the grave errors into which Lespès fell; and Müller in particular has nearly reached the solution of the problem of the nymphs of the second form on theoretical grounds, but has confined himself simply to suggestions.

The principal new points in the present work of Dr. Sandias and myself are the following:

"1. Numerous fully-winged examples emerge annually from the nests of Calotermes flavicollis and Termes lucifugus. A certain number of those belonging to the former species succeed in founding new colonies.

"Those of Termes lucifugus are all irretrievably lost in nature, at least in Sicily. This is shown by researches extending over some seven years.

"2. The males and females are accustomed to swarm separately, and consanguineous pairing is thereby rendered difficult.

"3. A certain number of winged Calotermes settle after swarming upon decayed spots on tree-trunks.

"There they skilfully get rid of their wings if not previously assisted by chance, and then begin to burrow into the decayed area. At this time the sexes meet and pair, and each pair begins to found a fresh colony.

[1] 'Ent. Nachr.,' xiii (1887), 177–8.

"The so-called amatory passages are not exhibited by Calotermes; they are seen in Termes, and possess no sexual significance, but are simply attempts to solicit dejecta.

"The pairs which begin to form fresh colonies have the antennæ mutilated; this is probably effected reciprocally on each other. Furthermore, these organs are never found intact in any royal example, true or neoteinic, of either Calotermes or Termes.

"4. Termes and Calotermes communicate among themselves chiefly by a jerking convulsion of the whole body, which may be accompanied (in the soldiers of T. lucifugus) by a readily perceptible crepitus, produced by friction between the head and pronotum.

"The tibial organ, discovered by Fritz Müller, is tympanic and most probably auditory. As far as we can make out, Termites hear the sounds produced by the convulsive movements.

"Members of the same nest recognise each other.

"5. The food of Termitidæ consists of—

"(1) Triturated particles of dead or decayed wood;

"(2) The material disgorged by their fellows, and composed of triturated wood mixed with saliva;

"(3) The dejecta of their fellows; this constitutes the favourite food of these insects, which show great skill in soliciting it from each other;

"(4) Dead examples of their own or other colonies, but of the same species,—moribund, healthy, but superfluous individuals (superfluous royal substitutes or soldiers, &c.);

"(5) The salivary secretion of their fellows (a transparent alkaline liquid).

"Termites also imbibe water.

"6. The colony can modify the development of a certain number of individuals which are destined normally to become perfect insects, by varying the proportion and quantity of their nutriment. It thus obtains workers, soldiers (which may be considered as further differentiated workers), and neoteinic forms, which may or may not exhibit special characters, such as long setæ. The neoteinic forms become sexually mature

without fully acquiring the perfect instar, and thus preserve the facies of the larva or nymph; they consist of substitute and complementary kings and queens.

"All this may be rigorously proved by the observation of colonies which have been deprived of the king, queen, or soldiers, &c.; and the insects can thus be forced to produce soldiers, workers, or neoteinic forms at will.

"These transformations can take place without limitation to a specified age in the individuals selected. Soldiers may be derived from larvæ and nymphs of different ages, and neoteinic forms from these or from perfect insects which are still white.

"Nevertheless the colony prefers to select examples for transformation at the ages specified in the present memoir.

"7. The larvæ and nymphs administer a large quantity of saliva to examples destined to become neoteinic; it causes the disappearance of the parasitic Protozoa. The importance of this disappearance is not clearly understood, but it is certainly insufficient by itself to produce neoteinia.

"Newly-born larvæ receive nothing but saliva, but little or none is administered later to those in progress of becoming workers or soldiers.

"8. The colony of Termes lucifugus is headed by hundreds of complementary queens; the existence of the complementary kings is precarious. Both appear invariably to be derived from the nymph of the second form.

"Orphaned colonies of Termes are found after a certain time to contain substitute instead of complementary queens. They are frequently derived from entirely wingless larvæ, but not seldom from perfect insects, which are more or less completely white.

"The colony of Calotermes flavicollis is headed by a royal pair derived from perfect insects. When they are absent a substitute pair is furnished. Strictly speaking, several such pairs are provided, but only one survives the ferocious conflicts and banquets of royal flesh which take place.

"Termes lucifugus readily migrates from one tree to

another, and carries eggs and young with it. But the complemental queens never change their situation, and are therefore not to be found in many of the dead trees inhabited by the species in different stages.

"If a detached offshoot established in a tree without royal forms loses its communication with the main colony, it immediately provides such forms in hundreds. New colonies of Termes lucifugus arise in this way.

"These phenomena depend entirely on the promptness with which Termites guard against the want of royal individuals.

"Calotermites readily receive strangers of the same species into a nest, even a royal pair, if they are orphaned. Jealousy is most conspicuously manifested between the royal forms, but much less rapidly than in bees.

APPENDIX I.

The Parasitic Protozoa of Termitidæ.

(Vol. 39, Plate 20.)

I propose to complete the account of my investigations on the protozoa Parasitic in Termitidæ by a concise description of the forms observed,[1] which all belong to the class of Flagellata.

Calotermes flavicollis harbours two species alone, one belonging to the family Cercomonadidæ, Grassi, the other to the Lophomonadidæ, Grassi.

In Termes lucifugus I have been able positively to determine six species, of which two belong to the Lophomonadidæ, two to the Cercomonadidæ, and the last two to a new family, Pyrsonymphidæ, mihi.

Adopting as far as possible the nomenclature employed by

[1] Similar parasites have been observed wherever Termites are found. Thus Leidy ('Proc. Ac. Sci. Phil.,' 1877 and 1881) and Saville Kent ('Manual of the Infusoria,' ii, pp. 551—556, pl. xxviii) have published incomplete descriptions of some species, and a new form has recently been described by Frenzel ('Arch. f. mikr. Anat.,' 1891, pp. 301—316). [A paper by Seip ('Am. Mic. Journ.,' ii, p. 288) is not referred to by Prof. Grassi.] These parasitic Infusoria were indicated in Termes by Lespès.

Kent and Leidy in their well-known works, I have employed the following names for the seven species studied.

Fam. Lophomonadidæ.

I. Joenia annectens, gen. et. sp., Grassi (in Calotermes flavicollis).

II. Trichonympha agilis, Leidy (in Termes lucifugus).

III. Microjoenia hexamitoides, gen. et sp., Grassi, = immature Trichonympha of Leidy (in Termes lucifugus).

Fam. Cercomonadidæ.

IV. Monocercomonas termitis, Grassi (in both Calotermes flavicollis and Termes lucifugus).

V. Dinenympha gracilis, Leidy (emend.); probably = Pyrsonympha vertens, Leidy (pro parte) (in Termes lucifugus).

Fam. Pyrsonymphidæ.

VI. Pyrsonympha flagellata, Grassi (in Termes lucifugus).

VII. Holomastigotes elongatum, gen. et sp., Grassi (in Termes lucifugus).

I.

JOENIA ANNECTENS, gen. et sp., Grassi.[1] (Pl. 20, figs. 6—9.) Atti Acc. Lincei (5), p. 36 (1892).[2]

Of relatively gigantic size, usually exceeding 130 μ in length and 40 μ in width. Variable in shape, sometimes pyriform with the broad end anterior, sometimes constricted in the middle with the anterior extremity the smaller.

Like Lophomonas, it has a large tuft of numerous flagella at the anterior extremity, is devoid of cytostome or

[1] The genus Joenia is dedicated by me to the memory of the distinguished naturalist Cavaliere Gioeni.

[2] [Although the genera and species described by Professor Grassi are indicated as new in the original of the present memoir, the descriptions were actually published in the preceding year.]

contractile vacuoles, and possesses a large nucleus in the neighbourhood of the flagellar tuft. It exhibits, however, the following important differences:

1. Instead of the denser and more opaque protoplasmic region occupying the anterior half of the body of Lophomonas, it possesses a complex endoskeleton, which seems of cuticular substance, and lies nearly in its long axis. This consists of—

(*a*) A rod similar to that found in Trichomonas, and tapering posteriorly. Anteriorly it is widened and bilaterally symmetrical, owing to the presence of a recess which lodges a large part of the nucleus.

(*b*) Numerous curved and claviform rodlets, which appear to be inserted into the anterior end of the rod by their lesser extremities, so as to form an encircling ring, which is incomplete, owing to their absence over a small portion. This circle of rodlets sometimes exhibits a bilateral symmetry, which does not, however, correspond with that of the rod. At times a filament may be observed apparently to connect the rod with the base of the flagellar tuft; I do not know whether this should not be considered part of the endoskeleton.

2. The posterior part of the body is ciliated in Joenia, and the cilia are never motile.

Joenia feeds on particles of wood, which it ingests in a manner not yet determined, and which may exceed half its body in length or bulk. Ingestion and elimination of dejecta probably take place at the anterior half of the body, except over the flagellate area, and are accompanied with amœboid movements.

II.

Trichonympha agilis, Leidy (Pl. 20, figs. 1—5.) Leidy, 'Proc. Ac. Sci. Phil.,' 1877, p. 147.

Reaching the dimensions of Joenia, variable in shape, sometimes oval, sometimes transversely constricted into two unequal portions at the level of the front of the nucleus. The

anterior is the smaller, and may show traces in its turn of one or two transverse constrictions.

The species is more commonly mammiform (shaped, e. g., like the udder of a goat), with a small nipple-shaped anterior process, which more or less resembles a teat, even when the general shape is not mammiform, and will be briefly referred to as the mamilla.

The Protozoon, therefore, exhibits a mamilla and a base from which it springs and appears to be delimited by an evident constriction. This mamilla may be curved towards the body in various ways, or may be spirally twisted.

Most of the varieties of form here described appear to be constant, and no change of shape is discernible under the microscope. But this may be due to the unfavorable circumstances for observation, and it must be left undetermined whether this diversity of form is dependent on contractile phenomena or not.

The mamilla and the anterior half of the body are certainly much more variable in form than the posterior half; and Trichonympha in general is far more variable than Joenia. In any case the mamilla is undoubtedly flexible.

Both ectoplasm and endoplasm are distinguishable. The ectoplasm of the mamilla and the anterior half of the body may conveniently be spoken of as the striated zone (although the apex of the mamilla is not striated); it differs from that of the posterior half, which constitutes the unstriated zone. The limit between these two zones may be median, or may be situated at the junction of the anterior third or fourth part of the body with the remainder.

The striation of the striated zone is longitudinal, and clearly due to the alternation of shallow sulci and ridges in the cortical layer of ectoplasm; that of the mamilla is coarser and more remote than that of the body mass. The ectoplasm of the striated zone usually appears dense and homogeneous, and its inner layer is traversed towards the front of the body by circular transverse lines; their meaning is obscure, but they are probably myonemes.

At the summit of the mamilla the ectoplasm is reduced to a very thin layer, like a structureless membrane, without trace of striation : this is more fully explained below.

Passing to the unstriated zone, the ectoplasm of the posterior extremity appears somewhat different from the rest, and forms a layer variable in thickness and aspect, but as a rule not dense. I suppose that this hinder extremity can execute amœboid movements for the purpose of ingesting the food, which ordinarily consists of more or less bulky particles of wood, sometimes more than half the length of the Protozoon.

The ectoplasm over the rest of the unstriated zone is dense and homogeneous, forming a thinner and more granular layer when the endoplasm is loaded with food particles. Externally it presents a distinct double outline, which is never distinguishable in that of the posterior extremity.

There is no distinct demarcation between the ectoplasm and endoplasm of the unstriated zone; but the ectoplasm of the striated zone encloses a clear space, which I regard as a lacuna filled with liquid or semi-fluid protoplasm. The arrangement of this lacuna will be made clearer by a further description of the mamilla. Its anterior unstriated extremity is separated from the rest by means of a transverse diaphragm, which gives it the form of a skull-cap. This diaphragm is membranous, and possesses a circular median orifice, which is occupied by the rounded and closed apex of a cylindrical tube, situated in the long axis of the striated portion of the mamilla. This tube does not completely occlude the orifice, but is separated from it by an annular space.

Under the thin membranous ectoplasm of the apical portion, which has been compared to a skull-cap, is a clear space, which I take also to be a lacuna filled with liquid or semi-fluid protoplasm, like the one previously described. And these two lacunæ communicate by means of the annular space.

The walls of the above-mentioned cylindrical tube are also membranous in appearance, and contain a liquid or semi-fluid protoplasm, like the lacunæ. Behind, at the base of the mamilla, this tube widens out considerably ; its walls become

thinner from this point until they are no longer distinguishable, and the contents of the enlarged portion do not remain liquid or semi-liquid, but change abruptly to a very dense protoplasm, full of fine uniform granules.

This enlargement gives rise to a structure which may be compared with a bottle, of which the cylindrical tube forms the neck. The bottom of this bottle corresponds posteriorly with the limit between the striated and unstriated zones, and is hollowed out, exactly as in a wine-bottle, to receive the nucleus.

Continuing the comparison, it will be seen from this description that the neck of the bottle is filled with a liquid or semi-fluid substance, whilst the body contains a granular protoplasm; but it fails in the fact that the bottle is devoid of walls at the part corresponding to the bottom, or rather that its walls become so attenuated towards the foot as to be indistinguishable. It will also be seen that this bottle-shaped structure is surrounded with the liquid or semi-fluid protoplasm of the lacunæ except at its base.

The concave base protects the nucleus by reception of its anterior half. The posterior half lies in a sort of cage of curved rodlets, somewhat remote from each other and connected by granular protoplasm. I am not certain as to their exact arrangement, but they terminate almost in connection with the periphery of the foot of the bottle, and most, if not all, are curved in apposition with the posterior half of the nucleus. I cannot give fuller details, but I may add that I believe them to be somewhat asymmetrical, and not alike in all individuals. They appear to consist of very dense protoplasm, and resist the action of acetic acid for some time.

The nucleus is very large, and oval or rounded. It may measure as much as 14 or 16 μ in diameter; when oval its major axis may measure from 16 to 18 μ, its minor axis 14 μ. It has a well-marked limiting membrane, and usually contains numerous small deeply-staining bodies, resembling large, thick, and more or less curved bacteria.

I have mentioned that nutrition and elimination of fæcal matter most probably take place at the posterior extremity,

and that the endoplasm may contain more or less solid food material; when this is not the case it is abundantly filled with granules of different sizes, usually very minute. The apparatus described at the anterior pole cannot possibly be regarded as a buccal organ.

The flagella remain for description. They arise from the striated zone in distinct longitudinal rows, and I believe from the examination of many preparations that they take origin from the longitudinal ridges which separate the sulci. Their number is very large; they are shortest in front, and gradually increase in length from before backwards, so that the longest posterior flagella greatly exceed the body in length.

The anterior flagella are variable, and may be directed either forwards or backwards, whereas the posterior are always directed somewhat obliquely backwards, so as to describe a spiral figure round the body: in spite of their length, they rarely arise from the posterior extremity. The animal's movements are rapid, and may follow a helical course.

The dense granular protoplasm forming the mass of the bottle-shaped structure is very scanty in many examples, so that the nucleus lies in a more forward position.

III.

Microjoenia hexamitoides, gen. et sp., Grassi (Pl. 20, fig. 10). 'Atti Acc. Lincei' (5) i, p. 36 (1892).

Of relatively small size, never exceeding 45 μ in length, and consequently less than the shortest specimens of Trichonympha agilis. Oval in shape, prolonged behind into a more or less prominent mucro. Intermediate in characters between Joenia and Trichonympha.

As in the latter genus, the anterior extremity is destitute of flagella, and is delimited by a very thin layer of dense ectoplasm, beneath which is a clear space or lacuna. The flagella originate from a striated zone corresponding with that of Trichonympha. A subaxial skeletal rod can be exceptionally made out, as in Joenia. The nucleus is in the neighbourhood of the anterior pole.

Leidy observed the species here briefly diagnosed, but regarded it, without evidence, as a young Trichonympha.

It also assimilates solid food.

IV.

Monocercomonas termitis, Grassi. 'Atti Acc. Lincei' (5), i, p. 36.

The anterior pole of this Monocercomonas is furnished with at least six very long flagella, of which one is directed backwards and the others forwards. A subaxial skeletal rod is present, as in Trichomonas. Average length 15μ. Solid food is ingested.

V.

Dinenympha gracilis, Leidy. (Pl. 20, figs. 11—17.) Leidy, 'Proc. Ac. Sci. Phil.,' 1877, p. 148.

Body uniaxial, dissimilar at the poles, elongate, subcylindrical or vittate, often -shaped or readily becoming clavate, the posterior extremity being the larger. Movements usually very rapid, consisting of alternate flexion and extension in the long axis of the body. Locomotion usually helicoid in direction.

The ectoplasm and endoplasm are contradistinguished only by the absence of solid food-particles in the denser peripheral layer. There is no trace of contractile vacuoles or mouth. The nucleus is situated anteriorly, and is pyriform or clavate, with the larger part posterior; there is no paranucleus.

Flagella are absent, and their place is taken by delicate undulating membranes similar to those of Trichomonas, or more closely, Paramæcioides, their flagellar origin being equally doubtful. The free margin of the membrane is certainly thickened, but as it is not united to the body by a very delicate attachment, as in Trichomonas, it never becomes separated so as to look like a flagellum. These apparently homogeneous membranes extend from one pole to the other, sometimes in straight lines, at other times so as to form one or more spiral turns. When their direction is straight, or

forms a single turn, it is easy to see that there are four, but I cannot say that this number is constant in all individuals. They are delicate in some and thicker in other examples.

Lastly, as in Trichomonas, a rod extends backwards from the anterior extremity without being strictly axial; it is extremely elastic, and appears to be bent by the rapid sinuations of the body.

Either or both extremities or the entire surface may also be furnished with appendages which may be taken at first sight for flagella, as has been done by Leidy. But after much hesitation I have satisfied myself that they are parasitic Spirilla, such as occur free in the intestinal contents of Termitidæ. Their shape, size, inconstant presence, and irregular distribution all justify this statement.

VI.

Pyrsonympha flagellata, Grassi. (Pl. 20, figs. 18—20.) 'Atti Acc. Lincei,' (5), i, p. 36.

This and the following form are assigned to a new family, the Pyrsonymphidæ, characterised by the body covered with flagella, disposed more or less exactly in spiral lines, the situation of the nucleus towards the anterior extremity, the absence of a paranucleus, mouth and contractile vacuoles, and lastly by the elliptic monaxial body, with antero-posterior asymmetry, moving, at least as a rule, in a helicoid path. In the family I distinguish two genera, each with a single species: Pyrsonympha flagellata, Grassi; and Holomastigotes elongatum, Grassi. Leidy considers these forms to be young Trichonymphæ, a view I absolutely reject.

Pyrsonympha flagellata, which will first be dealt with in detail, is subelliptical, and may measure as much as 98 μ in the major and 40 μ in the minor axis. The anterior extremity terminates in a more or less evident papilla; the posterior is variable, and may be rounded or produced, &c.: it probably serves for ingestion and elimination, as in Trichonympha. Nutrition is effected as in that genus.

The surface of the body is covered with flagella about 18 μ in length; if it is viewed lengthways, the flagella are seen to be arranged in oblique lines which run in opposite directions on the two faces, as an alteration of the fine adjustment shows. Appropriate observation suggests that they are disposed uniseriately in spiral lines, but their mode of origin at the anterior extremity and the number of turns made by each line appear impossible to determine, on account of the close juxtaposition of these turns over the anterior papilla. The spirals may or may not extend to the posterior extremity, and the lines of origin of the flagella appear subelevated throughout.

The ectoplasm differs from the endoplasm only in its greater density and freedom from food-granules.

In connection with the anterior extremity is a tubular organ, as in Trichonympha.

All the larger examples possess five or more rodlets, which may be somewhat bent at the level of the nucleus, as if to embrace it; they converge in front of it and lie in close proximity to each other, being disposed according to a slightly curved surface, and thus giving the animal a bilateral symmetry.

VII.

Holomastigotes elongatum, gen. et sp., Grassi. (Pl. 20, figs. 21—24.) 'Atti Acc. Lincei' (5), i, p. 36.

Reaching a length of 60 μ, and a corresponding width of 20 to 24 μ. Oval, very elongate, with the posterior extremity usually the more attenuated. The anterior papilla wanting; the flagella arranged as in the preceding species in spiral lines, easily seen, during contraction of the body, to start from the anterior pole. During elongation other spiral lines, which do not extend to the anterior extremity, are seen to be intercalated between those bearing the flagella.

Ectoplasm as in the preceding species: the endoplasm constantly contains very numerous, scarcely refringent granules, which might be taken at first sight for nuclei; they disappear with acetic acid, and do not stain. Similar granules may

sometimes be found in the endoplasm of Pyrsonympha: their nature is unknown.

General Notes.

It seems incredible, even to myself, but it is nevertheless true that, in spite of long and fruitless search, I have never been able to find any of these Protozoa encysted, or evidently in process of reproduction.

On a single occasion I found two examples of Trichonympha agilis fused together by the hinder extremities. They separated under my eyes after being united for an instant by a slender thread of hyaline protoplasm. This thread became much attenuated in the middle, and then broke, leaving each of the examples with a peduncle which was quickly retracted. They were of medium size, and both belonged to the form in which the nucleus is situated far forward.

This may have been either a casual act of fusion, brought about perhaps by the method of preparing for examination, or a process of division. But never having been able to find any other example in similar circumstances, or in any other stage connected with reproduction, I must adopt the former supposition.

In Dinenympha gracilis I have observed a condition which may be connected with reproductive phenomena.

Motionless examples are common, with or without food-particles, sometimes full of vacuoles and with the marginal membranes thrown into folds and quiescent, or even absent. The nucleus and skeletal rod appear to be unchanged.

These examples are attached to the intestinal epithelium, like Gregarines, by a kind of cuticular peduncle arising from the anterior extremity.

It was not till I had completed these observations on the Protozoa that it occurred to me that they must be ingested in large quantities by the Termitidæ with the fæces; they may possibly pass without encystment into the stomodæum and

chylific ventricle, or may perhaps multiply therein before entering the proctodæum.

I propose to return to this point in a future work, in which I shall deal more particularly with the minute structure of the protoplasm and nucleus in these Protozoa.

The various rods and rodlets of these parasitic Protozoa, as well as the dense and granular protoplasm of Trichonympha, evidently serve as an endoskeleton for the support of the body and protection of the nucleus. This is shown by their entire absence in Holomastigotes, the only form in question that does not take in solid food-particles (fragments of wood), which would seriously endanger the nucleus.

The special apparatus at the anterior extremity of Trichonympha allows it freely to thread its way through the surrounding swarms of its own and other species without compression of the nucleus, to which the mamilla acts as a sort of buffer. I have sometimes thought that the structure in question may have the further function of a sucker, by which the animal can attach itself to the intestinal walls. The papilla of Pyrsonympha is probably similar in function.

As I have previously said, all these parasitic forms must be classed among the Flagellata, and cannot be referred to the Ciliata, chiefly owing to the absence of a micronucleus (paranucleus).

The Lophomonadidæ, in which a large area of the body is covered with flagella, evidently form a family osculant between the other Flagellata and the Pyrsonymphidæ, in which the entire surface is thus covered.

The presence of the endoskeleton is the principal reason which leads me to associate all these forms with the group of Flagellata.

Catania; October, 1890.

APPENDIX II.

Contributions to the Study of the Embiidæ.

(Vol. 39, Pl. 19).

The Embiidæ have been consigned by systematists to a position in the zoological scale scarcely a degree lower than that occupied by the Termitidæ in the Corrodentia. If this is their position, they might be expected at least to manifest something of the extraordinary perfection of qualities exhibited by the Termitidæ, and for this reason I decided to include them in my studies.

A preliminary survey of the literature on the subject, which is fully given in Dr. Hagen's "Monograph of the Embidina,"[1] at once showed me that almost nothing was known about either the anatomy or the biology of the Embiidæ, and I was therefore compelled to make original investigations. These, as will be seen, force me to conclude simply that the family forms a separate branch of the Orthoptera (s. lat.) of uncertain position, and in any case without direct relationship to the Termitidæ. I propose, therefore, briefly to epitomise the results of my studies, and to limit myself to a sketch of the anatomy and biology of the Embiidæ.

The species I have investigated is widely distributed in Italy, and is probably, as I shall show later, Embia solieri, Rambur.[2] Hitherto the larva alone of this species has been known very imperfectly; and that it has even been doubtful whether it acquired wings or not.[3]

The external features of the species in question will first be dealt with.

[1] 'Canad. Ent.,' xvii (1885), pp. 141—155, 171—178, 190—199, 206—229.

[2] ['Hist. Nat. des Insectes—Neuroptères,' p. 313, No. 4.]

[3] [The fact that the larva only of E. solieri has been previously recorded from various parts of Europe appears to have depended on the unfounded hypothesis that the insect must necessarily be winged in the perfect state.]

The adult female attains a maximum length of 12 mm.; the adult male is usually a little smaller; neither possesses the slightest trace of wings.

The adult male is a dull ferruginous-brown, except the head, which is sometimes lighter; the flanks, which are markedly lighter; and the prothorax, which is reddish-yellow. The legs are concolorous with the prothorax, the dorsum of the posterior femora alone being red-brown. The antennæ and cerci are ferruginous.

The adult female is similar in colour to the male, differing only by the lighter ferruginous tint of the dorsal and ventral surfaces, though the former is sometimes as dark as in the male.

The larva is ferruginous above except for the prothorax, which is more clearly yellow, and sometimes the head, which approximates to the prothorax in colour, and the abdomen, which may be bright castaneous. The ventral surface is always lighter; the antennæ, legs, and cerci are testaceous or ferruginous.

Both adult and larva are pilose, with sparse and more or less long hairs; some longer setæ are present, especially at the side of the body. The basal joint of the cerci is clothed at the sides with long, fine, vibratile hairs, and at least one such hair is present at the middle of the distal joint on its dorsal side.

The body is somewhat flattened. The head is nearly horizontal, flattened and subhexagonal, with rounded angles, and the Y suture is conspicuous in the larva. The compound eyes are pyriform, scarcely prominent, and situated anteriorly in approximation with the anterior angles of the hexagon; each consists of more than thirty facets. Ocelli are absent. The antennæ are twice the length of the head, filiform, and inserted in front of the eyes. Their joints are rather stout and elongate, cylindrical towards the base, subelliptical towards the apex; the basal joint is markedly broader and often a little longer than any of the others; the fourth is generally the shortest, the fifth a little longer than the fourth, the second than the fifth, but occasionally it is the shortest of all.

The remainder are relatively long and differ little from each

other, but careful comparison shows the last to be a little longer than the penultimate, and the third to be very variable. The antennæ, which do not differ in the sexes, are very fragile. The maximum number of joints in the adult is nineteen, and is less in the larva.

[The translator has studied the arrangement of the antenna joints in a very large species of Embiidæ from Trinidad, recently described by M. de Saussure ('J. Trinidad Club,' ii, p. 293, 1896) under the name Embia urichi, but really discovered by Mr. J. H. Hart, F.L.S. Their number does not exceed twenty-five in any example; they are blackish in the male, fuscous in the female with pale articulations; the apical joints are white, but the last is tipped with black and differs slightly in shape. Its presence serves to show whether the antenna is intact at the time of examination. The number of joints present in the intact antenna may vary, irrespective of sex, from fifteen to twenty-five, and may differ on the two sides; when it is small there is an increase in the length of the individual joints, so that the average length of the organ does not vary proportionately with their number. It may, perhaps, be assumed that antennæ presenting unusually few joints have been broken at an early period, and subsequently regenerated; but it is a curious circumstance that the number of white apical joints is roughly proportional to the whole number present. Antennæ of 15—18 joints have only two white apical joints (counting the last); antennæ of 19—20 joints have three (rarely four) white joints; and antennæ of 22—25 joints have four (rarely five) white joints. So generally is this the case that in one example the left antenna has twenty-three joints, of which the apical half of the nineteenth is pale, and the twentieth to the twenty-third white; and the right antenna has twenty-two joints, of which the entire nineteenth is pale, and the last three are white. The blackish (basal) and white (apical) portions thus possess a certain constant ratio of length to each other independently of the number of joints, which may vary in each portion; and it therefore appears probable that if this

variability is due to regeneration, it cannot be explained by any process so simple as that of proliferation by division of the third or fourth joints, but that when the shaft of the antenna is regenerated, its division into a basal dark and an apical light part must be antecedent to the meristic division.—W. F. H. B.]

The buccal organs are of the Orthopterous type. The epistoma is short and broad, and is connected with the labrum by a membranous rhinarium. The labrum is broader than long, rounded in front and furnished beneath with two rows of strong spines; it possesses a median suture.

The mandibles are three-toothed, the two posterior teeth being more or less distinctly denticulate. In the male imago they are slender, elongate, and curved. But in the female, and the larva of both sexes, they are robust, short, and not curved; and the teeth are generally stronger.

The inner lobe of the maxillæ (Pl. 19, fig. 8) is somewhat thin, bidentate at the apex, and furnished on its inner portion with numerous species like those of the labrum. The outer lobe (galea) consists of a single joint, which carries, as far as I can see, a fringe of uniseriate hairs along its margin, and is supported by a well-developed base. The maxillary palpi are five-jointed, and somewhat exceed the maxillæ in length; the first joint is a little the widest, and the last is subconical; the first, fourth, and fifth are nearly equal in length, and a little longer than the second and third, which are subequal. The palpiger is scarcely distinguishable. The stipites of the labium (Pl. 19, fig. 7) are fused together in the middle line, which merely presents a hairless sulcus. There are two pairs of lobes, the inner being very much the smaller. Both pairs are separated from the stipes by an evident suture. The labial palpi are three-jointed, the apical joint being much the longest, and reach, when directed forwards, to the anterior extremity of the outer lobes; they are carried by a well-developed base which simulates a fourth joint. The ligula

is well developed, somewhat conical, and flattened at the tip. The mentum is subtriangular, the submentum subquadrangular; both are broader than long.

The pronotum is narrower than the head, subquadrate, and deeply sulcate behind the anterior fourth, at right angles to a shallow median sulcus which extends backwards to the base. The intersegmental membrane between the pronotum and mesonotum is well developed, pilose, and somewhat thickened, except at the anterior and posterior margins.

The mesonotum is broader than the pronotum, subquadrate with rounded angles. The intersegmental membrane between it and the metanotum is very similar to the previous one. The metanotum is subrectangular, as wide as, but shorter than the mesonotum, with rounded angles.

The thoracic sterna exhibit various transverse and oblique sulci. The mesosternum is longer than the prosternum, and the limits between the metasternum and the first abdominal segment are not clearly recognisable.

The legs are long and robust, the middle pair being the least developed. The respective pairs are equidistant from each other, and arise from the hind part of their respective segments; the insertions of the anterior and middle legs are remote and almost lateral; those of the hind legs are somewhat approximated. In all the coxa is very short and conical; the trochanter is short, narrow, and subcylindrical, and the femur laterally compressed.

The anterior femur, tibia, and first tarsal joint are of nearly equal length. The latter joint (Pl. 19, fig. 9) is enlarged, and possesses a curved sulcus running from the middle to the apex of its upper surface; below it is furnished with a very large number of small spines, and a smaller number of setæ, tubular and slightly curved at the tip, which sometimes exhibits a minute drop of transparent secretion. The second and third tarsal joints are comparatively small; the former is widened and subtriangular, the latter narrow, cylindrical, and inserted on the upper side of the second towards its apex. The second is armed below

like the first with spines and setæ; the third is furnished with two claws, and has no plantula.

The femur and tibia of the middle legs are subequal; the latter is furnished beneath, towards its apex, with peculiar short spines. The first tarsal joint (Pl. 19, fig. 10) is slender, cylindrical, shorter than that of the anterior pair, and furnished below with numerous spines similar to those of the tibia, and with a bare[1] colourless papilla at its apex; the second is very short, subcylindrical, and similar beneath to the first; the third is about twice as long as the second, but markedly shorter than the first, and possesses two claws as in the anterior legs.

The hinder femora are stout and longer than the tibiæ. The tarsi (Pl. 19, fig. 11) resemble those of the second pair, except that (1) the basal joint is more robust; (2) it is shorter in comparison with, and therefore nearly equal to the third; (3) it has a median ventral papilla similar to the one near the apex; (4) the second joint is not spinose below, but the base of the distal papilla is furnished, as far as I have seen, in the larva and the adult female with small spines on its inner side, which are absent in the male; (5) the claws are more robust.

The abdomen is much longer than the thorax, and its tergites are better developed than the sternites; the former are ten in number, the last three being the smallest.

The tenth tergite of the female and of the larva of both sexes is triangular and very broad at the base. In the adult male it is produced backwards into two asymmetrical apophyses [titillatores], a right and a left (Pl. 19, fig. 6[2]), the former being the larger. As the smaller process is entirely covered below by the penis, it is not easily made out, except at its apex, which is spirally twisted.

The cerci are bi-articulate, symmetrical in the adult female and larvæ, asymmetrical in the adult male, in which

[1] With high magnification the base of the papilla shows a few minute spines.

[2] [In this figure the drawing is reversed; it is so in the original plate.]

the left cercus is excavated on its inner side for partial reception of the penis (vide infra).

Nine sternites, the second to the tenth, can be made out, but the tenth is scarcely distinguishable in the adult male. It exhibits the anal fissure in both sexes. Sexual appendices are wanting in the female. The male imago possesses a long, conical penis, traversed by an internal canal (Pl. 19, fig. 6); it arises from the ninth sternite and is prolonged on to the tenth, beyond which it projects some distance. It is asymmetrical in position, and directed to the left so as to terminate under the left apophysis of the tenth tergite and in the cavity of the left cercus. The right apophysis can be directed downwards and to the left, so as to be flexed under the penis.

Probably the excavation of the cercus and the two apophyses serve to fix the penis during the act of coitus, the left apophysis acting above, the right below, and the cercus laterally.

From the external characters I pass to a description of the habits of the Embia.

This insect is tolerably common at Catania, and inhabits localities which are neither very moist nor very dry; it is particularly fond of very stony ground. In some places it occurs singly, in others eight or ten together may be found under a stone.

From November to May they remain under stones in silken galleries constructed by themselves, much longer than their body and ramifying. These galleries are attached by means of threads or small webs to vegetable rubbish (dead grass or dry leaves), or to stones on the ground; every gallery may exhibit one or more exit-holes, and those of different examples living together under the same stone communicate with each other.

From May to July they live in similar galleries, but at a depth of 10 to 15 cm. below the surface of the ground, so as

to avoid the dryness of that part. They do not dig in order to bury themselves, but make use of natural cracks caused by drought.

In order to gain a clearer idea of the galleries and of the mode of life of these animals, I kept a certain number in a corked glass jar a third full of earth and vegetable rubbish, and in this they flourished for several months.

They constructed galleries in the middle of the contents, or between them and the inner surface of the jar, most of which below the level of rubbish was spun over with threads or small webs of silk, with occasional galleries. They generally remain motionless in these, and probably emerge only for pairing, feeding, defæcation, or to escape their enemies.

If an Embia is put into a glass jar with a little earth it runs about in search of a suitable situation, either in a corner or against a lump of earth; then in two or three minutes it begins to cast out silk threads in order to make its gallery. This is sketched out in about a quarter of an hour, but takes from twelve to fifteen hours to complete. The Embia clearly accomplishes the task with its fore-legs, standing still on the chosen spot and moving them in the most varied directions, downwards, sideways, forwards, and backwards, always with great rapidity. Frequently it alternates the work of one leg with that of the other, but at times both work together with intervals of rest.

The silk produced is seen under the microscope to consist of a network of subcylindrical fibres, which appears to the naked eye as a thin semi-opaque white membrane.

Here and there are scattered silk threads of variable length and thickness, which serve to fix it in position. These threads can often be seen to merge gradually into the network, of which they are clearly a modified form; under the microscope they are found to be composed entirely of fibres. The component fibres of the silk are more or less slender, and vary from extremely fine fibrils scarcely visible with a high power to fibres readily distinguishable with low magnification. A

distinct striation indicative of secondary fibrillation is entirely wanting.

The fibres of the web cross in every direction, but those of the securing threads are more or less regularly parallel.

The Embia, when moving in its gallery, bends up the apical joint of the hinder pairs of tarsi, in order that the claws may not come into contact with the silk, and thus progresses without entangling itself. This explains the physiological function of the papillæ I have described on the second and third joints of these tarsi; it is by means of them that the feet come into contact with the gallery.

The silk is probably extruded as a liquid, and its structure and the method in which the insect works lead me to conclude that it is secreted in the anterior legs, which, as will be seen farther on, are furnished with strongly developed glands.

As an additional proof of so unusual and unexpected a source for the silk, I made the following experiment.

Ten intact Embiæ were put into a glass vessel, and ten more, whose anterior legs had been very carefully cut off, into a similar one. The unmutilated examples all began to produce silk in half an hour to an hour, and had completed and were comfortably living in their galleries at the end of twelve or fifteen hours. The amputated Embiæ lived and managed to hide themselves between particles of earth, but produced no silk. If the silk issued from the head, as one would theoretically suppose, some threads at least should have been found in spite of the amputation.[1]

[1] [The translator has kept examples of the Trinidad Embia urichi alive in England for some time. Unfortunately they were sluggish, and would not feed on anything that could be procured for them, but the little that was observed of their web-producing methods was in accordance with Prof. Grassi's account. Mr. J. H. Hart, F.L.S., by whose courtesy the specimens were obtained, was incredulous as to this origin for the silk, but though he observed the species under more favorable conditions he has communicated no facts antagonistic to Prof. Grassi's observations. It may be added that the salivary glands of E. urichi are apparently far too small to produce the

The galleries obviously serve as a protection to the body against excessive loss of moisture, or to keep the insect surrounded by not too dry an atmosphere. They are made both by the young and adults.

The Embiæ all become adult about the middle of June. Pairing takes place about the end of the month, and they probably deposit their eggs a few days later, dying perhaps in the course of the summer.

I imagine that they then die, as this would explain why young ones only, at most from 7 to 9 mm. in length, were to be found from November to June. Unfortunately my observations were interrupted from July to October. That they can acquire wings is entirely out of the question. In fact, at the end of June I have found impregnated females with the receptaculum full of semen, and others which had already laid eggs, and at this time neither sex exhibited the slightest trace of wings.

The Embiæ cannot jump, but run actively forwards or backwards, and, unlike Thysanura, can climb with ease up a glass surface.

They feed on vegetable matter, but perhaps do not disdain small arthropods (this I have been unable to determine).[1]

I now pass to their anatomical characters.

The chitinous cuticle is mostly thin and soft, especially in the larva, and its external surface is furnished with numerous small points. The hypodermis is pigmented.

There are ten pairs of stigmata (Pl. 19, fig. 2) as in the

necessary amount of silk, and there are no other glandular structures in the body except those of the anterior legs to which its origin can possibly be attributed.]

[1] [No observations in England on the food of Embia urichi were successful; but, according to Mr. Hart, the species probably feeds largely on bark, lichens, or fungous growths, and also on Coccidæ. Specimens of the latter on leaves which accompanied the insects appeared clearly to have been eaten; the contents of the alimentary canal in preserved examples were mainly of vegetable origin, but contained nothing of which the structure was definitely to be identified.]

Lepismidæ, and the respiratory apparatus is therefore holopneustic. The anterior pair is situated latero-ventrally: seen from above, they lie on the line of junction between the pronotum and mesonotum; seen from below, they lie in front towards the hind margin of the prosternum. The second pair is also latero-ventral; seen from above they appear to lie behind the anterior margin of the metanotum, and from below behind the anterior margin of the metasternum. Further, the position of these two pairs varies with the degree of contraction of the body. The remaining pairs are also lateral, but lie nearer the dorsal than the ventral surface; the line connecting any given pair would cut the tergite at the junction of its anterior fourth with the remainder.

The tracheæ are very numerous, without any dilation. They anastomose by longitudinal and transverse branches, which are repeated in most of the abdominal segments. The transverse branches are ventral and unpaired; they unite the tracheal system of one side with that of the other, or rather form a transverse canal between each pair of bronchi (as I call, with many writers, the trunks which originate from the stigmata), e. g. between the sixth right and the sixth left bronchus. Similar anastomoses are met with in the Lepismidæ, Termitidæ, &c. The remaining anastomotic branches are paired and longitudinal, and of two kinds, ventral and dorsal. The former serve as a communication between the transverse anastomotic branches, their anterior terminations lying more laterally (externally) than their posterior. The dorsal branches connect the dorsal transverse tracheal branches, and form longitudinal dorsal trunks, such as exist in the Lepismidæ. These anastomoses will be more clearly understood by observing that each stigma (e. g. the sixth right) gives rise to a single bronchus, which turns forward, and after a short course gives off a branch to form the transverse ventral anastomosis with the corresponding branch of the opposite side (continuing the illustration, from the sixth left bronchus). A little in advance the same bronchus gives off a trunk which runs transversely to the dorsum, and furnishes branches of the second kind, forming

the longitudinal dorsal anastomoses. Thus the transverse anastomosis is formed by a branch springing directly from the bronchus, whereas the longitudinal dorsal and ventral anastomoses, on the other hand, are formed by branches derived indirectly from the bronchi. Similar anastomoses are found in other Orthoptera (s. lat.), including the Termitidæ.

On one occasion I thought I had found a second dorsal longitudinal anastomosis, but I have been unable to detect it a second time, and think I must have been mistaken.

The general arrangement of the thoracic tracheæ is a little different from that in the abdomen, as can be partly seen in the plates attached to this work. Two large trunks on either side, as in Thysanura, run to the head.

The anterior pair of stigmata are, as usual, larger than the others, and the cuticular margins are obviously more developed, so as to act perhaps as a sort of valve. I have been unable to detect anything of the kind in the other stigmata. The spiral thread of the tracheæ is very distinct.

I have found nothing of importance in the vascular system. The blood-corpuscles are usually subelliptical and crowded with shining granules.

The nervous system exhibits, as usual, a supra-œsophageal ganglion, a ventral ganglionic chain (Pl. 19, fig. 2), and a stomato-gastric system; the sympathetic is not distinct. The supra-œsophageal ganglion possesses the usual median constriction; the optic lobes are small, lateral, and give off a fine and rather long nerve to the compound eyes. The ventral ganglionic chain presents a sub-œsophageal, three thoracic and seven abdominal ganglia. The three thoracic ganglia are large, and correspond with the respective thoracic segments. The first six abdominal ganglia are moderately small, the seventh is larger. The first corresponds with the segment médiaire; the second, third, and fourth with the second, third, and fourth abdominal segments, the latter ganglion lying in the posterior part of the segment; the fifth segment has no ganglion, so that the fifth, sixth, and seventh ganglia lie respectively in the sixth, seventh, and eighth segments. The

commissures between the fourth and fifth ganglia are relatively very long.

There is a small frontal ganglion connected as usual with the supra-œsophageal ganglion. From it there comes off an azygos nerve which turns back to run along the œsophagus; along it is a series of small ganglia, some before the point where the œsophagus begins to dilate, another in the first thoracic segment where it is somewhat dilated, and lastly, one in the third thoracic segment where it is much dilated. This supra-œsophageal nerve gives off numerous small twigs to the œsophagus. There are special nerves to the salivary glands, but I have not been able to determine their origin. More minute researches will probably lead to the detection of other ganglia in the neighbourhood of the œsophagus; I simply suppose them to exist.

The eyes are compound and euconic; there is a retinal ganglion (ganglionic lamina). The cones and rhabdoms are very short, and the cornea very thin. Ocelli are absent.

The anterior portion of the alimentary canal (Pl. 19, fig. 3) is very narrow at its commencement, with well-marked longitudinal folds. It begins to dilate from about the posterior margin of the head, and the folds become less distinct. About the beginning of the mesothorax it becomes very large, often after presenting a slight constriction, and the folds are no longer discernible. It persists in this condition to the segment médiaire, where it again becomes very narrow by an almost sudden constriction to finish a little further on. Four longitudinal folds arise at the point of constriction, and are continued back over a short and narrow tract, where they are intercalated with others. The anterior three fourths of this tract are free, and the hinder fourth is invaginated into the chylific ventricle. The cuticle lining the dilated portion of the fore-gut is spinose.

The chylific ventricle is enlarged in front and narrowed posteriorly. Like the œsophageal portion it runs straight, and is continued to the seventh abdominal segment. Its epithelium is cylindrical, with a striated cuticular margin, and at

the base of the cylindrical cells are interposed scattered small cells (replacement cells ?). Ventricular cæca are absent.

The intestine is more or less dilated in front, rather narrowed and more curved in the middle (giving a curvature to the whole region), and dilated again posteriorly (rectum). The rectum possesses six longitudinal folds (plicæ rectales) of large size and rich in glandular cells; each of them receives the ramifications of a tracheal branch.

The Malpighian tubules are long, with a somewhat ample lumen filled with solid excrementitious matter. Their number varies with age, and as many as twenty may be made out in the adult; perhaps the number may be still larger. Further appendages of the alimentary canal are the two salivary glands. These lie on each side of the thorax, and are lobulated; each has a special efferent duct dilated towards the middle, the ducts uniting in front to form a common canal which opens on the labium. These efferent ducts possess a spiral thread like the tracheæ.

I may here mention the sericigenous glands of the anterior legs. As I have stated, the lower border of the first tarsal joint is set with abundant short spines, among which is a row of less numerous setæ, curved at the tip, and perforated by a very fine canal. These canals open externally, in my belief, at the somewhat blunt apex of the setæ, and extrude from time to time a drop of transparent secretion; at their base they terminate in a complicated manner in multicellular glands, a number of which fill the greater part of the joint. Tubular setæ are also found beneath the second tarsal joint, but I have not detected the corresponding glands; perhaps they communicate with glands in the basal joint.

Other special organs are the papillæ of the middle and posterior tarsi. They possess a rather thick cuticle, more or less clearly divisible into two layers, and marked in places with distinct striæ (pore-ducts?). Threads of silk may often be noticed adhering to these papillæ.

The function of these tarsal organs, which show a certain

analogy with the adhesive organs of the feet of many insects, has already been explained.

I pass to the generative organs. There are two ovaries (Pl. 19, fig. 4), and each is pectinate, presenting five unilateral ovarian tubes, which, when mature, occupy most of the abdomen except the posterior segments. In the larva they lie at a certain distance from each other, in about the fourth, fifth, and sixth abdominal segments.

The oviducts are long and straight, and join about the hinder part of the seventh abdominal segment to form an unpaired very short and wide canal (oviduct-uterus), which is unlined by chitin. To this succeeds the still shorter and wider vagina, which has a cuticular lining, and into it opens a large spermatheca furnished with a similar delicate lining.

This spermatheca lies on the dorsal side of the vagina, and extends before and behind it; it communicates with its dorsal wall by a very short duct, also with a chitinous lining. Colleterial glands are absent. The vulva, like the vagina, is unpaired, and belongs to the eighth sternite.

Each ovarian tube possesses a very delicate investing membrane, and each ovum is surrounded by a follicle which is united to its fellows, when the ova have reached a certain size, by short peduncles formed of small cells, which are a continuation of those composing the follicle itself.

The ripe eggs are subelliptical, relatively large, with a very large micropyle, which is developed at the anterior extremity of the egg as it lies in the maternal body.

The male has five testes on each side, or ten in all, each consisting of a certain number of capsules, provided with separate investing membranes (Pl. 19, fig. 5). They lie in the third, fourth, and fifth abdominal segments. This arrangement in the male and the very similar disposition of the ovaries in the female may possibly explain the apparent absence of the ganglion in the fifth abdominal segment. When mature the testes occupy a large part of the abdomen, and, like the ovaries, are arranged unilaterally on the vasa deferentia, which are paired, like the oviducts. Each vas is

dilated posteriorly to form a vesicula seminalis; it then becomes narrowed again, and opens finally behind into a common ejaculatory duct. Just before the origin of this duct each vas receives the discharge of two glandular sacs, which sometimes contain a transparent secretion, and at other times semen.[1] The male genital opening is evidently situated in the larva at the hinder part of the ninth sternite. In the adult it is less distinct, as the penis is developed beyond that point by a direct prolongation of the ejaculatory duct, the external meatus of which corresponds nearly with its apex.

The spermatozoa, like those of most insects, possess a readily distinguishable head and an elongate tail.

[The internal anatomy of Embia urichi, with the omission of the frontal ganglia, the structure of the testes, and the histological features, which the translator has not investigated, corresponds precisely with Professor Grassi's description, except in the following points. The stomato-gastric nerve ends exactly as in Blattidæ in a triangular ganglion at the back of the proventriculus, from which two nerves pass obliquely backwards under the invagination between it and the chylific ventricle (no other ganglia are evident along the course of the œsophagus). The arrangement of the abdominal nerve-cord is identical. The chylific ventricle is relatively much larger than in Professor Grassi's figure, and extends from about the mesothorax to the eighth abdominal segment. At its anterior extremity on the dorsal aspect there is a short rudimentary pouch, which projects forward over the deeply invaginated portion of the proventriculus, and looks like an imperfectly developed cæcum. The Malpighian tubules vary in number from about twenty to twenty-six.

The ovaries are identical in structure; they become very large in the fertile female, and their arrangement cannot then be made out, as the tubes become coiled in a complex manner, and may extend even up to the prothorax, from which ripe ova have been extracted. As many as twenty-six ripe eggs of equal size have been found in the same female. They are oval,

[1] The nature of their contents requires further investigation.

with a very large flattened micropylar extremity, which is placed obliquely; behind it the egg has a very slight twist, so that its shape is almost exactly that of the body of a retort which has been cut off obliquely at its junction with the neck.

The structure of the testes cannot be made out in the adult, and the larval forms available were not sufficiently well preserved.

As Embia urichi is as far removed from Professor Grassi's species as any form in this very small family, it may be inferred that there is no material difference between the internal organs of the existing known species.—W. F. H. B.]

The systematic position of the form I have studied now claims consideration. At the present time seventeen species of Embiidæ are known (a few appear to be doubtful); of these, fourteen are winged and three wingless. The latter may be considered in relation to the form investigated. They are Embia (Oligotoma) antiqua, Pictet, found as a fossil in Prussian amber; Embia (Olyntha) Mülleri, Hagen, from Brazil (known only by a single example, probably a female, in bad condition); and Embia solieri, Rambur, found according to various authors in the south of France and Spain, but as yet only in the larval state. I have already stated that my species is probably Embia solieri, which is in its turn not clearly distinguishable from Embia antiqua. But further comparison is necessary before its identity can be indisputably established. In the length of the antennæ and the asymmetrical hinder extremity of the male my species approaches the genus Oligotoma, and differs from Embia (s. str.) by the symmetry of the hinder extremity in the female. It is remarkable for the sexual difference in the mandibles, which does not appear to exist in any other species, and I therefore think it should be referred to a new genus.

[There is very great confusion about the sexual and generic characters of Embiidæ, which Professor Grassi has not unravelled, owing to his necessarily relying on Hagen's statements. An analysis of the descriptions, a comparison of all

available species, and his own dissections, convince the translator that in the known species of Embiidæ—1, no winged female is known; 2, the apex of the abdomen is always asymmetrical in the male; 3, it is never asymmetrical in the female. The number of described species has now reached twenty, of which ten are known by winged males alone, mostly described from single specimens. The only species in which a winged female has been positively asserted to exist is Embia mauritanica. Lucas[1] describes a winged imago and a wingless larva (as large as the imago), and states that he opened the abdomen of several imagos and found them to be females. As his very poor description of the ovaries is absolutely irreconcilable with Professor Grassi's, or with the writer's dissections of E. urichi, and as his figure of the winged form has palpably the asymmetry of the abdomen due to the presence of the penis and titillatores, it must be irresistibly concluded that he mistook the testes for the ovaries, and that his so-called larvæ were presumably the females of the species. Embia persica, McLach., is said to have a winged female, but only very doubtfully. On the other hand, wingless females (which are much rarer in collections than the males) exist in six described species. In Embia solieri the so-called wingless larvæ may have included imagos of both sexes, and the species is probably, as Professor Grassi conjectures, the one with which he has dealt. As for the remaining generic characters, the sexual differences of the mandibles can be evaluated only when they have been studied in both sexes of other species; antennal differences are absolutely fallacious in forms in which the organs vary so excessively in the number of joints, and the only remaining character of any value appears to reside in the neuration which distinguishes the wings of the males in Oligotoma and Embia (with which is included the somewhat different-looking subgenus Olyntha), and in the absence of wings in the males of Professor Grassi's species, Embia solieri, and (apparently) Embia antiqua. But this applies to one sex only, and the writer sees no

[1] 'Explor. Alger.,' iii, pp. 111—114; 'Neur.,' pl. iii, fig. 2, *a*—*n*.

present possibility of separating the females generically. It may be added that, like the antennæ, the neuration can vary within limits in the same species, and even on opposite sides of the same individual. Embia urichi, de Sauss., which has been several times referred to, belongs properly to Westwood's subgenus Olyntha.—W. F. H. B.]

The Embiidæ are very remote from the Termitidæ, as I have pointed out in the body of my memoir, and they have no more definite affinity with the Perlidæ. Even the much-invoked relationship with the Psocidæ is very problematical; the concentrated nervous system of the latter and their tracheal system (according to researches communicated by Dr. Rovelli) establish a wide difference between the two families. To sum up, the Embiidæ approach the Orthoptera (s. str.) more nearly than any other group, though they are separated by a series of important characters. In the present state of our knowledge I think that they should be ranked among the Orthoptera (s. lat.), as a special sub-order parallel with that of the Orthoptera (s. str.).

It seems important to note that my species of Embia has certainly degenerated, a process which has simplified the organism without bringing out any of the characters which I regard as primitive in Thysanura (eleven pairs of stigmata, ocelli with rhabdoms, &c.). Even if we admit that the Thysanura have undergone a certain amount of degeneration, and that they once have possessed wings, it must always be recollected that they are the degenerate descendants of Orthoptera which retained certain primitive characteristics, which are lost in the Orthoptera of the present day, as far as is known at present.

My species of Embia is degenerated, or rather, simplified by the loss of its wings. This is probably due to the fact that the species are inhabitants of hot countries, and at the same time of situations neither over-dry nor over-moist. As the lack of a sufficiently warm climate prevents the European Embiidæ from acquiring wings before the summer drought

sets in, there is in my belief a precocious maturation of the generative organs (neoteinia). This interpretation is supported by the fact that they become mature here in Sicily at a time when the summer heat is well marked, and before the ground has become unduly dry.

CATANIA; December, 1888.

[The transformations of the Embiidæ are practically unknown, and the few facts recorded have simply increased the confusion which has surrounded it. While reserving the details of the metamorphoses of Embia urichi, it is sufficient to say here that the females of Embiidæ are practically ametabolous, the only essential morphological change during growth consisting in an increase in the number of antennal joints. The male of Professor Grassi's species undergoes the changes in the form of the mandibles and the posterior extremity which supervene upon the last ecdysis. In addition, the males of the winged species, such as Embia urichi, are normally hemimetabolic, and pass through a nymph stage, as in other Orthoptera (s. lat.) The form described by Mr. McLachlan[1] as a nymph has been conjectured by Hagen to be a micropterous form. This is quite erroneous. The chitinous coverings thrown off at the ecdyses are extraordinarily thin and transparent, and quite pigmentless, except over the tergal and sternal plates. In the nymph of E. urichi the pterothecæ are so extremely delicate as to be indistinguishable without very careful examination, and the young wings which they enclose appear quite distinct and separate as in the imago, but of very small size. But if specimens preserved in alcohol, which causes the body to shrink away from the integuments, are examined, or if the nymph-skin is removed intact by a longitudinal incision, like the ecdysial fissure, the existence of the pterothecal coverings to the small wings becomes evident. The structure of the thoracic segments and the mode of attachment of the legs is alike in both winged and wingless forms, and is typical of apterous

[1] 'Journ. Linn. Soc.,' xiii (1877), pp. 383–4, pl. xxi.

insects, "so that the Embiidæ that have wings may be described as apterous-like insects provided with two pairs of inefficient wings" (Sharp), the attachment of which to the thorax is feebler than and different from that found in other winged insects. In these respects the Embiidæ must be regarded as absolutely the lowest type of winged insect, and the present writer is inclined to assign them a position intermediate between the Thysanura and the Orthoptera Cursoria. At the same time he is fully impressed with the fallacies which attend any endeavours to establish phylogenetic relationship between the loosely knit surviving families which make up the Thysanura, Orthoptera, and Neuroptera, and believes that these three orders, which have given rise to so persistent a difference of opinion among systematists, may best be treated by their combination into a single order, which will be somewhat more than the equivalent of any of the more specialised orders of insects, and its division into families, without recognition of any larger aggregates.—W. F. H. B.]

N.B.—A second species of Embia, of which I know only the larva, exists at Catania. Its habitat is similar to that of the species described, but it is readily distinguished by certain characters. Above it is uniformly dark red-brown, except for the intersegmental membranes between the thoracic segments, which are dull yellow; the antennæ, cerci, and the greater part of the legs are nearly concolorous with the dorsum. The antennæ are twenty-jointed. The body, especially the abdomen and the hinder thoracic segments, is relatively larger. The papillæ of the second pair of tarsi are furnished on the inner side with numerous small and easily distinguishable spines; the same arrangement is repeated in the hinder pair, except that the second joint has no papilla.[1]

Catania; June, 1889.

[1] Dr. Rovelli has found an undetermined species of Embia at Rome.

On the Structure of Hydractinia echinata.

By

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With Plate 1.

An investigation of the structure and relative positions of the cœnosarc and chitinous parts of Hydractinia echinata was suggested to me by Professor Weldon, for whose generous advice and assistance I cannot be too grateful.

From my observations on this hydroid I find that the chitinous skeleton is for the most part a continuous irregular crust attached to some foreign object, and overlaid by a cœnosarc consisting of two layers of ectoderm, enclosing between them a number of branching and anastomosing endodermal tubes, which are connected at intervals with the endodermal canals of the polyps, the upper layer of ectoderm being continuous with the ectoderm of the polyps.

This view is somewhat similar to that of Strethill-Wright, who describes the chitinous skeleton as forming a continuous crust below the cœnosarc, which, according to him, consists of two ectodermal layers enclosing a single and continuous layer of endoderm permeated by tubular cavities.

With other observers of Hydractinia the prevalent idea as to the structure of the colony seems to have been that the skeleton was tubular, the chitinous tubes enclosing the cœnosarc, which also extended as a continuous sheet over the surface

of the skeleton, and here connected the polyps with one another.

The earliest reference we have to Hydractinia is that comprised in a few lines by Fleming (1) in 1828; this observer, however, regarded the horny crust of Hydractinia as being a polyzoon ectocyst, and assigned to it the name Alcyonium echinatum.

This view of the nature of the hydroid was also held by Johnson (2) in 1838.

Van Beneden (4), in 1841, first gave the generic name of Hydractinia to the hydroid, and wrote a paper on the structure of the egg. He regarded the sporosacs borne round the sides of the reproductive polyp as "eggs," but gave no description of the structure of the colony.

Hassall (5), in 1841, described, under the name "Echinochorium clavigerum," a hydroid found in Dublin Bay adherent to empty univalve shells, and which is, according to Allman (13), the "Hydractinia, sp." of Van Beneden. According to Hassall the colony consists of a "polypidom muricated with rough, spinous papillæ about a line in height. There are numerous indentations on the surface of the polypidom, in each of which the base of a polyp is inserted; this latter is about a quarter of an inch in height, and is of a white colour; its head is somewhat enlarged, and is surrounded with numerous contractile club-shaped tentacula; the number of these varies considerably, but frequently amounts to between twenty and thirty. The tentacles are not arranged in any determinate order, but are variously disposed. Whether the polyps are separate or united at their bases I am unable to say." His accompanying figure represents nutritive polyps and ridged spines on a reticulated base.

Philippi (6), in 1842, described as "Dysmorphosa conchicola" a hydroid from the Bay of Naples; his description is short, but he recognised the fact that the polyps are connected at their bases.

De Quatrefages (7), in 1843, described Hydractinia under the name of Synhydra parasites in the most comprehensive

paper on this hydroid that had so far appeared. He mentions two polyps,—reproductive polyps without a mouth, and nutritive polyps with a mouth; but describes the chitin as an endoskeleton developing in the substance of the common cœnosarc. He says that in the nutritive polyps the tentacles vary in number, are distributed in two alternating circles, and are very contractile. The polyps are all connected with a common living tissue which is directly continuous with the exterior layers of the body, and which, towards the edge, only consists of a delicate pellicle enclosing no solid skeleton. Below the horny base, and protected by it, there are little anastomosing tubes, the central canals of which communicate with the digestive cavity of the polyps.

De Quatrefages is the first observer who describes the reproductive polyps in any detail.

Van Beneden (8), in 1844, published a second paper, in which he describes the two sexes of Hydractinia as two distinct species—H. rosea and H. lactea.

Johnston (9), in 1847, revived the specific name "echinata," used by Fleming. He did not accept De Quatrefages' view of the endoskeletal nature of the chitin; on the other hand, he conveys no distinct impression as to how the individuals are connected together.

Strethill-Wright (10), in 1856, published a paper on Hydractinia, containing the most correct account of the structure of the colony which had so far appeared. He describes the corallum (skeleton) as being secreted externally, and forming a raised network between the spines. The polypary (soft tissue) "invests the corallum and fills up the grooves "of its papillæ, the interstices between its reticulations, and "the cavities of its hollow spines. It is often absent at the "summits of the papillæ. It secretes, renews, and extends the "corallum, gives rise to new polyps, and is the seat of commu-"nication between the polyps of the colony. . . . I conclude "that the polypary of this zoophyte consists of a single layer "of endoderm enclosed between two layers of ectoderm. That "the lower ectodermic layer, as it grows over the shell, attaches

"itself by its colletoderm" (which is, according to Strethill-Wright, an epidermis replacing the corallum at the growing extremities of the branches), "and secretes the horny plate of "the corallum. On this plate, by a further process of secretion "from the lower ectoderm, the grooved spines are erected. "That the upper layer of ectoderm is naked over the greater "part of its surface, or only covered by a thin epidermis; but "occasionally this layer also takes its share in the secretion of "the corallum, and in that event produces the smooth conical "spines, the concavity of which it fills."

Strethill-Wright conveys the impression that the endoderm of the polypary is a continuous layer, permeated by tubular excavations, which are connected with the digestive cavities of the polyps.

With respect to the edge of the colony he says, "On the less exposed parts of the shell the polypary frequently passes beyond the papillary corallum as a thin membranous expansion, or breaks up into a loose network of delicate anastomosing tubes. . . . . Propagative stolons are given off by these tubes; . . . . . a delicate chitinous investment may also be detected on the creeping tubular fibres, from which the stolons of Hydractinia take their rise; but I have not satisfied myself as to its presence on the entire upper surface of adult polyparies."

Two kinds of nematocyst, differing in size, are mentioned by Strethill-Wright, who is the first observer of the spiral and tentacular polyps. He describes the spiral polyps as mouthless, with rudimentary tentacles and a highly developed muscular coat, large nematocysts being crowded in the ectoderm of the tentacles and the whole body. The tentacular polyps he regards as always present. "Their tips are covered with a dense pavement of the larger thread-cells, and a few of the same bodies are thinly scattered along their whole length."

Van Beneden (11), in 1866, published another paper on Hydractinia, in which he says that the tentacles are generally absent in the reproductive polyps. What he formerly

described as "eggs" he now designates "sporosacs," and asserts that the skeleton is external and similar to that of Campanularia and Tubularia. The reproductive polyps have no mouth and only rudimentary tentacles. He confuses Hydractinia with Podocoryne, and distinguishes three new species of Hydractinia.

Hincks (12), in 1868, also mentions tentacular polyps; he, as well as Strethill-Wright, describes fixed reproductive sacs. He speaks of the basal cœnosarc as consisting of "a number of anastomosing tubular stolons closely packed together, and filling in the tubular orifices of the chitinous skeleton, which latter appears to consist of a series of tubes laid side by side on a plate of chitin, and closely appressed one to the other."

Allman (13), in 1871, published a full account of Hydractinia, in which he affirms that the basal part of the colony consists of a number of closely approximated chitinous tubes containing cœnosarc, which consists mainly of endoderm with only a thin investment of ectoderm, the latter secreting the chitin. "At the free surface of the cœnosarcal expansion its intercommunicating canals are only partially invested by chitin, this excretion being in the superficial layer of canals confined to the deeper parts, thus forming open channels in which the canals are lodged, so that when the soft parts are removed the chitinous perisarc forms on the surface a multitude of intersecting ridges, having between them the channels which had contained the superficial cœnosarcal canals. Upon the whole of the free surface, however, the ectoderm of these canals forms a continuous and very conspicuous layer, having acquired increased thickness and developed in its substance abundance of thread-cells. The whole free surface of the common basal expansion of the colony thus presents an absolutely naked layer of ectoderm. . . . There can be no doubt that the whole hydrophyton of Hydractinia must be regarded as consisting of a set of cœnosarcal, freely intercommunicating tubes, which have excreted from their free surface a chitinous perisarc, and have intimately coalesced with one another."

An injured part of the hydrophyton repairs the injury by a network of cœnosarcal tubes invested by a chitinous perisarc, the meshes of which are ultimately obliterated by the thickening and coalescence of their chitinous walls. The ectoderm and endoderm of the hydranths is continuous with the ectoderm and endoderm of the basal cœnosarc.

The spiral zooids are long, cylindrical, mouthless hydroids, with a crown of rudimentary tentacles crowded with large nematocysts; they have a tubular endodermal cavity, and possess the power of coiling themselves into a spiral: the mesogloea fibres are strongly developed. The blastostyles may have a mouth.

Allman believes that the tentacular polyps are only rarely present, and regards them as abnormal modifications of other hydranths.

Weismann (15), in 1883, described the blastostyles and migration of the sex-cells in Hydractinia. He figures a blastostyle with a mouth and ciliated endoderm, and mentions the occurrence of food granules in the endoderm of the upper region of the body.

Miss Bunting (17), in 1894, published an account of the origin of the sex-cells and the development of Hydractinia. She first observes the ova in the endoderm of the blastostyle, and concludes that they are probably endodermal in origin.

My observations on the structure of Hydractinia echinata have led me to agree to a certain extent with Strethill-Wright with respect to the relations of the cœnosarc and chitin, and to differ from Allman and Hincks as to their views of the tubular nature of the adult skeleton.

Moreover I have found a dactylozooid with a mouth, the existence of which has hitherto been overlooked or denied, while other observations show that there is a migration of ova between ectoderm and endoderm in the blastostyle.

Colonies of Hydractinia are generally situated on the surface of shells, which are commonly whelk-shells inhabited by hermit-crabs. A large colony may cover the whole shell except for a small roundish patch where the shell rubs along the ground

as the hermit-crab drags its home about; a small colony is usually situated near the edge of the shell.

A Hydractinia colony is comprised of four kinds of polyps:

1. Gasterozooids, or Nutritive polyps.
2. Blastostyles, or Reproductive polyps.
3. Dactylozooids, or Spiral polyps.
4. Tentacular polyps.

The Gasterozooids are the most numerous of the polyps; in the early spring and in the summer, however, the Blastostyles increase greatly in numbers, and at these times give rise to the generative products, the colonies being either male or female.

Round the shell mouth are situated the Dactylozooids, which are capable of coiling themselves spirally, and may function as defensive polyps.

Strethill-Wright (**10**) and Hincks (**12**) also mention "Tentacular polyps," which are scattered on the outskirts of the colony, and which they regard as constant in occurrence. Allman (**13**), on the other hand, does not believe that they are universally present, and supposes that they are merely abnormalities when they do occur.

However, in all those colonies which I have closely examined with a view to finding these polyps I have been successful; in one specimen they were particularly abundant, occurring principally at the growing edge of the colony. In other specimens they were rare, while on one very flourishing colony I only found about half a dozen. I doubt if they are abnormal dactylozooids, as Allman suggests, for I have never found them situated on that part of the colony frequented by dactylozooids.

These various kinds of polyp are all connected together at their bases by a common cœnosarcal expansion, which is a white semi-transparent investment completely covering the chitinous skeleton.

I succeeded in keeping several colonies alive for some time by suspending the shells on which they were situated in small tanks, the other occupants of the shells being removed. One

of these colonies lived in this way for about three months, when the zooids gradually dwindled away, and finally all disappeared. This colony, as is general with Hydractinia, extended itself round the shell mouth into the interior of the shell, and apparently began to absorb this enclosed portion of the shell, which became thin and brittle.

In other colonies kept in confinement the dactylozooids which were congregated round the edge of the shell disappeared after a few days.

A point of some interest which I noted in examining whelk-shells tenanted by hermit-crabs and bearing Hydractinia colonies on their surfaces is that a Polychæte worm, Nereis bilineata, was always found living inside the shell in company with the hermit-crab. This worm lived in the coils of the tip of the shell, and could completely withdraw itself from observation.

The most successful killing reagents used were Flemming's solution and picric acid solution; Hermann's solution also gave good results for histological purposes. The staining reagents principally used for sections were Delafeld's hæmatoxylin and borax carmine. For surface views of the thin edge of the colony aniline orange was found to be an excellent chitin stain; the preparations were left for a few minutes in a 90 per cent. alcoholic solution of aniline orange, when the chitin was stained bright yellow, the protoplasm being but slightly stained.

General Anatomy.

The Skeleton.—This is a continuous unevenly deposited layer of horny chitin, so closely attached to the rough surface of the shell that the latter must be decalcified in order to isolate the hydroid colony. It is, for the most part, secreted by the lower layer of ectoderm which forms part of the cœnosarc of the colony. In the central parts of the colony it is in many places of considerable thickness, with irregular lacunæ, and thickly beset with small chitinous spinules, while it is frequently raised up into a number of large conical

grooved spines. Even in these mature parts of the colony, that part of the skeleton which connects the spinules and spines together is often exceedingly thin and inconspicuous.

The spinules are merely solid chitinous thickenings, irregular in shape and size, projecting upwards from the chitin which is attached to the whelk-shell. They occur all over the skeleton, and are very conspicuous towards the thin edge of the colony (Pl. 1, fig. 1, *a* and *b*).

The spines are one of the characteristic features of the colony; they are mostly deeply furrowed, having longitudinal serrated ridges between the grooves. The ridges are connected with one another by chitinous cross-bars, and fuse together at the tip of the spine. Central chambers or a single chamber communicate with the grooves. The whole spine is covered by the general cœnosarc, which is also continued into the meshes of the framework, though it may often become rubbed off from the tips of old spines where the shell has come in contact with the ground.

Allman (13) has figured and described a typical spine in his account of H. echinata. Sometimes the spines bifurcate towards their apex, or are otherwise irregularly shaped.

Between the spines and spinules the skeleton is in some places deposited as a single thin layer, but it is usually secreted in thin, irregular, reticulated layers, one above another. In the older parts of the colony these layers, in vertical section, have the appearance of chitinous strands varying in thickness and running horizontally or obliquely (Pl. 1, fig. 1, *c*), but towards the thin growing edge of the colony they are more regular.

At intervals throughout the skeleton there are spaces surrounded on all sides by chitin, and containing degenerating masses of cœnosarc; such masses have become constricted off from the rest of the cœnosarc by unequal growth of the chitin, the various stages of such constriction being demonstrable. In some cases where the constriction and isolation are not complete the degenerating cœnosarc is seen to be in direct continuity with the cœnosarc of the colony (Pl. 1, fig. 2). In other cases the spaces are empty.

The chitin which is situated beneath such a degenerating mass becomes somewhat cup-shaped by unequal growth (Pl. 1, fig. 2). Gradually the edges of the cup grow over, only leaving the mass in the cup attached by a narrow bridge to the upper cœnosarc, until finally the edges of the cup meet and coalesce, and so isolate the degenerate mass of cœnosarc. These constricted masses consist only of the deeper parts of the cœnosarc, i. e. the lower ectodermal layer and the endoderm, and as they degenerate the boundaries between the cells become obliterated, the protoplasm becoming very granular, and the nuclei losing their distinctive appearance.

The Edge of the Colony.—At the edge of the colony where growth occurs the skeleton is very thin, though thickened at frequent intervals into short, solid spinules; here, too, the cœnosarc is generally thinner, and the polyps are few and small.

Extending over the surface of the cœnosarc of this region is a thin membranous layer of chitin, which is connected at many points with the tips of the spinules of the skeleton.

In this region the skeleton is occasionally raised up into conical smooth spines, which are fenestrated at their bases, so that portions of the basal cœnosarc, consisting of lower ectoderm and of endoderm, can penetrate into the interior of the spines (Pl. 1, fig. 3).

These spines are partially covered by cœnosarc, which, however, does not extend over their tips; a thin layer of chitin spreads over the surface of the cœnosarc, but is not continued over the naked tips of the spines (Pl. 1, fig. 3).

Strethill-Wright (**10**) mentions hollow chitinous spines filled with cœnosarc, but he describes them as being derived only from the upper layer of ectoderm.

The colony is derived at its growing edge from a number of cœnosarcal tubes clothed with a chitinous perisarc; these tubes branch about irregularly over a large portion of the whelk-shell, into the cavity of which they extend; ectoderm covers their tips, and they appear to secrete their perisarc as they grow.

The lower surface of the perisarcal tubes is thicker than the upper surface; this and the side walls are frequently thickened into vertical, conical spinules, and form the foundation of the characteristic skeleton of older parts of the colony. The thin upper surface has no chitinous thickenings, and usually stains more readily than the rest of the perisarc. As the cœnosarcal tubes continue to grow they branch repeatedly and anastomose with one another until the tubular character of the chitinous investment is obliterated; in this way a sheet of cœnosarc, enclosed between two layers of chitin, is formed.

When two such tubes anastomose, it is probable that part of the internal lining of ectoderm in each tube which lies nearest to the region of contact absorbs the chitinous wall along this region; consequently the two tubes are here placed in communication with each other, and the ectoderm of the upper half of one tube becomes continuous with the ectoderm of the upper half of the other tube, while the ectoderm of the lower half of the one becomes continuous with the ectoderm of the lower half of the other. Hence arises the differentiation of two ectodermal layers so characteristic of the maturer regions of the colony (Pl. 1, figs. 1 and 4).

The endoderm retains its tubular character.

The chitinous spinules along the side walls of anastomosing tubes do not all become absorbed, but many remain as part of the permanent skeleton, and are attached at their tips to the thin upper membrane of chitin (Pl. 1, fig. 1, *a*, *c*, *b*).

As the colony grows this membrane gradually weakens, and finally is lost (Pl. 1, fig. 1, *b*); the cœnosarc then grows up over the tips of the spinules, and so becomes a continuous expansion over the surface of the skeleton.

Here and there are empty chitinous tubes from which the protoplasm seems to have withdrawn itself; frequently all that remain of these tubes are the small chitinous spinules.

At intervals a small polyp branches from this growing region, breaking through the upper chitin, which does not extend over the polyp above its base. In some of the colonies I examined I found many "tentacular polyps" in this region.

The Basal Cœnosarc of the Colony.—As mentioned above, this consists of two layers of ectoderm, enclosing between them a ramifying mass of endodermal tubes, and forms that part of the colony which connects the polyps with one another. The lower layer of ectoderm secretes the greater part of the skeleton, forming a continuous layer in contact with it; the upper layer of ectoderm also forms an unbroken expansion, and is continuous with the ectoderm of the polyps. The two ectodermal layers thus form two sheets of cells, one above the other, and are in close contact with one another, except where the tubes of endoderm are interposed between them, such tubes serving to place the cavities of the polyps in communication (Pl. 1, fig. 1, *c*).

These endodermal tubes are more or less oblong in cross-section, and are always surrounded by a narrow layer of mesogloea, which separates the endoderm from the ectoderm (Pl. 1, fig. 5), and is continuous with the mesogloea of the polyps.

At frequent intervals thread-like strands of mesogloea from the lower surface of this endoderm pierce the lower ectoderm, and attach themselves by slightly widened bases to the skeleton (Pl. 1, figs. 1, *c*, and 5).

Similar strands fix the polyps to the chitin, but these are thicker, more numerous, and, since the lower ectodermal layer is generally much shallower below the polyps, they are often shorter. These processes thus exhibit the singularity of being surrounded on all sides by ectoderm cells. Similar strands are figured by Weismann (**15**) as attaching the cœnosarc of Eudendrium ramosum to the perisarc.

The lower ectoderm and the endodermal tubes penetrate into and fill up the intercommunicating chambers and the grooves of the large spines, the superficial ectoderm extending over all.

Strethill-Wright (**10**) and Allman (**13**) both mention the fact that this basal cœnosarc is capable of communicating the effect of any shock it may receive to the various members of the colony.

The Polyps.

1. The Gasterozooids.—The external appearance of these polyps has been frequently described and figured; they are cylindrical in shape and very contractile, and may attain to a height of about a quarter of an inch. They are provided with a conical hypostome, terminating in a mouth, and having round its base two closely approximated series of tentacles, which increase in number with the age of the polyp, and vary from ten to thirty in different colonies. These tentacles are very contractile, and are provided with numerous nematocysts. The polyps are capable of everting their hypostomes. The outermost cell-layer of a Gasterozooid is a layer of ectoderm, which is continuous at the base of the polyp with the upper ectodermal layer of the basal cœnosarc; the alimentary canal is lined by a single endoderm layer, which forms a wide cylindrical tube, connected at its base with the plexus of endodermal tubes which permeates the basal cœnosarc (Pl. 1, fig. 1, *c*). Between the ectoderm and endoderm is the well-marked mesoglœa.

2. The Blastostyles.—These are specially modified reproductive zooids, giving rise to "sporosacs." The blastostyles are smaller than, and differ considerably in appearance from the Gasterozooids; they have a small mouth at the apex of a conical "head," and at a short distance from this terminal mouth there are two or more closely approximated circles of tentacles. These tentacles vary in number from ten to thirty; they are rudimentary knob-like structures, containing prolongations of the endoderm and mesoglœa of the polyp, and their ectoderm is crowded with nematocysts (Pl. 1, fig. 6).

Below the tentacles the body narrows gradually, becoming externally constricted into a narrow "neck;" then it widens considerably into a globular dilatation, from the walls of which arise the round or oval sporosacs. The sporosacs are outgrowths of the walls of the polyp, all three layers of the polyp being involved in their formation: generative cells arise in the body of the blastostyle and migrate into the sporosacs.

Below the globular region the body again narrows and becomes connected with the basal cœnosarc. Miss Bunting (17) has fully described and figured the sporosacs and generative cells of Hydractinia. She mentions that she was unable to trace egg-cells in the ectoderm or mesoglœa of the blastostyle. I have, however, observed small cells situated in the ectoderm of the blastostyle close to the mesoglœa, and indistinguishable from undoubted egg-cells in the endoderm of the same region of the blastostyle (Pl. 1, fig. 6).

Also in two cases I have found egg-cells in the mesoglœa; the endoderm of the same region already contained several eggs (Pl. 1, fig. 7). In one of these cases the blastostyle had not yet formed sporosacs.

3. The Dactylozooids.—These are cylindrical throughout their length, and are furnished at the distal extremity with a circle of knob-like rudimentary tentacles, from ten to sixteen in number, and crowded with nematocysts.

They have no hypostome, and are described by Allman (13) and Strethill-Wright (10) as having no mouth. I have, however, found a distinct mouth situated in the centre of the tentacle circle, and leading down through a short tubular canal bounded by ectoderm into the endodermal cavity (Pl. 1, fig. 8). These polyps are exceedingly muscular, and are capable of coiling and uncoiling themselves.

In one colony which I examined at Plymouth the dactylozooids were frequently branched once or twice, each branch terminating in a typical dactylozooid head.

4. The Tentacular Polyps.—These polyps are principally situated towards the outskirts of the colony, though they may also occur in the older regions; most frequently I have found them arising from the network of tubes at the growing edge. They are exceedingly slender, though often longer than the other zooids comprising the colony, and are contractile, for their ectoderm is frequently marked by transverse folds. They possess a tubular internal cavity lined by large endoderm cells: I have not succeeded in demonstrating a mouth in these individuals.

The distal extremity is slightly enlarged into a club-shaped tip, covered all over with nematocysts, which also occur in considerable numbers throughout the ectoderm of the polyp.

In examining colonies of Hydractinia for these polyps I came across two abnormal individuals. One of these had the appearance of a tentacular polyp which had given rise to two branches situated at different levels, and each terminating, like the main polyp, in a rounded tip covered with nematocysts. (Process, Fig. 1.)

The other abnormality was a gasterozooid from which, just below its tentacles, branched a tentacular polyp. (Process, Fig. 2.)

Fig. 1.

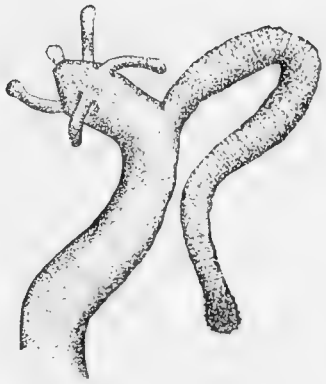

Fig. 2.

Histology.

A. Histology of the Lower Ectoderm.

This layer extends, renews, and secretes the greater part of the skeleton; occasionally, in very limited areas, it is more than one cell deep.

The cells comprising it are not uniformly equal in size; beneath the polyps and towards the edge of the colony they are more or less square and regular in shape, but in the adult parts they are frequently deeper than they are broad. As a consequence of the great variation in the depth of the cells of

this layer the thickness of the cœnosarc varies considerably throughout the colony; over the tips of the chitinous spinules and the jagged ridges of the spines this ectodermal layer is often so shallow as to be almost inappreciable.

The nucleus is large, and situated in the centre, or rather towards one side of the cell; it is oval in shape, and has a dense, deeply staining reticulum with several nucleoli.

In some regions of this ectodermal layer the cells are very vacuolated; the nuclei of these cells are easily discernible, and are surrounded by a mass of coarsely granular protoplasm, from which protoplasmic strands extend to the cell borders (Pl. 1, fig. 5).

In other places, however, the cells are closely crowded with deeply staining roundish corpuscles, which are often present in such numbers as to obliterate from view the boundaries between the cells and hide the nuclei. Probably the presence of these corpuscles is accounted for by the fact that the lower ectoderm apparently secretes some of the nematocysts which frequently occur in great abundance in these parts of the lower ectoderm (Pl. 1, fig. 1, *c*).

As was mentioned by Strethill-Wright (**10**), there are two kinds of nematocyst in Hydractinia, small and large; both kinds are found in the lower ectoderm.

The ectoderm of the gasterozooid tentacles is crowded with the small variety, while large nematocysts occur in the ectoderm of the blastostyle and dactylozooid tentacles, and in the ectoderm of the tips of the tentacular polyps; nematocysts are also found in small numbers throughout the ectoderm of the bodies of the gasterozooids, blastostyles, and dactylozooids, and in large numbers in the ectoderm of the bodies of the tentacular polyps, but I have never found both the large and small variety occurring in the same individual.

B. Histology of the Upper Ectoderm.

1. Ectoderm of the Basal Cœnosarc.—This forms a continuous and regular layer of cells, which are approximately equal in size, and appear cubical in section.

These cells are apparently not characterised by muscle tails, but interstitial cells are sometimes wedged in between them at their bases (Pl. 1, fig. 5). There are no sensory cells in this layer, but nematocysts are occasionally found. The nucleus is situated in the centre of the cell; it is round or slightly oval, and has always one large nucleolus: surrounding it is a mass of finely granular protoplasm which almost fills the whole cell.

2. Ectoderm of the Polyps.—This layer is in many respects similar to the basal ectoderm; the cells are often somewhat narrower, more columnar in shape, and more variable in size.

They are characterised by exceedingly long muscle tails, which can be demonstrated by macerating a hydroid for about an hour in $\frac{1}{5}$ per cent. solution of acetic acid, washing in water, staining with picro-carmine for an hour or more, and examining in weak glycerine.

The protoplasm of the muscle tails appears more homogeneous and less coarsely granular than that of the body of the cell. In several of these isolated ectoderm cells large vacuoles were observed. (Process, Fig. 3.)

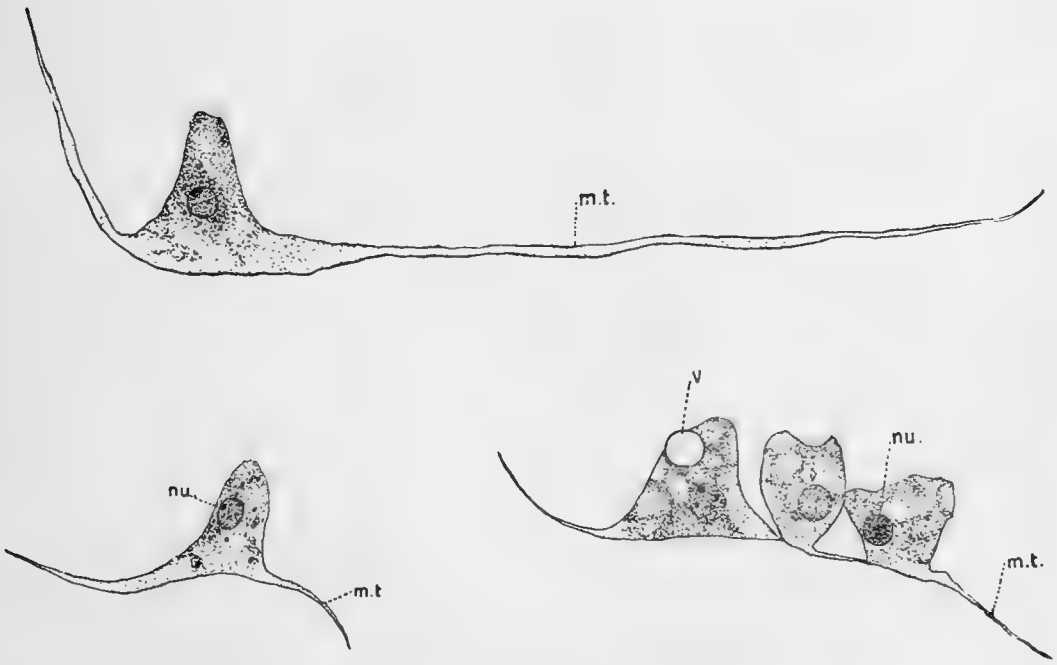

Fig. 3.

The muscle tails in non-macerated material are most easily demonstrable in sections of dactylozooids, where they are greatly developed (Pl. 1, fig. 8).

c. Histology of the Mesoglœa.

The mesoglœa extends between ectoderm and endoderm throughout the colony as a structureless, homogeneous layer. In the basal part of the colony it is very thin, and, as mentioned above, thin strands of its substance serve to fix the cœnosarc to the chitinous skeleton; in the polyps it forms a much thicker layer (Pl. 1, fig. 1, *c*).

d. Histology of the Endoderm.

1. Endoderm of the Basal Cœnosarc.—The endoderm lining the cavity of the ramifying tubes consists of a single layer of cubical cells, with large central oval nuclei. The protoplasm is coarsely granular and abundant, and there are many food granules. Other deeply staining gland-like cells occur at intervals throughout the endoderm of these tubes (Pl. 1, fig. 1, *b*); these are probably similar to certain so-called gland-cells which occur in the endoderm of the Gasterozooids.

2. Endoderm of the Gasterozooids.—This endodermal layer consists of long narrow cells, closely pressed against one another, and somewhat irregular in shape; they vary in length, so that their free ends are not all on the same level. They are greatly vacuolated, especially towards their free ends, where they widen slightly. The nucleus is situated in the middle region, and close to the border of the cell; it is oval, and has a granular reticulum, with one or more nucleoli.

In the hypostome the endoderm is thrown into longitudinal ridges, which project into its cavity; there are usually from four to eight of these ridges, which then pass downwards and lose themselves in the endoderm of the stomach. A transverse section of the hypostome consequently represents the lumen of the gut as being star-shaped. Allman (14) mentions that similar endodermal ridges occur in the endoderm of other gymnoblastic hydroids.

In a gasterozooid which has had no food for some time the protoplasm of the endoderm cells is exceedingly vacuolated, there being only a small mass of protoplasm congregated round the nucleus, and a thin protoplasmic cell lining; no other endodermal elements can be demonstrated, at all events in dead material.

On the other hand, an examination of the endoderm of a gasterozooid which is digesting food clearly shows that there is a certain amount of differentiation between the cells. The ordinary vacuolate endoderm cells are crowded with food vacuoles; the nuclear plasm appears more granular than in non-digesting endoderm cells. But besides these vacuolate cells, there are numbers of pyriform cells which are situated singly between every two or three of the vacuolate cells, and which stain very readily. These cells are rather wider in their middle region than the vacuolate cells, and taper off basally where they come in contact with the mesoglœa; the nucleus is situated towards the base of the cell and is surrounded by a dense mass of protoplasm. The rest of the cell is crowded with small spherical bodies, which are so abundant as to hide the protoplasm in which they are embedded (Pl. 1, fig. 9).

Miss Greenwood (16) describes similar cells in the endoderm of Hydra under the name of "gland-cells." Among the cell contents of the endoderm are numerous roundish bodies, smaller than nuclei, and especially abundant in the endoderm of the hypostome; some of these stain equally throughout, but others only stain partially, a large slightly stained region being left. They stain deep blue with hæmatoxylin but yellow with borax carmine; they are colourless in the living animal, and stain with iodine less than the other cell contents. The most likely hypothesis seems to be that they are proteid in nature; they may correspond to the "nutritive spheres" of Hydra described by Miss Greenwood (16). Endoderm is continued into the tentacles as a single row of cells which are very vacuolated and regular in shape; the nucleus is central and surrounded by protoplasm, which forms a central band in the cell.

3. Endoderm of the Blastostyle.—The endoderm lining the cavity of the blastostyle is mostly richly ciliated with very long cilia; the cells of the "head" are large, and each contains a nucleus like that in the endoderm cells of the rest of the colony; these cells digest food material, apparently becoming amœboid at their free ends (Pl. 1, fig. 6), the blastostyles undoubtedly taking in nutritive material by their mouths.

The cells of the "neck" are very long, regular, and narrow, and there are numerous small nuclei, each containing several distinct nucleoli in this region, which is richly ciliated; egg-cells are here present in abundance (Pl. 1, fig. 6).

Below this region the body of the blastostyle dilates, and sporosacs arise; the cells here are smaller, have large nuclei, and cilia are not so apparent. Gland-cells are nowhere present in the endoderm of blastostyles. The tentacles have a solid endodermal core as in the gasterozooids. Egg-cells have been observed in the endoderm of the base of the blastostyle.

4. Endoderm of the Dactylozooid.—This endoderm is very vacuolated; the cells are approximately equal in size, but are rather smaller towards the distal extremity of the polyp. The nuclei are situated in the middle of the cells. The endoderm of the upper half of the body is often crowded with food granules, but I have never observed food masses in the lumen of the endodermal gut, nor are gland cells present.

5. Endoderm of the Tentacular Polyps.—This endoderm is also crowded with food granules throughout its extent. The cells are very regular, long, and narrow, and the lumen of the gut widens considerably towards the base of the polyp.

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EXPLANATION OF PLATE 1,

Illustrating Margaret C. Collcutt's paper "On the Structure of Hydractinia echinata."

(Several different colonies were examined, hence there is a slight variation in the size of cells, &c., figured.)

LIST OF REFERENCE LETTERS.

up. ect. Upper ectoderm. *l. ect.* Lower ectoderm. *end.* Endoderm. *mes.* Mesoglœa. *i. c.* Interstitial cell. *n.* Nematocyst. *n. c.* Nematocyst cell. *gl.* Gland-cell. *plp.* Polyp. *spn.* Spinule. *p. m.* Perisarc membrane. *c. t.* Cœnosarc tube. *p. t.* Perisarc tube. *e.* Egg-cell. *f. m.* Food material. *mo.* Mouth. *sk.* Skeleton. *v.* Vacuole. *m. t.* Muscle tail. *mes. p.* Mesoglœa process. *d. c.* Degenerating cœnosarc. *nu.* Nucleus.

Fig. 1.—Series of vertical sections illustrating the transition from the growing edge to the older parts of the colony. *a.* Section at extreme edge, showing to the left a cœnosarc tube surrounded with perisarc, and towards the right tubes which have anastomosed. *b.* Section of somewhat older part of the colony, where the perisarc membrane tapers off and disappears. *c.* Section of old part of colony passing through portion of the wall of a polyp, and showing the communication between the polyp and an endodermal tube of the basal cœnosarc. The section also shows the tabulated growth of the chitinous skeleton. D. 3 cam.

Fig. 2.—Vertical section of base of colony, showing the degeneration of cœnosarc and a degenerating mass isolated in a chitinous lacuna. D. 3 cam.

Fig. 3.—Diagrammatic section of a smooth spine at the colony edge. A. 3 cam.

Fig. 4.—Optical horizontal section of two anastomosing tubes, showing both commencing anastomosis and complete anastomosis. The left-hand tube has given off a branch. D. 3 cam.

Fig. 5.—Vertical section through a growing chambered spine illustrating the relations of the basal cœnosarc to the skeleton, and showing a chamber of the spine containing cœnosarc. D. 3 cam.

Fig. 6.—Longitudinal section of upper part of blastostyle; on the right-hand side is an egg-cell, e', in the ectoderm, and two egg-cells in the endo-

derm of the "neck." Food is being digested by the endoderm cells of the "head." D. 3 cam.

Fig. 7.—Transverse section through a blastostyle showing an egg-cell, e'', in the mesoglœa of the blastostyle, and four egg-cells in the endoderm of the sporosac. D. 3 cam.

Fig. 8.—Longitudinal section of a dactylozooid, showing food granules in the endoderm of the upper part. D. 3 cam.

Fig. 9.—Longitudinal section of the endoderm of gasterozooid. D. 3 cam.

On the Histology of the Ovary and of the Ovarian Ova in certain Marine Fishes.

By

J. T. Cunningham, M.A.Oxon.[1]

With Plates 2—4.

The Development of the Yolk.

In my paper in the 'Journal of the Marine Biological Association,' vol. iii, No. 2, p. 154, I have described the development of the yolk in the plaice and dab as commencing by the deposition of yolk granules in a thin layer near the surface of the egg, and proceeding by the continual extension of this layer towards the centre. I have also described and figured the appearance of minute refringent globules distributed sparsely throughout the protoplasm of the egg, in specimens of the sole which were examined a little time before the commencement of the spawning season. Such globules were also seen in spent ovaries of the sole, and seemed to indicate that the formation of yolk occupied more than a year in some fishes, while in the plaice and dab no sign of the development of yolk was seen until some months after the spawning season.

I have since ascertained that among fishes with pelagic ova the one type of development is characteristic of eggs which have oil globules, the other of eggs that are not provided with such elements. Certain fishes, as for example the gurnards, have an extended spawning period, and ripen their eggs not

[1] The researches described in this memoir were carried out in the service of the Marine Biological Association in 1895 and 1896.

simultaneously but in succession. In the ovary of a gurnard accordingly, at the commencement of the spawning season, ova in all stages of development are found. In figs. 1 to 5 are shown the appearance under a low power in the fresh state, of a number of eggs of the common or grey gurnard (Trigla gurnardus) at successive stages in the development of the yolk. In fig. 1 the protoplasm is transparent, and contains a small number of globules scattered singly. In the next stage a dark zone is seen commencing to form around the nucleus, and in the outer region of the egg are globules similar to those of the previous stage, but smaller and more numerous. The contrast between the two concentric layers or regions is most marked in the next stage, fig. 3, in which around the light central region indicating the position of the germinal vesicle, there is a very opaque layer sharply delineated externally from an outer more transparent layer. In the next stage, fig. 4, the contrast between the two layers has disappeared, and except for a somewhat lighter ill-defined central region, the whole is nearly uniformly opaque. Eggs which are larger than this, begin to grow transparent again, and the appearance they present is shown in fig. 5. It is easy to perceive from examination of this last stage, that the internal globules are oily, the external vitelline. As the egg approaches the ripe condition, both the oil globules and the vitelline globules begin to fuse together. The large oil globules in the interior of the egg, formed by the fusion of the smaller, are seen in fig. 5. The vitelline globules in fusing form a continuous liquid, in which the still unfused globules remain suspended. The opacity of the egg is due to the small size and great abundance of the globules at the earlier stages, and we learn, therefore, that the greater opacity of the inner layer is due to the smaller size and greater refracting power of the fat globules, which are formed close to the germinal vesicle.

Similar features have been described by Emery in the development of the ovum in Fierasfer acus. The mature ovum of this fish resembles that of the gurnard, having a homogeneous transparent yolk, and a single large oil globule.

Emery figures a portion of an immature ovary as it appears under a low power in the fresh condition. In the largest eggs, scattered, highly refringent globules, exactly like those described by me in the eggs of the sole, &c., have begun to appear, and are identified by Emery as adipose globules. Sections of later stages from material prepared with picro-sulphuric acid are figured, in which the oil globules have become larger by fusion, and the yolk globules are developing, the latter forming an external layer, the former situated near to the germinal vesicle. There is a difference, however, to be mentioned. In Fierasfer the eggs are not spherical, but oval, and the germinal vesicle is situated nearer to one pole of the oval than to the other. At the pole which is farthest from the germinal vesicle the yolk globules are formed first, and the vitelline nucleus is situated there also, so that the yolk globules are formed around it. The yolk layer during its increase continues to be thickest at the same pole.

Emery describes the course of development in the ovary which culminates in the annual spawning. He found that from the commencement of autumn to the end of spring, the ovary contained transparent eggs only, of which the largest contained a few small adipose globules, but no vitelline; in all the ova the vitelline nucleus was still visible. As the time of spawning—which extends over July, August, and September—approached, the vitellus in the larger eggs was rapidly formed. When the mature eggs had been discharged the ovary was collapsed, it showed traces of hæmorrhage in the form of extravasated blood, or masses of pigment red or yellow in colour, and besides young ova contained others which, not having reached perfect maturity in time for the spawning process, were not expelled, and were undergoing adipose degeneration.

Dr. Robert Scharff published a paper on the development of the egg in Teleosteans some years ago (this Journal, vol. xxxviii), in which he refers especially to the ovarian eggs of Trigla gurnardus. He discusses somewhat briefly the formation of the yolk spherules and oil globules. His figures

represent the appearance of eggs and sections of eggs with considerable accuracy, but he interprets them as leading to the conclusion that the vitelline elements are derived from the nucleus, a conclusion which I hold to be entirely erroneous.

I will proceed now to a detailed description of the development of the yolk in the various species which I have examined. I will deal first with—

Pelagic Eggs containing one or more Oil Globules.

In the same family of fishes, some species may be characterised by eggs with oil globules, others by eggs in which these structures are absent. In the Pleuronectidæ it is curious to note that all the left-sided species have a single oil globule in the egg, namely, the turbot and brill (Rhombus), the megrim (Lepidorhombus megastoma), and the topknot (Zeugopterus). The soles, which are right-sided, produce eggs in which there are numerous small oil globules, and in the remaining right-sided species forming the genera Pleuronectes, Hippoglossus and Hippoglossoides, the eggs are without oil globules. In the family Gadidæ a similar state of things occurs, in certain genera, e. g. Molva, the ling, an oil globule being present in the eggs, while in the genus Gadus it is absent.

In immature specimens of the brill examined during the spawning season, the scattered globules are seen in the larger eggs. These oil globules first appear in contiguity to the membrane of the germinal vesicle. Fig. 6 shows the appearance of a portion of the ovary of a specimen of this species $12\frac{3}{4}$ inches long, examined at Grimsby on May 30th, 1895. The same condition was observed in a number of specimens examined on this date; they were from $10\frac{3}{4}$ to $12\frac{3}{4}$ inches long, and captured near the German coast off the Sylt Island. A piece of one of these ovaries was preserved in a mixture of picro-sulphuric acid and spirit, and in sections of it stained with Mayer's carmalum the oil globules, or the vacuoles in the cytoplasm in which the oil was originally con-

tained, can be at once recognised, having retained their form and position.

Even in the brill the oil globules at their first appearance are not entirely confined to the neighbourhood of the germinal vesicle, some of them being scattered in the rest of the cytoplasm. In the gurnard as seen in fig. 1, they are irregularly scattered through the cytoplasm, while in the sole the greater number are situated near the surface of the ovum. For the present I will confine myself to pelagic eggs with a single oil globule, leaving the sole to be considered separately.

In the turbot in ripening ovaries examined in the fresh state the same contrast between inner and outer zone in the developing eggs is observed as in the gurnard; I believe it occurs also in the brill. The fact that the outer more transparent zone consists of yolk spherules, indicates that, as might be expected, the yolk is formed first in these cases, as in the species of Pleuronectes, at the periphery. More minute examination of the process must be made by means of prepared sections.

Different modes of preparation make great differences in the visibility of the vitelline elements in sections. Thus portions of the ovary of a brill $17\frac{1}{4}$ inches long were preserved immediately after death, at sea, on September 29th, in chromic acid $\frac{1}{4}$ per cent. This ovary is evidently in a much more advanced condition than that previously mentioned, it contains eggs which have reached a much larger size than any in the latter, and in which the yolk is considerably developed. Yet in the younger eggs, of sizes corresponding to those in the specimen previously mentioned, the oil globules are quite invisible. The chromic acid appears to have the effect of closing up the oil-containing vacuoles.

From what has been stated above concerning the ovary of Trigla gurnardus, it is evidently possible to study all stages of the development of the yolk in this species, in sections from the same ovary taken at the beginning of the spawning season. The earlier stages are better preserved in material treated with picro-sulphuric acid and spirit. I have sections from material

so prepared taken from a specimen obtained in Grimsby market on April 26th, and from portions of an ovary preserved directly after the death of the fish at sea on July 22nd. Comparison of the two shows that the material from the market specimen is in fairly good condition, the vitelline elements being well preserved. The principal defect due to the staleness of the material is the contraction of the protoplasm of the younger yolkless eggs, and the presence of a layer of granular matter around them.

The yolk globules are first recognisable in eggs ·23 mm. in diameter, and have the form of minute granules situated near the surface of the ovum. The yolk granules can be distinguished from the oil globules by the fact that they are solid. The process of preparation coagulates the yolk substance, and it retains the staining matter used to a greater or less degree, while the oil globules are not coagulated but dissolved, and the spaces they occupied appear merely as empty vacuoles. At the stage now under consideration there is a zone of such vacuoles of rather large size separated by a zone of relatively homogeneous protoplasm, both from the surface of the nucleus and from the yolk zone. This stage corresponds to that of fig. 3, among those representing the appearance in the fresh condition.

Since in earlier stages, before the appearance of yolk granules, oil globules occur quite close to the wall of the germinal vesicle, it would seem that some change has occurred which has driven them outwards. This might be explained by supposing that new protoplasm was forming in the neighbourhood of the germinal vesicle. The egg is rapidly increasing in size, and it seems probable enough that the deposit of food material is taking place at the periphery in the older protoplasm, while the growth of the protoplasm is taking place in the neighbourhood of the germinal vesicle. The zone around the latter, however, has a granular appearance, and contains I believe very numerous but extremely minute globules of oil, although they cannot be recognised with certainty as such in the sections.

It is improbable that the great opacity of the inner zone in the ova seen in the fresh condition, could be due to the somewhat large and not very numerous oil globules which are most conspicuous in the sections, and in fact the appearance in the fresh condition indicates very numerous minute globules. Fig. 7 represents the appearance of an egg in the stage here described. The preparation from which it was taken was made from portion of an ovary of Trigla gurnardus, obtained in the market on April 26th. The material was preserved with a mixture of picro-sulphuric acid and spirit, and the sections were stained with Delafield's hæmatoxylin. Sections from material similarly treated, preserved at sea immediately after the death of the fish on July 22nd, contain eggs in a similar stage, and show in them the same structure, but for some reason or other the outline of such eggs in these sections is distorted, and I have therefore preferred to draw from the others. In the sections from the fresh material the inner zone of protoplasm shows more distinctly the minute cavities which I believe to be the smallest oil globules. In sections from some of the same material preserved in chromic acid $\frac{1}{4}$ per cent., the structure at this stage is not so well shown. The yolk and oil globules are less distinct, and there is a broader internal zone of more homogeneous protoplasm with a rather distinct boundary just within the zone of larger oil globules.

In the later stages, i. e. in larger eggs as seen in the sections, the zone of yolk globules has increased in thickness and extends to the zone of oil globules. Fig. 8 shows the appearance of a section of an egg ·37 mm. in diameter, preserved immediately after death in picro-sulphuric acid and spirit. The yolk globules themselves are now larger, and under a high power are seen to be solid, i. e. coagulated spheres, often with granules in their interior; usually some slight space is seen between the outline of the globule and that of the vacuole which it occupies. The yolk globules are stained a light yellow by the picric acid, but do not usually take much colour from the staining reagents used. The outer oil globules are

considerably larger than the yolk globules, and are seen as empty vacuoles in the protoplasm; they are not very numerous, forming an irregular ring, and within them is a protoplasmic zone for the most part destitute of yolk globules, and containing the minute vacuoles which probably were occupied by small oil globules in the fresh condition. Here and there, however, scattered yolk globules are seen between the larger oil vacuoles, and in the inner zone of protoplasm.

I have found that the reason why it is difficult to recognise the smaller oil globules in the prepared sections, is because the oil is entirely dissolved and removed in the process of preparation. The reagents used for removing the paraffin from the sections, and for clearing and mounting, namely, benzole or turpentine, necessarily remove the oil originally contained in the eggs themselves. When a portion of an ovary is preserved with osmic acid, either alone or in combination with other reagents, the oil in the eggs is blackened. I found that some of the blackened oil remained in the sections after the processes of dehydration, soaking in benzole, imbedding, cutting, and dissolving the paraffin from the cut sections on the slide, but by the time the sections were mounted with Canada balsam all trace of the blackened oil had disappeared. By washing the sections with alcohol after the paraffin had been removed, and then mounting them in glycerine, a portion of the blackened oil was kept permanently in *situ*. In order to preserve it completely it would be necessary to cut the sections by an aqueous method, e.g. by the freezing method, and mount them in glycerine, without dehydrating them at any stage.

The thickness of the zone of yolk spherules continues to increase until it includes the whole of the egg except the germinal vesicle, and a small number of large oil vacuoles, into which the numerous small oil globules of former stages have united. These vacuoles are situated close to the surface of the germinal vesicle. The condition here described is illustrated in fig. 9, which is taken from a preparation made from material preserved at sea on July 22nd in chromic $\frac{1}{4}$ per

cent., the sections being stained with safranin. The longer diameter of the egg represented is ·53 mm.

My sections from the gurnard do not show any later stages of the egg in which the changes immediately preceding the ripe condition are taking place, namely, the fusion of the yolk globules, and the passage of the nuclear elements to the periphery. I will pass on now, therefore, to the consideration of eggs of similar kind in other species.

The eggs of the turbot and brill are so similar that they may be described together. They differ from those of the gurnard in three particulars: (1) their smaller size; (2) the greater uniformity of condition among the eggs in a single fish; (3) the less conspicuous appearance of the oil vacuoles in prepared sections.

I have prepared sections from a portion of the ovary of a turbot, 1 foot $9\frac{1}{4}$ inches long, which was obtained from Grimsby market on April 12th, 1895. The material was preserved with picro-sulphuric acid. The condition of the yolked ova in these sections is good, the protoplasmic ova show the contracted condition usually seen in sections from market material. When fresh this ovary was found to contain opaque white eggs advanced in development, but none in the ripe transparent condition. The contrast between the internal and external zone was not visible, the stage of development being too advanced.

Figs. 10, 11, 12 represent eggs in three different stages as they appear in these sections. The great majority are in the condition seen in fig. 12, in which the extra-nuclear region is everywhere crowded with round yolk globules, excepting the space occupied by the oil vacuoles near the nucleus. The egg section drawn is ·35 mm. in diameter. This is the stage a little before the fusion of the yolk globules commences. A proportion of younger eggs in successive stages are present in the sections. In the stage represented by fig. 11, the large size of the yolk globules is remarkable. This stage in the turbot corresponds with that of fig. 8 in the gurnard, and it will be seen that in the turbot the yolk globules are larger and

less numerous, and the oil globules in the zone next to the nucleus much smaller and less conspicuous. Fig. 10 represents the stage at which the formation of yolk granules at the periphery is just commencing, small oil globules being visible in the inner zone; eggs in this stage are comparatively few in number. There are, as in all stages of the ovary, a certain number of yolkless ova.

Sections from the ovary of a specimen $19\frac{3}{4}$ inches long, obtained in Grimsby market on April 24th, show a less advanced condition of maturation. The majority of the ova are in a stage a little earlier than that of fig. 12, the oil vacuoles being smaller and more numerous.

I have sections from another stage in the history of the turbot's ovary, the material having been taken from a fish 2 feet $1\frac{1}{2}$ inches long, on July 23rd, and preserved in picrosulphuric acid and spirit at sea immediately after death. The preservation is not quite satisfactory, but the sections show many points worthy of mention. With the exception of a small number in young stages, all the eggs are in two stages, namely, ripe, with the yolk in a continuous mass surrounded by a thin envelope of protoplasm, and nearly ripe, with the yolk spheres still separate. There are scarcely any intermediate stages between these two; but in the nearly ripe condition the protoplasmic strands separating the yolk globules are not visible toward the centre of the egg. It is evident, therefore, that the fusion of the yolk spherules takes place almost simultaneously. The nearly ripe eggs in these sections are nearly all ruptured on one side, the zona radiata being much thickened on the opposite side, and the contents partially escaping. This may be attributed to the effect of the picro-sulphuric acid, but whether its action has been to contract the zona radiata, or to burst the latter by swelling the yolk, I cannot say. Curiously enough the ripe eggs are not burst, but in them the zona radiata is thinner, and the diameter of the egg larger. It is evident that the yolk swells considerably during the final stage of maturation.

The sections of the ripe eggs exhibit an internal homo-

geneous mass of transparent almost unstained yolk surrounded by a thin envelope of stained protoplasm. The latter contains numerous vacuoles, and its inner surface is very uneven; but I have failed to detect in it any structures representing the nucleus of the ovum, which ought to be present in it somewhere, and which is distinct enough in the nearly ripe eggs in the same sections. The oil globules, one or more in number, are generally visible just within the protoplasmic envelope.

Sections of a portion of the ovary of a brill preserved in chromic acid $\frac{1}{4}$ per cent., on September 29th, show an early stage in the development of the eggs for the next spawning season. The largest eggs are ·3 mm. in diameter, and are in a condition somewhat more advanced than that of the turbot's egg represented in fig. 10, which is only ·17 mm., and similar to that of the gurnard's egg represented in fig. 7. There is in these eggs a broad inner layer of rather large oil globules, and a narrower external zone containing minute yolk granules.

Although the eggs of the mackerel, like those previously considered, have a single oil globule and homogeneous yolk, I have not seen any indication in them of the presence of scattered small oil globules before the commencement of yolk formation. I examined several mackerel 12 to 13 inches in length at Lowestoft, on October 8th, and although they were evidently mature fish which had spawned the previous season (May and June), under the microscope all the eggs were yolkless, and quite transparent, without any refringent globules.

On April 27th, 1896, I examined the ovary of a large mackerel in London. This ovary was much enlarged and approaching the ripe condition, but had not begun to discharge its ova. Microscopic examination in the fresh condition showed that the majority of the eggs were much advanced in development. They were full of spherical yolk globules, and in the central region could be seen in many the oil of the egg already fused into one large globule. In younger and smaller eggs, a central more opaque zone contrasted with an outer more translucent, as in the eggs of the gurnard, but there were no transparent eggs with separate scattered oil

globules. The earliest stages seen were nearly transparent, with minute globules at the periphery, and also minute globules not much more opaque around the nucleus. I concluded that the latter consisted of oil, and that in the mackerel the deposition of oily substance and yolk commences in these different regions of the egg simultaneously, and does not take place in any eggs until some time after the previous spawning season. In the eggs of the sole, I have not observed at any stage the marked contrast in the fresh condition between the darker and lighter zones which is seen in the eggs of the gurnard and other species. In immature and spent specimens a considerable proportion of the transparent young eggs contain numerous scattered globules which are certainly oil globules. They have a yellowish colour, and most of them are situated near the periphery of the egg, forming a somewhat well-marked layer. They can be seen even in the earliest stages in prepared sections, but are much more conspicuous in the fresh condition.

The yolk proper begins to be developed after the spawning season. In a specimen examined at Lowestoft, on September 20th, it had made considerable progress. It begins as usual at the periphery of the egg outside the oil globules, and the latter in portions of the ovary examined in the fresh state remain in all stages visible through the yolk, and are conspicuous on account of their somewhat large size, refringent character, and yellowish colour. Fig. 13, Pl. 2, shows the condition of the most advanced eggs, as seen in prepared sections, in the ovary of the specimen mentioned above, which was 16 inches long, and was obtained in the market. The external portion of the ovum contains yolk in the form of minute granules; on the inner side of the zone of yolk are the oil globules in groups projecting into the protoplasmic zone, which is rather deeply stained.

In the nearly ripe egg of the sole as seen in sections prepared from an ovary which was in process of spawning, oil globules are not easy to distinguish. In the figure of such an egg which I have given in my Treatise on the Sole, they have

been overlooked altogether. They are separate small vacuoles distributed singly in the yolk. The yolk itself consists throughout of rather large spherical globules, which in my sections contain in their interior refringent granules.

Pelagic Eggs containing no Oil Globules.

In figs. 14 and 15 are represented two stages in the development of the yolk as seen in sections prepared from the ovary of a large plaice taken from the aquarium, and killed on August 25th. The zone in which the yolk is already developed is much more sharply defined from the inner protoplasmic zone than in eggs which contain oil globules. The yolk layer continues to become thicker in proportion to the layer of cytoplasm internal to it, until it reaches the surface of the germinal vesicle. The formation of yolk globules commences at the periphery of the cytoplasm, and in consequence of the absence of oil globules, when eggs of this kind are examined in the fresh state, no dark inner zone is seen. The eggs become more and more opaque, but their opacity is nearly uniform, except that the centre is usually more transparent in consequence of the presence of the germinal vesicle. In the ovaries of plaice and other fish, whose eggs do not contain oil globules, transparent ova containing scattered refringent globules are never seen. In the immature fish all the ova are perfectly transparent, with the exception of the occasional aborted ova described in a subsequent part of this paper. In the spent ovary, too, all the ova are transparent, except those whose maturation has been arrested, and which are dead and in process of absorption.

Structure of the Spent Ovary.

A spent ovary, that is one from which the annual crop of ripe eggs has just been extruded, may be recognised from several symptoms, of which the most certain is the presence of a few ripe eggs detached from the walls of the ovary, but still remaining in the cavity. When a portion of the germinal tissue of an ovary in this condition is microscopically examined

in the fresh state, the most conspicuous characteristic is seen to consist in a variable number of opaque, granular bodies of irregular shape and structure. Investigation has proved that these are eggs which have not fully matured, which have died *in situ*, and which are not discharged from the ovary by the bursting of their follicles, but are removed by absorption. In the fresh material, when a number of fish are examined from time to time, various stages in the disintegration of these eggs are seen, from the condition in which the original structure is but slightly altered, and which can only be distinguished from that of the healthy eggs by the great opacity, to a condition in which nothing is left but a small mass of opaque granules. The appearance of two such dead yolked eggs as seen in the spent ovary of a sole is shown in fig. 16.

The presence of dead aborted eggs is not, however, the most essential characteristic of ovaries which are spent. Such eggs occur, as will be explained below, in other stages of the ovary. Aborted eggs are absorbed *in situ*, but healthy ripe eggs escape from the ovary by the bursting of their follicles, and only ripe eggs escape in this way. The essential characteristic of spent ovaries is, therefore, the presence of empty collapsed follicles from which the eggs have escaped. So far as my observations go, these empty follicles are not to be detected by microscopic examination of fresh material; in this all conditions and stages of the eggs are easily seen, but the condition of any part belonging to the connective tissue of the ovary is very difficult to distinguish. In mounted sections, however, the history of the follicle, after the escape of the egg, can be studied with some success.

In the fish ovary the follicle, after the escape of the matured egg, passes through changes similar to those which are known to occur in the so-called corpora lutea of the mammalian ovary. In the fish ovary the degenerating follicle is always found in connection with the superficial membrane of the germinal lamellæ. When the egg escapes, the interior of the follicle opens on to the surface of the ovarian lamella, and the wall of the follicle is thus restored to the condition from

which it started when the egg first began to develop—namely, that of a portion of the superficial tissue of the germinal fold or lamella.

The opening of the follicle, however, soon closes up, and the whole cavity disappears by the contraction of the walls. In follicles from which the eggs have only recently escaped, a somewhat indefinite cellular tissue is seen, containing numerous round nuclei. The appearance of the collapsed follicles in a newly-spent plaice, as seen under a low power, is shown in fig. 17, Pl. 3, while fig. 18 shows a single follicle of the ovary of the grey gurnard more highly magnified. The internal cellular tissue is detached from the inner surface of the wall of the follicle, and presents a different appearance from that of the wall itself. I think there can be little doubt that this tissue is the remains of the follicular epithelium. I have not observed any indication in later stages of hypertrophy or proliferation of this epithelium—in fact, it soon ceases to be distinguishable. It has recently been maintained by J. Sobotta that the corpus luteum of the mammalian ovary is produced chiefly by the hypertrophy of the follicular epithelium, but this conclusion, if correct, does not appear to me to apply to the Teleostean ovary.

The wall of the empty follicle has the same structure as the outer layer of the stroma of the ovary, of which it forms part. It consists of connective tissue, somewhat fibrous in appearance, containing numerous nuclei, and furnished with blood-vessels. In its final stages, the follicle forms merely a globular projection inwards from the surface membrane of the ovarian tissue. At this stage it is a solid mass, consisting of fibro-cellular connective tissue, quite similar to that which forms the wall of the follicle at the earlier stage. I have not observed anything in its structure at the later stages which appears to be derived from the follicular epithelium.

The absorption of the empty follicles proceeds rather rapidly, and all trace of them has disappeared in ovaries which have begun to mature the ova for the next season. On July 15th I killed at Plymouth a plaice 16 inches long, of which the

ovary appeared from its size and flaccid condition to have previously spawned. The tissue was opaque white, and the microscope showed it to contain numbers of partially yolked eggs, the largest of which was ·5 mm. in diameter. In sections prepared from this ovary no trace of degenerating follicles can be seen. They are also absent in sections from an ovary of a large plaice killed on August 25th, which was known to have spawned the previous season, having been kept under observation in the aquarium.

A female flounder which spawned in March in the aquarium was killed on July 11th. The ovary contained only transparent yolkless ova—that is to say, the formation of yolk in the eggs to be shed in the following spawning season had not commenced. But in prepared sections a few remnants of the degenerating follicles from the previous spawning could be discovered. It may be concluded, therefore, that in plaice and flounder the "corpora lutea" are entirely absorbed in about three or four months.

In preparations from portions of the ovary of a spent sole, preserved on board a fishing vessel fifty miles eastward of the Humber, on July 24th, the degenerating follicles are numerous and distinct. They are, however, small and solid, the cavity having been obliterated. This ovary contains only a comparatively small number of yolkless ova, the production of new eggs having not yet taken place to any considerable extent. The spawning of soles in the North Sea takes place chiefly in May and June. In preparations from a sole taken off Lowestoft, on September 24th, the degenerating follicles cannot be detected, but there are distinct though small remnants of them in sections from a specimen obtained on September 20th.

In a haddock 19 inches long, portions of whose ovary were preserved at sea immediately after death on July 23rd, the empty follicles had been entirely absorbed. The haddock spawns in the North Sea principally in March, and there are no immature specimens over 16 inches long.

Reabsorption of Aborted Eggs in Spent Ovaries.

I will proceed now to the consideration of the opaque granular masses which are so frequently seen in the fresh germinal tissue of fishes' ovaries. As was previously stated, these masses are eggs in which the development of yolk has proceeded to some stage, and which have then died, and are in process of reabsorption. On August 1st, 1895, I made a careful examination of the ovary of a sole 17 inches long, obtained in Grimsby market, A sole of this size is invariably mature, and it may be presumed, therefore, that this specimen had spawned in the preceding spawning season—that is, about May or June. The roe was thin and narrow, though of considerable length, and when cut open presented the appearance suggestive of the spent condition. It was red and congested, and here and there were opaque yellow spots, which were evidently dead yolked eggs. Microscopic examination of a portion of the fresh tissue showed that it consisted chiefly of transparent eggs, the larger of which were ·16 mm. in diameter, and contained scattered minute oil-globules. There were no eggs containing yolk, except those which were evidently dead and had been left behind in an incomplete state of maturation when the spawning process was over. Fig. 16 represents the appearance of a portion of the tissue under a low power. The dead yolked eggs were from ·29 to ·33 mm. in diameter, and were in various stages of yolk development, the largest being quite opaque. As seen in the figure, they had shrunken away from the walls of their follicles very considerably.

I have not been able to make a complete histological study in prepared sections of the history of these dead eggs and the process of their absorption. I can only describe briefly the condition in which I find them in a few specimens of different species. The appearance they present in sections differs very much according to the stage at which their development has been arrested. In cases where they have ceased to develop at

an early stage they are much less conspicuous in sections than they are in the fresh condition. Among my preparations they are most conspicuous in sections from a specimen of Trigla hirundo caught on September 24th; the portion of the ovary from which the sections were made was preserved in chromic acid ¼ per cent., at sea, immediately after death. In these sections the dead eggs are very numerous, and contain a large quantity of yolk. Each consists of a mass of yolk globules of various sizes, contained in a follicle; between the yolk globules are nuclei and cells, the latter not distinctly defined. The process that is taking place in these dead eggs is evidently closely similar to that which I have described as occurring in an empty follicle. A proliferation of cells has taken place from the walls of the follicle towards the interior, the cells penetrating into the interior of the mass of yolk, and doubtless effecting its absorption. The question arises whether these cells are derived from the follicular epithelium or from the connective tissue of the wall of the follicle, and I consider the latter alternative is probably correct. In these sections none of the diminishing empty follicles are to be seen, and therefore I am not sure that the specimen was normal; possibly for some reason or other the discharge of ripe eggs had not taken place, and they were all being reabsorbed in situ.

In sections from spent ovaries of the plaice preserved in February, as in portions of the same examined in the fresh condition, small ova in which the formation of yolk has commenced are to be seen. It is known from the condition of the ovary of the fish in later months that such eggs die and are absorbed. But I have no preparations showing stages of the absorption in the spent ovary in this species.

In preparations from part of the ovary of a weever (Trachinus draco), preserved immediately after death at sea on September 25th, both empty follicles and aborted eggs can be seen in process of absorption. The empty follicles are much reduced, and small, and consist of thick-walled capsules full of cellular tissue. Here and there among the rather small ova at the surface of the ovarian lamellæ is seen

one which is undergoing retrogressive change. Neither in these nor in the neighbouring normal ova can yolk granules be distinctly recognised, but there are a few vacuoles which probably represent small oil globules. The chief abnormality in the degenerating ova is the condition of the germinal vesicle, which consists of one large nucleolar mass; the rest of the vesicle, membrane and clear contents, having disappeared almost entirely. In the central part of the ovarian lamellæ were distinct masses of irregular shape and yellowish colour, which had not taken the stain of the hæmatoxylin. These consisted of yolk-like substance, with nuclei scattered through it. Each was surrounded by connective-tissue fibres, but not by a distinct follicular wall. These masses are the remains of eggs which had reached an advanced stage of maturation when spawning took place, and which have been in great part reabsorbed. It is evident, therefore, that in the spent ovary, eggs in various degrees of development die, and are absorbed.

On September 17th I examined, in the fresh condition, an ovary of the greater weever in which spawning had more recently occurred, and in which, therefore, the reabsorption of abortive ova had scarcely begun. Its condition supports the interpretation given above. It contained (1) perfectly transparent yolkless ova, (2) slightly opaque, apparently healthy eggs containing a zone of granules, (3) dark yellowish granular masses, without refringent globules, evidently dead eggs which had not been ripe when spawning was completed, and (4) large rounded masses, quite opaque, and containing large oil globules; these were evidently eggs which had nearly reached the ripe condition before they died.

Reabsorption of Aborted Eggs in Immature Ovaries.

I at first supposed that the opaque granular masses, identified as partially matured but aborted ova, only occurred in spent ovaries. But it was afterwards found that they occur

very commonly, if not always, in immature ovaries. I have seen them in abundance in numbers of specimens of plaice examined in November and December, in which no healthy yolked eggs were to be seen; and at that time of the year the ovaries of all mature specimens are full of eggs in which the development of yolk is far advanced. At the same time none of the plaice have begun to spawn, and no spent fish are to be found. The specimens, therefore, whose ovaries have not begun to mature in those two months, could not spawn until the next season, and are therefore immature.

I have prepared sections from an ovary with dead yolked eggs belonging to a plaice obtained in Grimsby market on August 3rd, 1895. The specimen was $11\frac{7}{8}$ inches long, and was caught on grounds not far from the Humber. As no specimens less than 13 inches long from that part of the North Sea have been found to be mature, it may be concluded that this specimen had never spawned. In the sections, eggs in three different conditions are seen. (1) There are small eggs of healthy appearance, entirely protoplasmic, and, as is usual with such young eggs, deeply stained with hæmatoxylin. These are from ·04 to ·11 mm. in diameter, and have a normal and healthy structure. The germinal vesicle in them has a regular definite circular outline with nucleoli arranged round the periphery. These young ova are not very numerous; they are distributed singly just within the surface of the ovarian lamellæ. (2) There are next a number of larger eggs much less deeply stained, and having a granular appearance; the largest of these are about ·18 mm. in diameter. There can be no doubt that these are the largest transparent eggs seen in the fresh condition of the same material. No yolk was visible in the fresh condition, and none can be seen in the sections. But the granular condition of the protoplasm, especially at the periphery, indicates that these eggs have reached that stage of maturation at which the deposition of yolk is about to commence. The material was obtained from the market, and therefore not preserved till some time after the death of the fish. This doubtless accounts for the fact that in many of

the eggs of this kind the protoplasm has separated from the germinal vesicle, leaving a space containing scattered granules, and in some cases the germinal vesicle is absent altogether, having been washed away in the process of preparation. But where it is present it shows the usual structure, having a distinct membrane, on the inner side of which are the nucleoli. In many of these eggs the vitelline nucleus is very distinct, situated at the periphery of the protoplasm.

The remaining eggs are in a remarkable condition, which is illustrated in fig. 19. The shape is variable. The remains of the egg are seen forming a shrunken dense mass within the follicle. The mass is more deeply stained than the eggs in the condition previously described, and less granular in appearance. Within the mass is a more translucent area containing a single nucleolus. The nucleoli of the healthy condition have apparently fused together, and the germinal vesicle has degenerated. On the outside of the mass can be seen a shrunken and crumpled membrane, evidently the membrane of the ovum which had begun to form at the time when the maturation was arrested. Within the wall of the follicle is seen a distinct granular lining with nuclei here and there, but no definite cell outlines. This I take to be the follicular epithelium in a degenerating condition, although it is thicker and more distinct than in either of the conditions previously described. In the walls of the follicle are seen fibres of connective tissue and nuclei. There can be no doubt that these structures are the opaque dead eggs, or "masses" seen in examination of the fresh material, and it is evident that the egg having reached the stage of maturation at which yolk formation commences, has died, and forms within its follicle a contracted opaque mass which is undergoing a process of absorption.

That the condition of the germinal vesicle seen in these aborted eggs in prepared sections is not artificially produced by the process of preparation is proved by the fact that the same condition has been observed in aborted eggs in the fresh state. In fig. 20, Pl. 3, is shown the appearance presented

by an aborted egg in the ovary of a plaice 12¼ inches long, examined at Lowestoft on September 21st, 1895. The egg has evidently died at a very early stage, when scarcely any yolk had been deposited, and consequently it is not very opaque. It appears also to have only recently died, and therefore to have undergone but little of the process of retrogressive change. The nucleus, it will be seen, shows an irregular indistinct outline, and a single nucleolus towards the centre. The appearance of this aborted egg presents a marked contrast with that of the three normal younger eggs figured with it.

The History of the Germinal Vesicle and Yolk Nucleus.

To give a complete history of these structures and their relations to one another, it would be necessary to follow continuously the history of the egg from its origin in the germinal epithelium onwards. But unfortunately it is very difficult to obtain a distinct and definite view of the parts of the young ova in their earliest stages, in consequence of their small size and the indefiniteness of the limits between different eggs and different parts of each egg. I have endeavoured to trace the origin of the ova in the germinal epithelium, both in the ovaries of very young immature fish and in the spent ovaries of mature fish, at the stage in which the new crop of ova is beginning its development. Up to the present I have not succeeded in tracing satisfactory indications of the division of the germ-cells by which the ova must be produced, nor have I been able to detect the yolk nucleus in the very young ova still within the germinal epithelium, or but recently separated from it.

If indirect or mitotic division of the nucleus were universal, or if it occurred at least in all undifferentiated rapidly multiplying cells, it ought to be the mode in which the germ-cells divide in the germinal epithelium. We know that it occurs and is obvious and conspicuous enough in the division of

spermatocytes. The question of the occurrence of direct or amitotic division in the multiplication of germ-cells is discussed by O. vom Rath, in a paper published in 1894 (**13**). He states that many authors consider that this mode of division occurs in genital cells. He points out that a decision of the controversy is of great importance, since all modern views on the nature of fertilisation and heredity are based on the assumption of the continuous succession of mitotic or indirect divisions in the history of genital cells. He argues that if amitotic division did take place, an exact division of the chromatin between the two daughter-cells would be impossible, since the division of the threads does not occur in that process as it does in mitosis. Nevertheless, vom Rath has himself seen phases of amitotic division in genital cells in the ovary of the Salamander, especially in very young females, together with stages of regular mitosis; in his figure, however, he shows none of the latter. He considers that all the ova or germ-cells which undergo amitotic division are abortive, are undergoing retrogressive changes, and are about to be absorbed. In the ovaries of older specimens of Amphibia, amitosis is more seldom seen, but degeneration of ova without amitosis is common. Vom Rath considers that the amitoses observed by other investigators in generative organs were in many cases divisions of follicular cells, which come to an end of their history when the genital cells are ripe or are absorbed, and not of true germ-cells destined to become ova or spermatozoa. In fact, he maintains the general theory that amitosis only occurs in cells which are approaching the termination of their capacity for division, and that when amitosis of the nucleus once occurs none of its descendants ever again pass through the processes of mitosis, but soon cease to divide at all, and ultimately die. Mitosis is, according to this view, essentially connected with the continuity and persistence of cell life.

The youngest stage of the Teleostean ovary which I have examined is that of a plaice 3 inches long. The specimen was killed in March, and must have been hatched in the previous

year. The ovary was preserved with a mixture of chromic acid $\frac{1}{10}$ per cent., and osmic acid $\frac{1}{10}$ per cent. The eggs in the sections from this ovary measure from ·01 to ·06 mm. in diameter. In none of these eggs have I been able to distinguish the yolk nucleus with certainty. All of the eggs mentioned have passed the stage of subdivision, they are no longer multiplying germ cells, but definite eggs in the stage of maturation. The germ cells and germinal epithelium in these sections are too much shrunken by the preserving reagents, and too little differentiated by the staining to be studied.

The nucleus of the smallest eggs exhibits a single rather large nucleolus 2·5 μ in diameter in the central part, though not exactly at the centre, and some smaller, not very distinct nucleoli which appear to be thickenings of the nuclear membrane. In the larger eggs there are several nucleoli of very different sizes; usually one is very large, reaching 7·5 μ, or even ·01 mm. in diameter, while the rest are much smaller. All the nucleoli are situated close to the nuclear membrane, and they are usually spherical in shape, but occasionally they are hemispherical, the flat side being in contact with the nuclear membrane. It is possible that the large nucleolus divides, but I have only once seen a condition which could be considered a stage of division, namely, two nucleoli of equal size almost in contact. Occasionally in these, as in other sections, a nucleolus is seen outside the nuclear membrane, but whenever I have seen this I have seen evidence that the external nucleoli had been removed from their proper position mechanically in the process of preparation. The central space of the nucleus is occupied with a network of fine threads, according to the prevalent view representing the chromatin, the nucleoli being composed of a material somewhat different in properties.

The sections I have next to describe were prepared from the ovary of a plaice $7\frac{1}{2}$ inches long, killed in March. The tissue was fixed in a mixture of chromic and osmic acid, each to the strength of $\frac{1}{10}$ per cent., the tissue remaining in this mixture

for about fifteen hours. The largest eggs in these sections are ·15 mm. in diameter. In these the vitelline nucleus is distinct. The form of the egg is well preserved. The cytoplasm is very homogeneous in appearance, and exhibits no trace of yolk or of granular texture. The vitelline nucleus is situated close to the periphery of the egg, and consists of a rounded stained mass containing vacuoles in its substance. Its limit from the cytoplasm is not perfectly definite, as it is evidently continuous with the substance of the latter. The germinal vesicle is somewhat excentric in position in the egg, has a distinct membrane, and a number of small nucleoli arranged at nearly equal intervals inside the membrane. The reticulum of the internal cavity of the nucleus is extremely fine, and takes stains very slightly. The smallest egg in which I have seen the vitelline nucleus in these sections is ·08 mm. in diameter, and in this case it is also situated at some distance from the germinal vesicle. I have not been able to make it out in any egg in contact with the membrane of the germinal vesicle, although it is seen in that position, in what is evidently its earlier stage, in fresh material treated with acetic acid on the slide. I am obliged to conclude, that although the preservation of the material in the sections above described appears to be excellent, yet the mixture of chromic and osmic acid mentioned is not capable of differentiating the vitelline nucleus in the earlier stages of its history, and of demonstrating its origin. In these sections, as in those previously mentioned, the germinal epithelium is thin, flat, and indistinct, so that it is vain to attempt to trace the origin of the ova from it.

In the ovary of a mature flounder which had spawned in March, and which was killed on July 11th, it is evident from the sections that the production of the new crop of ova from the germinal epithelium was going on. At numerous points in the epithelium are seen groups of germ-cells, consisting of two or more, each having an oval shape in section, and containing a large nucleus, which in structure is similar to that of the smallest eggs described in the ovary of the plaice 3 inches

long. These germ-cells are about 8 μ in their larger diameter, but some of them are somewhat larger. Around them can be seen the small nuclei of the ordinary non-germinal epithelial cells, which in most cases extend over the surface of the groups of germ-cells. The epithelium in the intervals between the spots where germ-cells are seen appears to be only one cell thick, and the cells composing it are extremely small. They are not, however, so flat and thin as they are in other conditions of the ovary, but show some thickness in section.

Some of the sections now under consideration are from material fixed with corrosive sublimate and acetic, some from material fixed with the strong mixture of Flemming. In none can I distinguish actual figures of mitotic division in the germ-cells, although I am strongly inclined to hold that vom Rath's view is correct, that the germ-cells always divide by typical mitosis. I have seen division figures in these cells in the germinal epithelium of Myxine and Conger. The preparations of the generative organ of Myxine to which I refer were obtained from very young specimens, in which the organ was a very thin narrow fold. It contained anteriorly minute eggs, and in the posterior part small testicular capsules. The material was fixed in Flemming's mixture, the sections stained with hæmatoxylin. The germinal epithelium in these Myxine sections is limited to the extreme edge of the genital lamina, and in section is somewhat crescentic. It is several cells deep, but the cells are irregularly arranged, and are polygonal in shape, not flattened. The germ-cells in the resting state are about 13 μ in diameter, the greater part of which is taken up by the nucleus; the interstitial cells are somewhat smaller.

No investigator appears yet to have traced out completely the division of the germ-cells in the germinal epithelium of the vertebrate ovary, and their conversion into definite ova surrounded with follicle cells. Vom Rath figures only two sections, one of the ovary of a young female Salamander, one of an undifferentiated reproductive ridge of the same animal. In the former the structure represented seems to me to corre-

spond to the stroma of the ovary containing definite ova, and not to include the germinal epithelium properly so-called.

G. Born (7) mentions briefly that in the ovary of Triton tæniatus, between the larger eggs are primitive ova, or nests of them; that he often saw in these primitive ova stages of mitosis, and that he also saw lying in contact with the resting nuclei of primitive ova a mass of finely granular protoplasm containing some more distinct granules. But he gives no further consideration to these points, his paper being devoted to the later history of the ovum.

R. Fick (10) and Rückert (8) also confine themselves to the discussion of the history of the definite ovum after it has left the germinal epithelium and ceased to divide.

I have not been able to trace the centrosome in the primitive ova still within the germinal epithelium, nor have other observers described it in this stage in Teleosteans. It is still a disputed point whether the centrosome is a permanent organ of the cell, that is to say whether it is always present in the cytoplasm throughout all the successive phases of cell-life, dividing before the nucleus when division takes place. The majority of authors believe in the persistence of the centrosome, and hold that it is always outside the nucleus. But O. Hertwig maintains that this is true only in certain cases, and that usually after mitosis the centrosome passes into the interior of the nucleus, and only emerges again into the protoplasm when the cell is preparing to divide again.

Beneath the epithelium in the ovarian lamellæ in these sections is a layer of young ova, all destitute of yolk, of various sizes, the largest being ·14 mm. in diameter. In the larger of these the vitelline nucleus is very definite and conspicuous, forming a rounded or elliptical body, somewhat deeply stained and having a granular appearance. Sometimes the granular appearance is seen to be due to vacuoles, and occasionally I have fancied I saw a minute corpuscle in the centre of the mass. As usual, the edges of the body are not sharply defined, but are seen to be connected with the strands of the surrounding cytoplasm. In the smaller eggs there is

no body separate from the germinal vesicle, but there is frequently to be detected a cap of differentiated cytoplasm applied to the external surface of the membrane of the germinal vesicle. There can be no doubt that this cap of cytoplasm, which is granular and deeply stained, is the earlier condition of the vitelline nucleus. The two stages described are represented in fig. 21, as they are seen in two contiguous ova in one of the sections. The smaller ovum is ·037 mm. in diameter, the larger ·098 mm.

In a previous paper (**20**) I have briefly described the relations of the vitelline nucleus in the ova of the common pipe-fish, Syngnathus acus, so far as they can be ascertained by the examination of fresh material. Although I have not been able to trace the history of the ova in this form completely, I am able, after examining several ovaries by means of sections, to confirm and extend the results previously recorded. The ovary of Syngnathus is a cylindrical tube of narrow diameter, and its structure is remarkable on account of the narrow limits to which the proliferating germinal epithelium is confined.

There is but one germinal lamina which extends along the ovarian tube lengthwise, and germ-cells are present only at the extreme edge of this lamina. The ova, when separated from the germinal epithelium, pass in succession towards the base of the lamina, and then into the wall of the ovary as they grow larger. At least, it is certain that the largest and most advanced eggs are found in the wall of the ovary, and that the free projecting lamina contains a row of eggs diminishing in size, and descending in degree of maturation towards the extreme edge. The arrangement may be partly or wholly due to the growth of the germinal lamina outwards, and not to the passing of the ova inwards, as the fact that the oldest parts of a herbaceous plant are nearest to the root is due to the fact that growth takes place at the apex. I have not attempted hitherto to determine positively the mode of growth in the ovary, but the resulting arrangement is that represented by the section of the germinal lamina shown in fig. 22. The section from which the figure was taken was prepared from

material preserved in a mixture containing $\frac{1}{10}$ per cent. chromic acid and 40 per cent. picric acid. The condition of the yolk in the three largest eggs is the effect of the picric acid, which destroys the original form of the yolk globules, and in sections prepared with this reagent the vitellus usually exhibits the laminated appearance shown in the figure. But it generally happens that when the yolk in the larger eggs is well preserved, the young protoplasmic eggs are shrunken and distorted, and vice versâ. In one of the younger eggs there are seen two very distinct vitelline nuclei or corpuscles. The sections were treated with the triple stain recommended by Flemming—gentian violet, safranin, and orange G,—and the vitelline nuclei are brightly coloured with the safranin, while the remaining parts show only a light tinge derived from the orange. The vitelline nuclei are round, and differ from those seen in the ova of Pleuronectes in their sharpness of contour. Their outline is very definite, and appears to have no connection with the surrounding cytoplasm.

The ovum in which the two vitelline nuclei are seen is ·16 mm. in diameter. Although the presence of two of these bodies in one ovum is not uncommon, it is more usual to find only one, and there can be little doubt that the presence of two is due to the division of a single one. In the examination of fresh material, treated with acetic acid, I have sometimes seen three, and even four of these bodies; but when there are several they are proportionally smaller than when only one is present. The earliest condition in which I have been able to demonstrate the structure is, as in other cases, that in which it is in contact with the outer surface of the membrane of the germinal vesicle. Fig. 23 shows the smallest ovum in the section represented in fig. 22 more highly magnified. The greatest diameter of this ovum is ·049 mm. The vitelline nucleus is seen as a little, hemispherical, highly-stained body attached to the membrane of the germinal vesicle; around it is a region of denser cytoplasm. It may be remarked that even if it should prove that the vitelline nucleus is derived from the germinal vesicle, it is evidently not of the same com-

position as the nucleoli, as in these sections it has taken a very different colour in staining. In none of my sections of ovaries of Syngnathus are the germ-cells sufficiently differentiated to enable me to trace the centrosome in them, or decide as to the origin of the vitelline nucleus.

Henneguy (**11**) has recently studied the vitelline nucleus in the ova of Teleosteans and other classes of Vertebrates. In young ova of the trout he finds it is a round body at some distance from the germinal vesicle, and consisting of a central part deeply stained, and an external zone in which the stain is less intense. In Syngnathus acus he finds its earliest condition in very young ova is that of a refringent corpuscle in contact with the germinal vesicle. In his text he does not connect the structure with anything previously existing in the germ-cells either in a state of division or in the resting state, but concludes that it arises from the germinal vesicle, and gives to it a very far-fetched and fanciful interpretation—namely, that together with the nucleoli of the germinal vesicle, it represents the macro-nucleus of the Infusoria, the chromatic network representing their micro-nucleus. But Henneguy gives a figure of a section from the ovary of a newly-born kitten which goes far to prove the identity of the vitelline nucleus with the centrosome. At the time he wrote his paper, Balbiani's paper (**12**) mentioned below was not published, and the doctrine of the centrosome was not so far developed as it has been since. It is not surprising, therefore, that the author overlooked the significance of the figure to which I refer.

In this figure are shown germ-cells in process of mitosis with a centrosome at each end of the spindle, and others in the resting-stage with a minute stained corpuscle at the side of the nucleus. There is nothing to distinguish this corpuscle from the centrosome of the dividing cell on the one hand, and on the other from the vitelline nucleus or corpuscle shown in other figures of the same plate in ova contained in definite follicles, except that the vitelline nucleus is somewhat larger.

The history of the structure has also been studied by Mr. Jesse W. Hubbard (**15**) in the ova of Cymatogaster aggre-

gatus, Gibbons, one of the viviparous Embiotocidæ of the Pacific coast. The eggs of this fish are very small, and develop scarcely any yolk. The earliest stage of the vitelline corpuscle which Hubbard could discover was, like that which I have described, a crescent-shaped body, fitting closely to one side of the nucleus. The eggs in which this condition was observed were 20 μ, or ·02 mm., in diameter. In later stages the body was found at a distance from the germinal vesicle. It travelled towards the periphery of the ovum, and remained visible even in the period of segmentation. Hubbard concludes that the body originates from the nucleus.

The later history of the vitelline nucleus in Pleuronectidæ is as follows:—It moves away from the germinal vesicle towards the periphery of the ovum, as seen in fig. 21. When the deposition of yolk commences in the peripheral layer of cytoplasm, the vitelline nucleus is seen close to the inner limit of the yolk, and the body then assumes somewhat the form of a sphere at the apex of a cone. The conical portion is in contact with the yolk layer at its base, and careful examination shows that it is continuous with the strands of cytoplasm which separate the yolk globules. This condition is shown in figs. 10 and 14. Usually the cone can be seen to give off divergent strands which pass into the cytoplasmic network, and then the vitelline body reminds one forcibly of the form of an octopus, with its arms extending into the yolk. In the spherical portion of the body vacuoles appear, and these appear to be similar to those which contain the yolk globules, but I am not certain whether they actually contain yolk substance or not. As the yolk layer increases in thickness, the vitelline body becomes completely surrounded by it, and is then detected with some difficulty as a little island of cytoplasm rather more deeply stained than the rest, and comparatively free from yolk. After the yolk has reached the surface of the germinal vesicle, I have not been able to detect the vitelline nucleus.

The structure in question is very much less conspicuous in the ova of the grey gurnard in my preparations than in those

of Pleuronectidæ. I have been able after some trouble to convince myself that it exists, and has similar relations in this species, but have only been able to detect it occasionally in the larger of the yolkless ova, which were lightly stained. My material was fixed with picro-sulphuric acid, and with chromic acid alone; the latter is not very effective for the demonstration of the body in Pleuronectid ovaries, but the former brings it out very clearly in them in certain conditions of the ovary. It would perhaps be more easily seen in the ova of the gurnard after the use of a fixing mixture containing acetic acid.

In Syngnathus I have not seen any trace of the vitelline body or bodies after the appearance of the yolk. As described above, the yolk globules in this form appear uniformly throughout the cytoplasm from the first, not as a peripheral zone increasing in thickness, and I have been unable to discover the remains of the vitelline bodies among them. The largest ovum in which I have seen it was ·25 mm. in diameter, and in this the formation of the yolk was just commencing.

According to Boveri, both the centrosomes of the first division spindle in the fertilised egg arise from the spermatozoon,—that is to say, the spermatozoon becomes in the egg a male pronucleus and a centrosome, and the latter divides to form the two centrosomes of the first segmentation spindle. The ripe egg on this view possesses no controsome, and for this reason is incapable of self-division.

Balbiani, the original discoverer of the vitelline nucleus, in 1893 identified this body as the centrosome of the ovum. He concludes that it arises from the nucleus as a little bud at the moment when the ovum quits the germinal epithelium. He points out that the vitelline nucleus condenses around it a portion of the cytoplasm more dense than the rest; and this surrounding layer he compares to the archoplasm or attractive sphere around the centrosome. The vitelline nucleus may be double, and the centrosome is also often seen to be double in resting cells. The increase in size of the vitelline nucleus is interpreted as hypertrophic degeneration. Balbiani considers

that the degeneration of the vitelline nucleus, that body being the centrosome of the egg, accounts for the absence of the centrosome in the fertilised egg; and for the fact, asserted by Boveri, that the centrosomes of the segmenting egg are both derived from the spermatozoon.

These views are open to several serious objections. There is first the uncertainty with respect to the origin of the vitelline nucleus. Balbiani argues that since the vitelline nucleus arises from the nucleus proper, the centrosome in an ordinary cell has a similar origin, a fact which he thinks explains the important part taken by the centrosome in the division of the cell. But the latest and most definite researches indicate the constant presence of the centrosome in the cytoplasm, and if this is correct then it ought to be possible to trace back the vitelline nucleus into continuity with the centrosome of the germ-cell. An equal difficulty presents itself in the interpretation of the later history of the egg. There is ample evidence that the vitelline nucleus disappears in the yolk, and takes no further part in fertilisation or segmentation. Yet the polar bodies are formed by mitotic division before fertilisation. If the vitelline nucleus is the effete centrosome of the ovum,—and if centrosomes are such a constant and important feature in mitotic division,—how is it that the nucleus of the ovum is able to divide twice in the formation of the polar globules? Boveri (3) concluded that the centrosome of the ovum disappeared before fertilisation, and pointed out that in some eggs the directive spindle was destitute of polar radiations at its extremities. But numerous observers have seen radiations and centrosomes at the extremities of the directive spindle, and Boveri suggests that the female centrosome, when present, is no longer capable, after the formation of the polar globules, of performing its functions, i. e. of taking part in cell division; it disappears, and the male centrosome takes its place. This suggestion has been demonstrated to be actually correct by A. D. Mead (16) in a paper published in 1895. This observer states that he followed the degeneration and disappearance of the egg centrosome,—that is to say, the inner centrosome of the

second directive spindle. These facts, assuming them to be firmly established, have a very important bearing on Balbiani's theory of the significance of the vitelline nucleus. Since, at any rate in the eggs of many animals, the directive spindles are provided with centrosomes, of which the one belonging to the female pronucleus disappears before the formation of the first segmentation spindle, it is clear that the disappearance or degeneration of the vitelline nucleus can have nothing to do with the absence of the female centrosome in fertilisation. We must consider the question of the identity of the vitelline nucleus with a centrosome on other grounds.

If the centrosome were a permanent organ of the cell, and the vitelline corpuscle were identical with the centrosome of the ovum, the vitelline corpuscle having disintegrated, or, at any rate, having been removed from the neighbourhood of the nucleus, fixed in a distant part of the cytoplasm, and usually surrounded by yolk, the directive spindle could not possess centrosomes. For it is certain that the vitelline nucleus does not return to the vicinity of the germinal vesicle and again take part in its changes. If follows, therefore, either that the centrosome is not a permanent part of the cell, or that the vitelline nucleus is not to be identified as the centrosome. Now if the vitelline nucleus is not the centrosome it is certain that there is no other body visible in the ovum outside the germinal vesicle which can be identified with the centrosome. The suggestion that the centrosome exists within the germinal vesicle is inconsistent with the theory of the persistence of the centrosome outside the nucleus. The history of the ovum, then, between its first definite constitution and the formation of the polar bodies, actually disproves the theory of the unbroken continuity of the centrosome as an extra-nuclear body. It is an observed fact that the directive spindle, whether first or second, possesses centrosomes, and yet no body can be discovered in the egg at an earlier stage which can be identified as a centrosome, except the vitelline nucleus, which is known to degenerate and disappear. The centrosomes of the directive spindle, then, must be formed as such at the time of their

appearance—must be a new modification of some part of nucleus or cytoplasm.

If centrosomes can be formed anew thus on the occasion of one mitosis, there is apparently no reason why they should not be newly formed at each mitosis. Further, we know that the centrosome of the ripe egg—that is to say, the inner centrosome of the second directive spindle—degenerates. It may be urged that this is a special case, that this centrosome degenerates as a preliminary to fertilisation, because the centrosomes of the first segmentation spindle are supplied by the sperm. But since the centrosomes of the directive spindle are formed anew, the preceding centrosome must have disappeared. Therefore there is nothing to contradict the suggestion that this preceding centrosome is the vitelline nucleus, which degenerates. And if the centrosome degenerates after one mitosis, there is no reason why it should not degenerate after every mitosis. On these grounds the theory might be suggested that the centrosome is not persistent, but is a structure formed by the changes which precede mitosis, and that after division it disappears, more or less gradually in the cytoplasm. This theory does not, however, appear to be applicable to all mitoses, for in the segmentation of the fertilised ovum, according to the latest observations, the centrosome and archoplasm persist after each division, remain during the reconstitution of the resting nucleus, and then divide to form the new spindle of division and the centrosomes of its two extremities. It is definitely and particularly stated by A. D. Mead that this occurs in the segmentation of the ovum of Chætopterus.

We must conclude, then, that although the extra-nuclear persistence or continuity of the centrosome has been observed in the segmentation of the ovum, and may occur in the mitosis of all somatic cells, it certainly does not occur in the maturation of the ovum. The centrosomes of the directive spindles are not formed from a pre-existing extra-nuclear body. In this case the centrosome of the ovum must have passed into the interior of the germinal vesicle, or must have degenerated in the cytoplasm. There is no evidence of the inclusion of

the centrosome within the germinal vesicle, while there is some, although not complete, evidence of the identity of the vitelline nucleus with the centrosome left at the last division of the germ-cell. The centrosome of the ovum then degenerates and disappears before the two polar divisions, and this suggests the question whether there is anything in the process of spermatogenesis which corresponds to this occurrence in the ovum.

It has been found that the reduction divisions of the spermatocyte take place in the same way as in the ovum—that is to say, before the last two divisions there appear half the proper number of chromosomes, each of which consists of four distinct particles, and after the divisions one of these particles is contained in each spermatozoon.

According to O. vom Rath there are four generations or divisions of the male germ-cells before the last two, or reduction divisions, which take place by what he terms "heterotypical mitosis," because of its special character. He finds the cell of the fourth generation larger than that of the third, and believes that there is a resting and growing phase after the third generation. But he does not mention any degeneration or disappearance of the centrosome between the fourth division and the first reduction division, and this is the interval where such an occurrence is to be sought. He figures one of the large resting cells of the third generation, and says that often near the resting nucleus at a spot where the cytoplasm has a coarse granular appearance and dark colour, he has seen two round bodies, which he takes to be two attraction spheres with their centrosomes, as they are too large for centrosomes alone.

According to vom Rath, the tetrads or groups of four chromatin bodies which are present at the commencement of the reduction divisions in the spermatocyte are formed directly without a resting phase from the dyaster of the previous division. Thus the process does not agree with that which takes place in the ovum. But he points out that in the growth-period of the ovum, according to certain observations, the

nucleus does not pass through a genuine resting phase. Thus Val. Hacker believed that in the ova of certain fresh-water Copepods throughout the growth-period a double chromatic thread could be traced in more or less evident form, and that it was directly derived from the dyaster of the preceding division, so that the division of the chromosomes (tetrads) in the first reduction division was prepared in the previous mitosis. Rückert has reached similar conclusions in the study of the ova of Selachians. No further information being apparently available concerning the history of the centrosome in the spermatocyte in the period just before the reduction divisions, we may proceed to consider more precisely the history of the germinal vesicle in the ovum, and may conveniently refer to Rückert's observations on the Selachian ovum as affording the most definite and comprehensive view of the subject, and then inquire how far they harmonise with what I have been able to see in the Teleostean egg.

Many authors have maintained that the nuclear network temporarily vanishes in the germinal vesicle. Rückert remarks that the reticulum which is considered to be the vehicle of heredity, and has been studied to the farthest possible detail in the final stage of its development, was not known with certainty, when he began his investigation, even to exist in the young stages of the egg. In 1890 Boveri, considering each tetrad as a single chromosome, proved that the reduction to half the normal number of chromosomes had already taken place when the first directive spindle appeared, and maintained that the reduction must take place in the germinal vesicle, if not at some earlier stage. But he had not minutely traced the fate of the chromatic substance in the germinal vesicle.

O. Hertwig, on the other hand, considered that the number of chromosomes was doubled, which means, of course, that each member of the tetrad represents a chromosome. Then the reduction follows from the two divisions without the interposition of a resting phase or further splitting of the chromosomes.

Rückert's investigations were carried out principally on ovaries of Pristiurus at Naples, though he also examined ovaries of Scyllium and Torpedo. For fixing he employed Hermann's osmic mixture, sublimate, and sublimate with 5 per cent. acetic. The youngest ova he describes are 28 μ in diameter. In these eggs a membrane was not seen around the germinal vesicle in material fixed with sublimate, but was distinct enough in osmic preparations. The cavity of the vesicle contained some small gleaming nucleoli, and a distinct and still stainable chromatin reticulum. The latter consisted of separate unbranched chromosomes of fairly uniform thickness and bent into undulating curves. These formed a tangle (knaüel). In this respect the structure of the germinal vesicle differed from that of the ordinary resting nucleus, and approached the tangle phase of mitosis; from which, however, it was distinguished by the less compact form of the chromosomes. There was never in the growing egg a branched chromatin network like that which can be demonstrated in the ordinary resting nucleus, although this period in the history of the egg extends over a long period of time; in the case of eggs which ripen late in the fish's life, over several years.

Rückert was not able to count the chromosomes at this stage exactly, but estimated their number at thirty to thirty-six, which was about the same as in the somatic cells of the embryo of the same species. The chromosomes in the germinal vesicle were not all of the same size, some being remarkably small and slightly stained.

The first period of the development of the egg is from the stage described above to that of eggs which are 1½ to 2 mm. in diameter, in which the germinal vesicle is at its maximum size, namely, mm. in diameter. The membrane of the vesicle becomes thicker, and is thrown into grooves and folds, which last, if examined in section, might be taken for outgrowths of the vesicle. The nucleoli increase in number and size, and after being at first chiefly peripheral, gradually aggregate in a particular part of the vesicle, usually that towards the surface of the egg. The network of the vesicle becomes almost in-

visible, and stains scarcely at all. It is this condition which has led to the conclusion that the chromatic network disappears; but Rückert says that the chromosomes are converted into much expanded structures, consisting of numerous transverse threads along an axis, so that each chromosome resembles the cylindrical brush used for cleaning lamp chimneys. The change is effected by the microsomes first becoming elongated into transverse rods, or perhaps discs, and then further breaking up into the thin curved threads. In eggs $\frac{3}{4}$ to 1 mm. in diameter the tangle of chromosomes again becomes more distinct. To see the general distribution of the chromosomes, whole germinal vesicles must be isolated and examined, because a section takes in only a very thin slice of the large structure. It is now found that the chromosomes are arranged in pairs, and there are as many pairs as there were chromosomes in the earlier stage, which shows that each pair has been produced by the division of a chromosome. The position of the members of each pair indicates that the division of the chromosome has been longitudinal.

The second period of the development is to be followed in eggs from 2 or 3 mm. in diameter to the full-grown condition when they are 14 to 16 mm. in diameter. The vesicle becomes smaller, and reaches the surface of the egg, against which it flattens itself to some degree. The chromosomes remain in pairs, but become much shorter and thinner, and they concentrate towards the centre of the vesicle.

In eggs of 14 mm. diameter the coil or tangle formed by the chromosomes is only 36 μ by 8 μ in dimensions, while the whole vesicle is 296 μ by 148 μ. The external part of the feathery structure of the chromosomes becomes stainless, while the central part stains more deeply. Rückert regards the changes in this and the preceding period as to a great extent due to a divergence and convergence of particles. Finally, the chromosomes become converted into portions of chromatin, consisting of distinct granules, but having the form of dense masses, not elongated loops or cords; in some of the masses there are indications that each consists of four rods.

The author finds that during the enlargement there is a great increase of substance, during diminution a great loss of substance, in the chromosomes. He regards this temporary substance as the somatic plasm, whose function is to govern or cause the growth of the egg and the accumulation of the yolk; when this object is accomplished it merges in the cytoplasm, i. e. becomes ordinary cytoplasm, and the chromatin which is left is that which is concerned in fertilisation and heredity.

The nucleoli experience in the second period of the development of the egg a reduction in mass, which proceeds at a rate equal to that of the diminution of the chromosomes. The nucleoli diminish in size, become paler, and then vanish altogether, following the chromosomes during the process towards the centre of the vesicle.

According to B. Holl, in the human ovum the chromosomes disappear altogether, and the nucleolus (here single) takes their place, breaking up into little spheres which become the chromosomes of fertilisation. But Rückert considers that this is an error.

The third period in the history of the ovum, according to Rückert, is the formation of the polar bodies. At the beginning of this period the chromatin-figure is surrounded by a remnant of the germinal vesicle without any membrane, and much smaller than the original vesicle. The chromatin forms a dense heap, 6 μ in diameter. It seems at first to be homogeneous, but is really a dense coil formed of the rods of the previous stage. The heap separates again into separate corpuscles, and these form the equatorial plate of the first directive spindle.

In a later paper (1892) Rückert has described how he endeavoured to ascertain the stage at which the doubling, or longitudinal division of the chromosomes took place. He found that they were already double in eggs of $\frac{1}{2}$ to $\frac{1}{3}$ mm., in which the chromosomes are most indistinct, and, indeed, in ova fixed with sublimate, invisible. They can, however, be traced in the form previously described in eggs fixed with Flemming's mixture. He points out that in younger eggs the

chromatin network, although distinct and well stained, is so dense, and exhibits so many crossings in all directions, that it appears to have the structure characteristic of the resting nucleus. He declares, however, that by means of the oil-immersion lens he was able to resolve the network into a tangle (knäuel) of chromosomes, and that in thin sections of the germinal vesicle some of these being included in their whole length can be seen to be double. He was even able to see traces of doubling in the daughter tangles of the last mitotic division of the germ-cells, and comes to the conclusion that the germinal vesicle in the egg during its enlargement and growth is not a resting nucleus, but a daughter tangle of the germ-cell enlarged to enormous dimensions.

The history of the germinal vesicle in Teleostean ova, according to my observations, agrees in many respects with that described by Rückert in Selachians. There are certain well-known features which are common to both cases, in particular the conspicuousness and deep staining of the nucleoli, and the indistinctness and unstained condition of the nuclear network during a considerable period of the egg's growth. As I have already described, in the smallest ova in my preparations, about ·01 mm. in diameter, the germinal vesicle exhibits one large conspicuous nucleolus, with others much smaller, and a nuclear network. The structure of these germinal vesicles or nuclei appears to resemble that of a resting nucleus in an ordinary cell, and I have not been able to distinguish chromosomes in them as described by Rückert in the ovum of Selachians. I cannot assert that the network does not consist of separate chromosomes, but although I have used sections from material fixed in Flemming's mixture, and have studied them with an apochromatic oil-immersion of 2·0 mm. by Zeiss, I have not succeeded in resolving the network into chromosomes. Rückert maintains that with sufficient magnifying power and careful scrutiny the apparent network can always be seen to consist of a tangle of chromosomes, and that the germinal vesicle is, in fact, an enormously enlarged tangle-phase or spirem, and therefore essentially different from

an ordinary resting nucleus. But it may be suggested that possibly the individual chromosomes could be distinguished in the ordinary resting nucleus, if it was re-examined with sufficient care. It may, on further investigation, be found that the fibres of the network of the resting phase are only apparently branched and anastomosed, and that it really consists of chromosomes, the limits of which do not entirely disappear. On the other hand, if the network of the germinal vesicle consists of chromosomes, we have to inquire what is the origin of the membrane and nucleoli. The existence of these structures cannot be ignored. The theory that the chromosomes after the last division of a germ-cell place themselves in a convoluted series to form a tangle or spirem, without entirely losing their individual independence, does not account for the appearance of the nucleoli or of the membrane. There are two views of the reconstruction of the resting phase after the mitosis of an ordinary nucleus. According to the most detailed recent descriptions of the process in the blastomeres of a segmenting ovum, the chromosomes become vacuolated, and form little vesicles, which fuse together, and so give rise to the nuclear network, membrane, and nucleolus. According to Flemming's original scheme, the stages of reconstruction were the same as those of division, but occurred in the opposite order: the chromosomes united into a continuous thread, which acquired the intricate convolutions of the tangle-phase or spirem, and then resolved itself into nuclear network, membrane, and nucleoli. According to Rückert's views, neither of these modes of reconstruction occurs in the germinal vesicle, the chromosomes being always distinct. But perhaps while preserving their individual existence these bodies give up and afterwards regain a large portion of their substance, which goes to form the nucleoli.

Rückert describes the daughter-tangle after the last division of a germ-cell, as consisting of a number of bent chromosomes with their bends all converging towards a "polar region," in which a large nucleolus was often to be seen. He says nothing concerning the origin of this nucleolus, but it is certain that the chromo-

somes without a nucleolus are not the same as chromosomes plus a nucleolus. It is not probable that the nucleoli are formed from the achromatic elements; on the contrary, there is much evidence indicating their connection with the chromatin.

If the chromosomes are of great physiological importance, it must be because they influence the life of the cell, they must take part in the general metabolism of the cell. If they were merely fixed elements which were divided and transmitted in permanent form from generation to generation of cells, it would be difficult to understand how they could affect the properties and qualities of the cell. To say that the chromatin is the substance of heredity, merely means that judging from the history of the spermatozoon in fertilisation, all the peculiar qualities which are transmitted from parent to offspring must be contained in the chromosomes of the pronucleus. Even this is not strictly true, because there are the centrosomes to be taken into account. But the number and form of the chromosomes are nothing unless we know how they act upon the cell and determine its behaviour.

The small ovum in fig. 21, Pl. 3, shows the appearance of the germinal vesicle in ova of the flounder of ·037 mm. diameter. The reticulum is but slightly stained, more by hæmatoxylin than by safranin, the large nucleolus is deeply stained, and the cytoplasm also takes stains very deeply at this stage. In this respect the cytoplasm of the ovum at this stage differs from that of other cells, and from that of the ovum at other stages. The cytoplasm is very dense and almost homogeneous in appearance. The other ovum represented in fig. 21 is ·098 mm. in diameter. The germinal vesicle in it is much larger than in the smaller egg, there are several small nucleoli in contact with the membrane of the vesicle, and there is a nuclear network, some strands of which are more stained and more conspicuous than the rest. The cytoplasm is granular, and after the action of chromic and osmic acids scarcely stained, but in material fixed with picro-sulphuric or sublimate it stains considerably. In the stage here considered there is a distinct membrane.

The following stages are to be seen in sections made from the ovary of a plaice killed on August 17th. The development of yolk was advanced in many of the ova. The best preparations are from portions fixed with chromosmic mixture or Kleinenberg's picro-sulphuric acid. In ova a little larger than those last mentioned—namely, from about ·16 mm. in diameter upwards—in the interior of the germinal vesicle there can be distinguished a central and a peripheral region. In the central region can be seen, even with a low power, indications of stained fibrils, which are wanting in the peripheral region. Examination with higher powers shows that these fibrils form apparently separate lengths or loops, whose direction is very irregular; sometimes they can be seen to be in pairs, and with the immersion lens they have a feathery appearance. These structures clearly, therefore, correspond to those described by Rückert in the Selachian ovum, and identified by him as chromosomes. The impression I have obtained by comparing the ova in this condition with the younger is that the vesicle has expanded, while the fibrillar network has remained of the same size, so that a space has been left between the border of the network and the membrane of the vesicle. In the younger ova the nucleoli are all in contact with the membrane of the vesicle, but in later stages they travel towards the centre, and are found in the region of the chromosomes. The substance between the fibrils and in the peripheral region of the vesicle appears to be finely reticular. Fig. 24 shows the germinal vesicle of an ovum ·27 mm. in diameter from a chromosmic preparation, as seen with Zeiss immersion 2·0 mm., compensation ocular 8. The reticular appearance of the achromatic substance is not very evident, the ovum represented being one of those in the internal part of a piece of material, in which the achromatic substance is very pale. In the ovum from which this figure is taken yolk formation had not yet commenced, and only three small nucleoli are seen in the region occupied by the chromosomes.

When the period of yolk formation sets in, the following changes occur in the germinal vesicle. The membrane becomes thinner and less distinct, and loses its regular contour. In

sections it is seen to be more or less wrinkled, folds of it projecting inwards between the nucleoli. This is not a condition produced entirely by the action of the fixing reagents. I have often observed in perfectly fresh material that the surface of the vesicle instead of being smooth consisted of numerous bulbous projections, as seen in fig. 25. In consequence of this condition it frequently happens that a nucleolus appears in a section to be outside the vesicle, when examination of consecutive sections shows that it is really contained in a pocket or diverticulum of the vesicular membrane. This is, I believe, one of the circumstances that have led observers to describe the migration of nucleoli from the vesicle into the external cytoplasm. It is, however, certain that during the period now under consideration the nucleoli migrate from the periphery to the central region of the vesicle, where they are for the most part found around the tangle of fibrils, though some are scattered among the fibrils. Fig. 26 shows a section of a vesicle from the same preparation as fig. 25 in an ovum ·39 mm. in diameter, in which the yolk is at the stage seen in fig. 15, Pl. 2. The ovum figured being near the edge of the preparation, where the osmic and chromic acids have acted most strongly, the fibrils are not well stained, and are therefore less distinct; the reticular appearance of the achromatic substance is, on the other hand, conspicuous. Fig. 27, Pl. 4, shows the appearance of the vesicle of an ovum ·36 mm. in diameter, in a section fixed from material with picro-sulphuric acid, the material being a portion of the same ovary of plaice from which the chromosmic sections were derived. The vesicle itself is ·16 mm. in its longest diameter. The section was stained with Delafield's hæmatoxylin. The fibrils are distinct, but appear at this stage to form a continuous convoluted thread, a true spirem, not a number of separate chromosomes.

I have not been able to follow out the history of the germinal vesicle in the later stages in the plaice and flounder, and must therefore proceed to the description of certain stages which I have examined in other species. In figs. 10, 11, 12, Pl. 2, will be seen the appearance of the vesicle in various stages of

the egg of the turbot, all from one series of sections, the material for which was fixed some hours after the death of the fish in picro-sulphuric acid. The chromatic fibrils in these sections are very indistinct, in many of the oldest ova they are not to be distinguished, the structure appearing uniformly granular. In the younger ova, and in some of the older, however, distinct traces of the fibrils are to be seen. The sections from which figs. 28 and 29, Pl. 4, are taken were prepared from material taken from a fish just captured and fixed immediately; but the mixture used, picro-sulphuric acid and spirit, has not given very good results. The fibrils are very indistinct.

It will be seen that in the turbot ovum in the stage shown in fig. 12, when the whole of the cytoplasm is filled with yolk globules, the nucleoli are very large and deeply stained; in many cases each consists of a number of large coarse granules. In the ovary to which figs. 28 and 29 refer, the ova are nearly all in two stages, the ripe stage in which the yolk globules have fused together, and the nearly ripe stage in which the yolk is a little more dense than in fig. 12. In the former stage I have not been able to detect the germinal vesicle, or any remnant of it at all. In the latter stage the vitelline membrane has burst at one point, in consequence of the action of the fixing reagent, which has caused the yolk to swell, but the germinal vesicle is not much distorted. In fig. 28 it will be seen that the nucleoli are distributed irregularly about the nuclear cavity, with the exception of the centre, where there are one or two indications of fibrils. The nucleoli are more numerous than at the preceding stage, having presumably subdivided. In some of the eggs, although these are not quite the largest, the germinal vesicle presents the remarkable structure shown in fig. 29. There is a ring of stained bodies having the form of rods of various shapes, and within the ring are faint indications of fibrils. The stained rods must obviously be the transformed nucleoli, and at the same time they possess considerable resemblance to chromosomes. According to Rückert the nucleoli vanish altogether, and are not

converted into any of the structures of the directive spindle, but he suggests it as probable that they form reserves of chromatic substance, which they afterwards give up to the chromosomes. His own description is not very consistent with this suggestion, since he states that the nucleoli increase and decrease synchronously with the increase and decrease in size of the chromosomes. But although, according to various descriptions, the chromosomes of the directive spindle are very much shorter than the chromosomes of the earlier stages of the germinal vesicles, they are much thicker, and much more deeply stained. They contain, therefore, in all probability, more chromatin, and possibly they take up chromatin from the nucleoli when the latter disappear. Possibly the substance of the nucleoli is absorbed into the fibrils. The condition shown in fig. 29 may be a stage in such a process. The stained bodies in this condition have a resemblance to chromosomes, but as observers are agreed that the nucleoli are not converted into chromosomes, but that these are identical with the nuclear fibrils, and since the fibrils are present within the ring of stained bodies, it may be inferred that the substance of the latter is about to be transferred to the fibrils. This suggestion can only be tested by further investigations.

In figs. 7, 8, 9 are shown some stages of the germinal vesicle in the ova of the grey gurnard (Trigla gurnardus). Fig. 7 is a section of an ovum ·28 mm. in diameter. The preparation from which it is taken was made from material obtained in the fish market on April 26th, and fixed with picro-sulphuric acid. The largest yolked ova are for the most part burst, in consequence of the action of the fixing reagent on the yolk and the vitelline membrane, but the younger ova are well preserved. In those of the stage figured there are numerous small nucleoli on the inner side of the membrane, and very distinct feathery separate fibrils in the central region of the vesicle. These are of various shapes, sometimes V-shaped, sometimes even circular. The ground substance appears very finely granular. The feathery fibrils are even more distinct

in ova smaller than that figured, and are visible as separate elements in the larger up to those of a diameter of ·43 mm.

The sections from which fig. 8 is taken were prepared from material fixed immediately after the death of the fish in a mixture of picro-sulphuric acid and spirit. The feathery fibrils in these can only be faintly traced with difficulty, as they are scarcely stained at all. They are more distinct in the younger eggs. Fig. 30, Pl. 4, represents the vesicle of the most advanced egg in these sections. Although there are empty follicles showing that ripe eggs have escaped, there are no stages more advanced than that figured. Fig. 9 shows the structure of the most advanced egg in sections from part of the ovary of a gurnard fixed with chromic acid $\frac{1}{4}$ per cent. In the younger eggs the feathery fibrils can be seen, but in this stage in these sections they cannot be distinguished. The nuclear substance appears pale and granular. The ovum represented is ·53 mm. in diameter.

The history of the nucleoli in the ova of other animals has been much debated, and although the transformations of the germinal vesicle have been recently traced completely and in detail in certain cases, there is still room for doubt concerning the significance of the changes which are observed. In 1887 O. Schultze stated that in the nearly ripe egg of Rana fusca there was no chromatin network; that the nucleoli formed a rounded group in the germinal vesicle, in the centre of which were very minute corpuscles. He believed that these corpuscles were derived from the disintegration of nucleoli, and that they united together to form the chromosomes, which afterwards united into a convolution. He also saw some nucleoli dissolve into liquid.

Professor G. Born, in 1892, made an exact investigation of the development of the first directive spindle in the ovum of another Amphibian, Triton tæniatus. He found that the chromatic convolution was not derived from the nucleoli, but as Rückert described in Selachians, from chromatic fibrils previously present. These fibrils are very imperfectly seen after staining in mass with borax carmine, but are to be

demonstrated by fixing with chrom-acetic acid and staining with hæmatoxylin and orange G. After such treatment in the germinal vesicle, when the nucleoli are still at the periphery, there is seen a more deeply stained and more coarsely granular body in the central region. This is the appearance with a low magnification; with higher powers the body is seen to consist of broad winding chromatin cords, composed of threads transverse to their direction, i. e. having the structure which I have called feathery. These feathery threads contract into thinner, denser threads which break up into separate chromosomes: during this process the nucleoli move in a centripetal direction, surround the aggregate of chromatic fibrils, and gradually disappear, some breaking up into pieces, others becoming pale and dissolving.

In 1893 Rudolf Fick (**10**) described the corresponding transformations in the ovum of the axolotl. He observed that the nucleoli vanished completely, but leaves open the question whether there is any connection between their disappearance and the development of the chromosomes.

In a still more recent paper (1895) E. Korschelt (**17**) describes the maturation of the ovum in the chætopod Ophryotrocha puerilis, and states that the single nucleolus present vanishes by dissolution while the convoluted chromatic thread is developing, but leaves undecided the question whether the substance of the nucleolus is transferred to the thread.

In one of the Foraminifera the chromatin of the nucleus is in the form of elements which have a curious resemblance to the nucleoli seen in the germinal vesicle of the Teleostean ovum, but in the present state of our knowledge it is impossible to say whether any great importance is to be attached to this resemblance. Fritz Schaudinn (**18**) has described the history of the nucleus in Calcituba polymorpha, and the following is a summary of it. The protoplasm of the animal is multinucleate. The largest nuclei are 10 to 35 μ in diameter, and consist of a vesicle bounded by a membrane. The central part is filled only with structureless nuclear sap, while the chromatin lies on the inner surface of the membrane in the form

of homogeneous compact spheres of different sizes (1 to 5 μ) and numbers (20 to 100). The membrane of the nucleus in this stage disappears, and the chromatin masses wander apart, forming the smallest nuclei lying free in the plasma. These dense homogeneous nuclei send out processes and embrace vacuoles of the plasma, until they contain several of these, and then they again assume a smooth outline. The nuclei in this stage have thus a membrane of chromatin and a framework of partitions between the vacuoles which appears optically as a reticulum. This process recalls the vacuolation of nucleoli seen in the germinal vesicle of Teleostean and Amphibian eggs, and also the vacuolation of the chromosomes described in the reconstitution of nuclei after mitosis in segmenting ova. The chromatin is seen in granules in the network, and it accumulates into a single mass in the centre, or fixed to one side of the membrane. From the mass extend linin threads; then the mass of chromatin breaks up, granules passing in succession along the threads to the points where these join the membrane, and there forming the spherical masses; the achromatic threads then break or dissolve, and so we have the recurrence of the condition from which we started, and the process begins over again. All this suggests strongly that the multiplication of nucleoli in the Teleostean ovum, and their disappearance at a later stage, are effected by the streaming of chromatin along threads of linin.

The Muræniдæ.

In consequence of the peculiarities in their reproduction the generative organs of the conger and eel will be here considered separately. For a fuller discussion of their natural history I must refer to my papers in the 'Journal of the Marine Biological Association,' and the literature to which references are there given. It is sufficient to mention here that they do not spawn annually, nor periodically, but only once, after which they die, and that their adolescent or immature stage extends over several years.

In the eggs of the conger and eel, as in those of the gurnard, turbot, brill, &c., above described, the formation of oil globules in the cytoplasm precedes that of the yolk proper. But the deposition of fat occurs at a certain stage not merely in the eggs but in the stroma or interovular tissue of the ovary, and suggests that there is some important relation in the nutritive processes between fat and proteinaceous vitelline substances. Whether the fat is actually combined with other substances to form nitrogenous compounds, or is merely used up separately to supply the energy required by the body while the vitelline constituents are being produced from other sources, is a physiological question I am not prepared to discuss. Whatever the function of the fat, there are three distinct periods in the history of the ovary: (1) a stage in which the organ is very small, and contains only protoplasmic ova destitute of deutoplasmic elements, and in which the stroma of the ovary is small in quantity and destitute of fat-cells; (2) a stage in which fat is largely deposited in the stroma in the form of fat-cells, and the eggs are isolated from one another by this adipose tissue, and in which the eggs themselves contain oil globules; (3) a stage in which the fat in the stroma is gradually absorbed, and yolk globules are developed in the eggs: at the end of this stage a condition is reached in which the ovary resembles in the structure of the stroma the earliest stage (1), and consists almost entirely of eggs, now much larger and full of yolk, crowded together.

The first of the stages above defined is seen in the ovaries of female conger which are 2 feet in length. The ovary in such specimens is 3 to 7 mm. in width, and the breadth of the lamellæ, measured from the attached base to the free edge, does not exceed ·6 mm. These lamellæ contain small protoplasmic eggs entirely without secondary deposits (fig. 31, Pl. 4). The greater number of these eggs are of nearly uniform size, and these are the largest eggs present. Their diameter in my preparations from one specimen of 2 feet in length is ·05 mm. There are a smaller number of smaller eggs, and at the surface of the lamellæ is seen the germinal epithelium, which in a

great many places is proliferating, and so giving rise to large numbers of new eggs. These young eggs, or germ-cells, are collected in small groups, which project inwards from the germinal epithelium towards the interior of the lamella, and these groups are doubtless formed by the subdivision of one or a few cells.

Of the intermediate or second of the stages defined above I have only one preparation. It was made from material preserved in a mixture of chromic acid and platinum chloride. The appearance of a portion of one of these sections is shown in fig. 32, which represents part of the transverse section of a lamella. It will be seen that the lamella consists of adipose tissue in which are embedded two rows of cells, one corresponding to each surface of the lamella. The largest eggs are ·25 mm. in diameter. Near the periphery of these eggs there is a zone of vacuoles, which in the fresh state were oil globules. No yolk elements are distinguishable.

In his paper on the sexual organs of Murænidæ[1] J. Brock has given an interpretation of the structure of the ovary which differs from mine, and which I believe to be erroneous. Without mentioning the size of the fish whose ovaries he examined, he describes a stage of the conger ovary in which the lamellæ are no higher than they are broad. In each lamella, at this stage, there are eggs towards each surface in two or three layers, while in the axis of the lamella, cavities are beginning to appear in the supporting tissue. He then describes ovaries of Conger and Myrus, which were apparently advanced in development. The lamellæ in these were 2—3 mm. high. He found that when stained in the mass these ovarian lamellæ retained their opaque white colour, as though they consisted merely of fat, showing only small stained dots scattered at intervals. He states then that microscopic examination explained the mystery; that abortion of sexual cells must have taken place on a large scale, for the whole ovary consisted of a narrow-meshed network of empty follicular membranes, in

[1] "Untersuchungen über die Geschlechtsorgane einiger Murænoiden," 'Mitt. Zool. Stat. Neapel,' Bd. ii, 1881.

which here and there perfect eggs were distributed either singly or in small groups. Brock remarks that these specimens show that the abortion of sexual cells takes place in the female sex as well as in the male, but that at the same time the fact that the condition in question was only observed in isolated cases leads to the conclusion that it is not a constant and regular stage in the history of the ovary.

My own observations, on the other hand, show that the condition of the ovary here in question is a perfectly constant stage, and that it is due to the remarkable and abundant deposit of fat in the connective tissue of the ovary. The meshes of the network described by Brock are, in reality, fat-cells. In sections prepared by the paraffin method the fat has been dissolved out, but in the fresh state the oily nature of the contents of these cells is perfectly obvious. There is no indication at all of the abortion of ova. The process of such abortion and reabsorption, as it occurs in the ovaries of other species, has been shown in this paper to be manifested in particular appearances produced by the presence of the remnants of the disintegrated ova. No trace of such remnants has been seen in the ovary of the conger, and their absence is in accordance with the special history of the ovary in the eel family—namely, a slow but continuous development culminating in a single and final act of spawning.

In the conger the fatty condition of the ovary is found from the time when the fish is a little over 2 feet long to that when it is full-grown, 4 to 6 feet or more long, and the ovary begins to assume the ripe condition. In nearly all specimens that are caught in the sea, except the small, in which the ovaries are in the stage previously described, the ovaries are in this condition.

The third stage in the development of the ovary of the conger is that exhibited by the ovaries of specimens which have died in aquaria with these organs much enlarged, and evidently almost ripe. In these ovaries there are no fat-cells, they have all disappeared, and each lamella consists chiefly of two layers of large eggs not differing from one another very

much in size or stage of development. These eggs are close to one another, only separated by the thin membranes of the follicles containing them, the interstices being occupied by strands of connective tissue and blood-vessels. I have prepared sections from three specimens which died in the aquarium of the Laboratory of the Marine Biological Association at Plymouth, and the history of which is recorded in my paper on the conger in the 'Journal' of the Association, vol. ii (new series).

The structure of the largest and most advanced eggs in these sections is shown in fig. 33, which is from a preparation made from material preserved in corrosive sublimate and acetic acid. It will be seen that the vitellus has shrunken away from the vitelline membrane, which is very thin. The follicular epithelium also lying upon the vitelline membrane is very much thinner than in the eggs of other fishes at a similar stage. The yolk consists of small vitelline globules which extend throughout the extra-nuclear region, and are closely crowded together. There are also a number of scattered oil vacuoles, situated principally about midway between the germinal vesicle and the surface of the egg. The germinal vesicle is somewhat contracted, so that there is an artificial separation between it and the surrounding vitellus. There is no nuclear membrane, the nucleus consisting of a finely granular or really reticulate achromatic substance containing a small number of deeply-stained nucleoli.

It is an interesting question whether any inferences can be drawn from the structure of this nearly ripe egg, concerning the condition of the ripe egg after deposition. The fact that among pelagic eggs none have been obtained in British waters which could possibly belong to conger or eel, is an obstacle to the supposition that the egg of the conger may be pelagic. Yet the structure of the egg, as above described, certainly agrees rather with that of nearly ripe pelagic eggs than with that of those which are heavy and adhesive. Eggs of the latter kind generally have a thick vitelline membrane, showing a division into two layers, while that of the conger is remarkably thin.

The condition of the yolk in the conger's egg resembles that of nearly ripe pelagic eggs, and there is nothing in it to indicate that the yolk spherules might not fuse together more or less completely, and so give rise to the condition of a pelagic egg with one or more oil globules. But there is a third possibility, that the ripe egg is neither pelagic nor adhesive, but remains unattached on or near the bottom.

My preparations of the ovaries of conger do not supply any evidence of great importance concerning the more minute features in the structure of the ovum which I have discussed in connection with other species. They were not made for the purpose of minute investigation, and the material was fixed in either corrosive sublimate or Perenyi's mixture. I have not been able to trace in them the vitelline nucleus, and although the nuclear network is visible in the younger stages, in eggs up to a diameter of ·27 mm., I have only seen a faint trace of fibrils in the nearly ripe eggs. The presence of one, or occasionally two nucleoli of very great size is noticeable in the younger eggs up to the size just mentioned. In an ovum of that diameter I found that the large nucleolus measured ·03 mm. There are in addition very numerous small peripheral nucleoli.

The history of the ovary in the eel is very similar to that in the conger. I have not examined the earlier condition in very young eels, but I have preparations showing the fatty stage from a specimen 1 ft. 11½ in. long, killed in June. The ova in these sections do not exceed ·13 mm. in diameter, and they are surrounded by fat-cells, as in the conger ovary represented in fig. 32. On the other hand, in sections from the ovary of an eel 1 ft. 9½ in. long, killed on November 15th, the largest ova reach ·2 mm., and there is no fat. One of the ova in these sections is represented in fig. 34. As shown in the figure, the cytoplasm contains numerous oil globules, but no yolk.

The testes of the conger, like the ovaries, also at a certain stage consist in large proportion of adipose tissue, in which small islands of germ-cells are scattered. As in the case of

the ovary, J. Brock has described the fat-cells as empty follicles of which the germ-cells have aborted. He states that in a testis of Myrus he saw near the empty follicles altered germ-cells which were opaque and had lost their nucleus, and considers that this leads to the conclusion that in the empty follicles the germ-cells have disappeared altogether. He proceeds to state that in osmic acid preparations the conclusion becomes a certainty, for the follicles in these are usually not empty, but contain shrunken cells which have reduced the osmic acid very strongly as only fat and nerve-cells usually do. He infers that the germ-cells have undergone a fatty degeneration. It is clear enough that all this is better explained by the simple fact that what Brock calls follicles are merely true fat-cells, developed in the connective tissue, since evidence that abortive germ-cells turn into fat is entirely wanting. With the development of the adipose tissue, the testis, like the ovary, becomes much larger in comparison with the younger fatless stage, and therefore there is no reason to suppose that the germ-cells, although they are scattered, have been reduced in number. Fig. 35 shows the proximal portion of a section of a testis of the conger in the adipose condition, as seen under a low power. At the base of the section is seen the lumen of the vas deferens. The total breadth of the testis, whose structure is shown in this figure, from the attached base to the distal edge, was about 5 mm.

As the testis becomes more developed the narrow cords of germ-cells or spermatogonia, seen in section in fig. 35, become much enlarged, and form spermatic tubes filled with spermatocytes, of which those in the centre are smallest, and therefore most advanced in development. The tubes are still separated by a certain amount of adipose tissue, especially near the distal edge of the organ, while near its attached base they are more closely approximated. This is the condition observed in sections of an organ about 7 mm. broad. In a much larger organ, something over 1 cm. broad and 5 mm. thick from side to side, there is still some adipose tissue between the large tubes crowded with spermatocytes. But as I have shown in

my paper in the 'Journal of the Marine Biological Association' that the ripe conger lives for about six months without taking food, there can be no doubt that all the fat in the testis is absorbed long before the milt is all shed.

Summary and Conclusions.

In fishes which have pelagic ova and an annual spawning season, the formation of yolk in the developing ova to be shed at a given spawning season commences some months after the close of the preceding spawning season. The active development of the annual crop of ova does not take much more than six months.

The formation of yolk always commences near the surface of the cytoplasm and extends inwards.

In those eggs which develop separate oil globules, a few of these of small size are present long before the formation of yolk commences. The eggs of the mackerel form an exception to this statement. In immature specimens of sole, turbot, brill, &c., examined during the spawning season, the largest ova in the ovaries are found to contain scattered oil globules, and these are also present in the largest transparent ova in spent ovaries of these species. When the formation of yolk takes place in such eggs the oil globules form a zone internal to that of the yolk.

The essential peculiarity of the spent ovary is the presence in it of the ruptured follicles, from which the ripe eggs have escaped. The follicular epithelium in these appears to disintegrate and dissolve. The cavity is obliterated by the contraction of the follicle, which forms a mass of cells and fibres, and is finally absorbed soon after the commencement of the formation of yolk in the eggs for the following season.

In the spent ovary there are a number of eggs which have not reached the ripe condition, which die, and are not discharged from their follicles, but absorbed *in situ*. In the fresh state they are visible as opaque amorphous masses.

Similar opaque masses are also seen in immature ovaries in

which spawning has never occurred. Here also they are aborted dead ova, which are undergoing disintegration and absorption. They are scattered singly in the ovarian tissue, and their development is arrested at an early stage, before the formation of yolk has made any progress, if even it has commenced. In those in which death has only recently occurred the germinal vesicle is seen to be shrunken, and to contain a single large spherical nucleolus.

The vitelline nucleus is first seen as a stained corpuscle in contact with the germinal vesicle. I have not been able to follow the mitosis of the germ-cells, or to trace back the vitelline nucleus, but consider it most probable that the latter is identical with the centrosome which remains in the ovum after the last division of the germ-cell.

In ova of plaice and flounder the vitelline nucleus separates from the germinal vesicle, moves towards the surface of the ovum, and is afterwards found at the inner border of the yolk-layer. It becomes surrounded with yolk, and ceases to be visible.

In Syngnathus acus there are often two or even more vitelline nuclei in a single ovum. These are probably produced by the division of a single body.

If the vitelline nucleus is the centrosome, its disappearance forms an interruption to the persistence of the centrosome as an extra-nuclear body, since the directive spindle is provided with new centrosomes.

The germinal vesicle in the Teleostean ova examined consists at first of a single large nucleolus, a nuclear network, and a surrounding membrane. I could not resolve the network into a continuous filament, or into separate chromosomes.

At the next stage the vesicle is larger, and there are several nucleoli in contact with the nuclear membrane.

In still larger ova the nucleoli are still at the periphery, but there is a central region in the vesicle distinguished by the presence of separate feathery fibrils, the centrosomes of Rückert.

After the formation of yolk has commenced the membrane of the vesicle is wrinkled. The nucleoli migrate from the periphery of the vesicle, and are found around and among the central fibrils.

There are indications in the ova of the turbot that the substance of the nucleoli is absorbed into the central fibrils to form the chromosomes of the polar mitoses, but the actual formation of these chromosomes was not followed.

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DESCRIPTION OF PLATES 2—4,

Illustrating Mr. J. T. Cunningham's paper, "On the Histology of the Ovary and of the Ovarian Ova in certain Marine Fishes."

PLATE 2.

Figs. 1—5.—Ovarian eggs of Trigla gurnardus in various stages of growth and yolk formation, as seen in fresh condition with Zeiss's obj. A, oc. 3. From a specimen examined April 26th, 1895, at Grimsby.

1. Transparent ovum with scattered small globules, probably oily.
2. Large ovum with internal darker zone of oil-globules, external more transparent zone, containing yolk.
3. Later stage, in which the contrast between the yolk layer and the oil-layer is at its greatest.
4. Later stage, in which the yolk layer has become so opaque as to conceal the oil layer.
5. Stage in which the ovum is approaching maturity, and begins to grow transparent again.

Fig. 6.—Portion of ovary of immature brill, $12\frac{3}{4}$ inches long; examined in fresh condition May 30th, 1895, at Grimsby. Zeiss A, oc. 3.

Fig. 7.—Ovum of Trigla gurnardus, ·28 mm. in diameter. From roe containing some ripe eggs, obtained in the market, fixed with picro-sulphuric

acid. Sections stained with hæmatoxylin. Zeiss C C, oc. 3, camera. *y. g.* Yolk globules. *o. g.* Oil globules.

Fig. 8.—Ovum of Trigla gurnardus, ·37 mm. in diameter. From ripe ovary fixed with picro-sulphuric acid and spirit. Sections stained with Delafield's hæmatoxylin. Zeiss C C, oc. 3, camera.

Fig. 9.—Ovum of Trigla gurnardus, ·53 mm. in diameter. From rip ovary fixed with chromic acid ¼ per cent. Sections stained with safranin Zeiss C C, oc. 2, camera.

Fig. 10.—Ovum of Rhombus maximus, ·175 mm. diameter. From ovary of a fish 1 ft. 9¼ in. long, obtained in the market April 12th. Fixed with picro-sulphuric acid. Stained with Delafield's hæmatoxylin. Zeiss C C, oc. 3.

Fig. 11.—Ovum of same species, ·25 mm. diameter. From same series of sections as Fig. 10. Zeiss C C, oc. 3.

Fig. 12.—Ovum of same species, ·35 mm. diameter. From same series of sections as Fig. 10. Zeiss C C, oc. 3.

Fig. 13.—Ovum of Solea vulgaris, ·67 mm. diameter. From ovary of a fish 16½ in. long, obtained in the market September 20th, 1895. Fixed with picro-sulphuric acid and spirit. Stained with hæmatoxylin.

Fig. 14.—Ovum of Pleuronecles platessa, ·33 mm. diameter. From ovary of a large specimen taken from the Plymouth aquarium on August 17th. Fixed with Lobianco's chromosmic mixture. Stained with Ehrlich's hæmatoxylin. Zeiss C C, oc. 3. *v. n.* Vitelline nucleus.

Fig. 15.—Ovum of same species, ·34 mm. diameter, in which the development of yolk is more advanced. From the same ovary as that shown in the preceding figure, but fixed with Kleinenberg's picro-sulphuric acid and stained with Delafield's hæmatoxylin. Zeiss C C, oc. 3.

PLATE 3.

Fig. 16.—Portion of ovary of a spent sole, 17 in. long, from Grimsby market August 1st, 1895. Examined in fresh condition. Zeiss A, oc. 3. *d. o.* Dead eggs, in which yolk development has proceeded to a certain stage.

Fig. 17.—Portion of a section from an ovary of a spent plaice, 15¼ in. long, obtained in the market at Plymouth February 2nd. Fixed with Flemming's mixture. Stained with safranin. Zeiss A, oc. 3. *fol.* Empty follicles, from which the ripe eggs have escaped. *f. e.* Follicular epithelium.

Fig. 18.—An empty collapsed follicle from a section of the ovary of a specimen of Trigla gurnardus, captured July 22nd in North Sea. Fixed in picro-sulphuric acid and spirit. *f. e.* Follicular epithelium. *f. w.* Wall of the follicle. *b. v.* Blood-vessel.

Fig. 19.—A dead aborted ovum, from a section of the ovary of a plaice $11\frac{7}{8}$ in. long, obtained in Grimsby market August 3rd, 1895. Fixed in chromic acid $\frac{1}{4}$ per cent. Stained with Mayer's hæmalum. *f. e.* Follicular epithelium. *g. v.* Germinal vesicle in altered condition, showing one large nucleolus.

Fig. 20.—Ova from the ovary of a plaice $12\frac{1}{4}$ in. long, examined in the fresh condition September 21st, 1895, at Lowestoft. The largest ovum has just died at a stage when the formation of yolk has scarcely commenced. *g. v.* Germinal vesicle.

Fig. 21.—Two ova from section of the ovary of a flounder 11 in. long, taken from Plymouth aquarium July 11th. Fixed in Flemming's strong mixture. Stained with safranin. Zeiss F, oc. 3. *v. n.* Vitelline nucleus.

Fig. 22.—Portion of a section of an ovary of Syngnathus acus, fixed with chromic acid $\frac{1}{10}$ per cent. and picric acid 40 per cent. Stained with safranin, gentian violet, and orange G. Zeiss A, oc. 3. *g. e.* Germinal epithelium at the edge of the ovarian lamella. *v. n.* Vitelline nuclei.

Fig. 23.—Ovum ·049 mm. diameter, from the section shown in Fig. 22, showing the vitelline nucleus in contact with the germinal vesicle. Zeiss E, oc. 3. *v. n.* Vitelline nucleus.

Fig. 24.—The germinal vesicle of an ovum ·27 mm. diameter, in section of the ovary of a plaice from which Fig. 14 is taken. In this ovum yolk-formation had not commenced. Zeiss, apochr. immersion, 2 mm., compens. oc. 8. *ch.* Feathery fibrils, the chromosomes of Rückert. *n.* Nucleoli.

Fig. 25.—Ova from the ovary of a plaice $13\frac{1}{4}$ in. long, examined in the fresh condition September 16th. The formation of yolk has just commenced in the largest ovum, which also shows the wrinkled or mulberry-like form of the membrane of the germinal vesicle. Zeiss C C, oc. 3.

Fig. 26.—Germinal vesicle of an ovum ·39 mm. in diameter, from the same series of sections from which Fig. 14 is taken. Zeiss E, oc. 3. *ch.* Central fibrils. *n.* Nucleoli.

PLATE 4.

Fig. 27.—Germinal vesicle of an ovum ·36 mm. in diameter, from the same series of sections from which Fig. 15 is taken. *ch.* Central fibrils. *n.* Nucleoli.

Fig. 28.—Germinal vesicle of an ovum ·46 mm. in diameter, in a section from the ovary of a turbot 2 ft. $1\frac{1}{2}$ in. long. Fixed with picro-sulphuric acid and spirit. Stained with hæmalum. Zeiss C C, oc. 3.

Fig. 29.—Germinal vesicle of an ovum ·39 mm. in diameter, from the same series of sections as Fig. 28. Zeiss E, oc. 3. *n.* Nucleoli in the form of rods of various shapes.

FIG. 30.—Germinal vesicle of an ovum ·55 mm. in diameter, in a section of the ripe ovary of a specimen of Trigla gurnardus. Fixed with picro-sulphuric acid and spirit. Stained with hæmatoxylin. Zeiss C C, oc. 3.

FIG. 31.—Portion of a section of the ovary of a conger 2 ft. long, killed October 24th, 1888. The figure shows two ovarian lamellæ. Zeiss A, oc. 3.

FIG. 32.—Portion of a section from the ovary of a conger of moderate size. Zeiss A, oc. 3. *f.* Fat-cells. *o.* ova.

FIG. 33.—Ovum from section of portion of the ovary of a conger, which died with nearly ripe ovaries in the Plymouth aquarium March 21st, 1890. Diameter, ·65 mm. by ·56 mm. Fixed with corrosive sublimate and acetic. Stained with borax-carmine. Zeiss C C, oc. 2.

FIG. 34.—Ovum from section of the ovary of an eel 21½ in. long, killed November 15th, 1895. Fixed with chromic acid ¼ per cent. Zeiss C C, oc. 3.

FIG. 35.—Section of testis of conger in the intermediate condition. Zeiss A, oc. 2. *f.* Fat-cells. *sp.* Cords consisting of spermatic cells. *v. d.* Vas deferens.

On Ptychodera flava, Eschscholtz.

By

Arthur Willey, D.Sc.

With Plate 5.

Ptychodera flava has the distinction of being the oldest as well as one of the least known of the Enteropneusta, having been recorded and figured for the first and only time by Eschscholtz in 1825, from material obtained from the Marshall or Rumanzow Islands, in the Pacific, north of the equator.

Eschscholtz, as quoted by Spengel,[1] regarded his Ptychodera as a worm-like animal belonging to the group of the Holothuridæ. Otherwise, however, his description was very defective, and far from being a specific diagnosis. Still, in so far as Eschscholtz stated that the body of the animal was "der Länge nach gespalten," and also from the figure which accompanied his description, and is reproduced in Spengel's monograph, indicating the presence of genital pleuræ (see below), Spengel was enabled to retain the species in his amended genus Ptychodera.

Since 1825 this species has not been re-discovered in the true sense of the term, although Spengel makes the suggestion, which the present contribution provisionally[2] supports, that the fragments of a Balanoglossus obtained by Dr. François in

[1] J. W. Spengel, "Die Enteropneusten des Golfes von Neapel, &c.," 'Fauna und Flora des Golfes von Neapel,' Berlin, 1893.

[2] I say provisionally because absolute certainty can only be arrived at when the form from the first recorded habitat (Marshall Islands) comes to be re-investigated.

the vicinity of Nouméa, New Caledonia, belonged to P. flava. François[1] simply mentions that his native servant one day brought him some fragments of a Balanoglossus, and he makes no further reference to it.

I have found a Ptychodera which may probably be identified with P. flava, especially if it may be assumed that Eschscholtz's figure represents approximately the natural size of the object,[2] in great abundance near the low-tide mark on the small islet of Amédée, upon which stands the lighthouse, some twelve miles out from Nouméa and eight or ten miles inside of the great Barrier Reef of New Caledonia.

It occurred near the surface of the sand, chiefly underneath loose stones, often adhering to the latter, and creeping into the holes with which the coralline blocks are riddled. On a later occasion I found it to be, if possible, still more abundant on the rocky platform of coral limestone which surrounds a great part of the Isle of Pines. This platform is, in places, much excavated, and, while it is exposed at low water, there are numerous rock-pools scattered over it, in some of which many different kinds of seaweeds luxuriate. In the shallower pools Ptychodera flava occurs in great numbers in the sand at the base of or in the neighbourhood of the tussocks of seaweed, being often involved in the roots of the latter. Several species of Nemertines occur in the same locality,[3] but are much rarer than the Ptychodera.

External Features (cf. Fig. 1).

When first taken from their native habitat the individuals of P. flava average in length approximately from $1\frac{1}{2}$ to 2 or even 3 inches, and the intestine is often full of sand. But

[1] Ph. François, "Choses de Nouméa," 'Archives de Zool. expér.' (2), t. ix, 1891, p. 232.

[2] Spengel (loc. cit., p. 190) suspects that the figure given by Eschscholtz represents the animal on a reduced scale. Judging by the material obtained by me in New Caledonia, this need not have been the case.

[3] The exact spot on the Isle of Pines where I found the Ptychodera was situated at the point on the opposite side of the harbour to that on which the military buildings are placed.

after being kept for a short time in captivity they discharge the sand, and the larger specimens may then stretch themselves out to a length somewhat exceeding 5 inches. The collar region may be upwards of a quarter of an inch in length, while the proboscis is normally somewhat shorter. The relatively great length of the collar region is characteristic of the genus Ptychodera. The gill-region measures from half to three quarters of an inch in length, being about as long as the proboscis and collar regions put together. The hepatic region may measure about one and a half inches.

Eschscholtz correctly described the body as presenting numerous transverse folds or annulations. When the intestine is free from sand and the body is consequently not swollen out, these annulations are very prominent ridges, and are characteristic for the species. They are not continuous all round the body, but are interrupted along the dark yellowish or reddish-yellow coloured lines which mark the course of the dorsal and ventral nerve-cords. Here and there, especially in the posterior dorso-lateral region, the annulations are subdivided into islets. The more faintly marked ridges on the outer surface of the genital pleuræ are often similarly subdivided and also branched.

On either side of the dorsal nerve-cord may be seen a dark longitudinal band corresponding in position to the ciliated grooves in the intestinal wall, described by Spengel in other species, and more recently by Hill[1] in P. australiensis.

But these externally visible lines do not cause any interruption in the annulations or islets of the integument, as they do in P. australiensis, according to Hill's description, and as does the single asymmetrical band, present only on the left side, in P. minuta, as described by Spengel.

As indicated by the specific name, flava, the colour of this species is a nearly uniform dull yellow, somewhat deeper in

[1] Jas. P. Hill, "On a New Species of Enteropneusta (Ptychodera australiensis) from the Coast of New South Wales," 'Proc. Linn. Soc. New South Wales,' vol. x (2), 1894. Id., "Preliminary Note on a Balanoglossus from the Coast of New South Wales," ibid., vol. viii (2), 1893.

the more opaque regions of the proboscis and collar. Sometimes the body has a more brownish tinge. The anterior liver sacs, however, offer a relief to the general yellow ground colour, in that they are of a dark greenish-brown colour, while the sacs about the middle of the hepatic region are of a light brown, passing posteriorly into the usual yellow colour. The liver sacculations pass quite gradually behind into the ordinary annulations of the body-wall, and it is not always easy to say which is the last hepatic diverticulum.

In cases where the body has evidently been broken in two in the hepatic region, and the anterior portion of the body, including the whole of the branchial region has been lost at no very distant period, a new collar and proboscis have been added by regeneration immediately in front of the liver-sacs, while the branchial region would no doubt be regenerated later. In such regenerated individuals the collar and proboscis are white and unpigmented.

The proboscis in the normal condition is distinctly grooved in the dorsal middle line, and in this respect P. flava may be compared with Balanoglossus sulcatus, Spengel (cf. fig. 1).

The liver-sacs are not always simple smooth outgrowths, but the larger ones are distinctly lobed, and sometimes present a digitate appearance. An intensification of this lobed structure would probably lead to such a diffuse arrangement of the liver-sacs as is met with in P. erythræa, Spengel.

In only two individuals out of the many that have passed under my observation have I observed them to be infested with a curious parasite (? Ive balanoglossi, Paul Mayer), originally remarked by Spengel in P. minuta, and more recently by Hill in P. australiensis. As described by Hill in the latter species, the parasite occurs in one of the genital pleuræ (in the example here figured on the right side), where it forms a very prominent tubular enlargement" (cf. fig. 2). Hill states that in P. australiensis "a large proportion of the individuals" are infested with the parasite.

Anal Respiration.

I should like to direct the attention of those zoologists who may have future opportunities of observing living Balanoglossus, to the possibility of the occurrence of anal respiration. When the posterior end of the body is protruding from a mass of sand and seaweed I have observed the anal orifice in P. flava to open periodically, widely, and slowly for a second or two, and then to close up again. This may occur two or three times to the minute, and it has apparently no relation whatever to the evacuation of fæces.

In the case of the large Balanoglossus occurring at the Islands of Bimini, in the Bahamas (species not stated), whose development was studied through the Tornaria stage by Morgan,[1] the author states that generally the posterior end of the worm protrudes from the surface of the sand, sometimes as much as an inch. "If," he says, "the spade is thrust rapidly into the sand before the worm has been disturbed, it is easy to cut off from six inches to a foot of the hind end of the body, but impossible to get more of the worm." When it is so deeply embedded in the sand, it is conceivable that the branchial respiration would not entirely suffice for the needs of the animal, and that anal respiration may occur as an accessory to the former.

With regard to the tenacity of life exhibited by P. flava, it cannot compete with Balanoglossus Kowalevskii in this respect, according to Bateson's account (quoted by Spengel, loc. cit., p. 341). In a dish of P. flava, in which the water became slightly turbid overnight, the Ptychodera were nearly all dead, those that were not dead being moribund, and they were outlived by several Annelids.

Genital Pleuræ.

The genus Ptychodera is distinguished from the other genera of Enteropneusta, established by Spengel,—above all by the possession in the anterior region of the body (the branchio-

[1] T. H. Morgan, "The Development of Balanoglossus," 'Journ. Morph.,' vol. ix, 1894.

genital region of Spengel) of lateral wing-like expansions, which can be folded over so as to meet one another in the dorsal middle line, and so to completely embrace the branchial region and the most anterior portion of the hepatic region. These are the genital wings (Genitalflügel) of Spengel, so called because they contain the gonads. We may conveniently refer to them as the genital pleuræ.

In P. flava the genital pleuræ have a very low origin, arising from the ventro-lateral margins of the body, and they constitute remarkable structures. They are very mobile, and in life can be spread out laterally nearly flat; while, as already stated, they can meet over the pharynx in the mid-dorsal line, thus producing a most effective peripharyngeal cavity or atrium, opening to the exterior posteriorly in the neighbourhood of the anterior hepatic region. The genital pleuræ of P. flava attain their maximum development within the branchial region, and maintain it for some distance into the post-branchial region, behind which they gradually decrease in size, and finally die out on the outer sides of the liver-sacs (fig. 1). In P. australiensis, according to Hill, they reach their maximum size somewhat posterior to the gill region.

The Pharynx (cf. Fig. 3).

When, in the living animals kept under observation, the genital pleuræ are spread out laterally, a complete and beautiful view of the entire pharynx is to be obtained. The latter is then seen to stand up, erect and independent, in the middle of the peripharyngeal area, and the branchial bars are visible nearly if not quite throughout their whole length. Dorsally, on either side of and adjacent to the dorsal nerve-cord, two whitish pigmented bands extend throughout the length of the pharynx. These are the bands which in most Enteropneusta form the inner or median boundary of the longitudinal grooves into which the gill-pores open.

In P. flava, however, the U-shaped gill-slits open freely to the exterior throughout their whole extent, and their external openings are therefore not reduced to minute circular or ellip-

tical pores as they are in most other species of Enteropneusta, and indeed in most other species of the genus Ptychodera itself.

In the possession of this remarkable free pharynx P. flava exhibits its close affinity with P. erythræa, Sp., from the Red Sea, and P. bahamensis, Sp., as described by Spengel, especially, as it would appear, with the latter.

As in all species of Ptychodera, so here, the anterior portion of the alimentary is subdivided by a deep longitudinal constriction into a dorsal, branchial, and a ventral œsophageal portion.

From what has been said above it is obvious that P. flava is a very favorable species for studying the structure of the pharynx, since the latter can be easily removed and examined under the microscope.

As might be supposed, there is not much anatomical detail to be added to the exhaustive account given by Spengel of the Enteropneustan pharynx; but there is a point of importance in any comparison between the latter and the pharynx of Amphioxus, which is not emphasised in Spengel's monograph; in fact, so far as I can ascertain, he makes no reference to it whatever, and yet it is of prime significance.

On examining the pharynx of P. flava one cannot fail to be astonished at the relatively enormous size of the tongue-bars as compared with the primary or septal bars (fig. 3). The former are wide, opaque, dark brownish coloured structures, while the former are narrow and semi-transparent. The contrast between the primary and tongue bars in point of size and appearance could hardly be much greater than it is in P. flava.

In fact, it may be stated categorically that in the Enteropneusta in general (as shown by Spengel's figures), and in P. flava in particular, the tongue-bars are much larger than the septal bars; while in Amphioxus, as is well known, the reverse condition obtains, in that the primary bars are larger (but not so much larger) than the tongue-bars.

This is a most important difference, not only in an ana-

tomical and physiological sense, but in a morphological sense also, because while no zoologist would conclude from it that the corresponding structures in the respective types were morphologically different, yet it serves to explain most of those differences in detail which Spengel so elaborately enumerates.

In correlation with the great size of the tongue-bars in the pharynx of the Enteropneusta, it is not surprising to learn the important fact from Spengel that they, rather than the primary bars, are hollow, containing a wide prolongation of the cœlom. "In Folge dessen," says Spengel himself (loc. cit., p. 725), "kann die Zunge der Amphioxus-Kieme nicht, wie die der Enteropneusten, zwei Zungenzinken enthalten, sondern nur eine, die allerdings aus zwei gleichen Hälften zusammengesetzt erscheint."

Thus, according to Spengel's own assertion, the presence of two skeletal rods instead of one only, in the tongue-bars of the Enteropneusta, stands in correlation with their hollow character; while the latter, in its turn, is correlated with the great size of the tongue-bars. In consequence of the occurrence of two separated skeletal rods in the tongue-bars, the dorsal arcuate extremities of the branchial skeletal structures are not continuous as they are in Amphioxus, but are interrupted at each tongue-bar (cf. Spengel, loc. cit., Taf. ii, fig. 21).

The fact that the skeletal rod of the tongue-bar is single in Amphioxus and double in the Enteropneusta is an anatomical difference of importance, but not necessarily and, it may be confidently asserted, not in fact a morphological difference. But it accounts, on the principle of correlation, for other differences upon which Spengel lays such stress. It fully explains the difference which Spengel has had printed in spaced type—namely, that "beim Amphioxus gehört jede Skeletgabel einer einzigen, bei den Enteropneusten aber zwei Kiemen an."

Before coming to the conclusion that "die Kiemen der Enteropneusten und des Amphioxus . . . . wesentlich verschiedne, morphologisch einander nicht entsprechende Bildungen sind," Spengel makes a serious attack upon the synap-

ticula or cross-bars of the pharynx. He says (loc. cit., p. 726), "Bei den Enteropneusten sind die Synaptikel stabförmige Sprossen, welche zwischen einer Zungen- und einer Septalzinke ausgespannt sind. . . . Anders beim Amphioxus. Dort sind . . . . die Synaptikel . . . . zwischen zwei Septalzinken ausgespannt und der dazwischen liegenden Zungezinke nur angelagert."

A glance at fig. 3 accompanying this paper will, I think, show conclusively that the above quotation represents merely a subjective mode of expression. In P. flava the synapticula on one side of a tongue-bar are approximately often quite opposite to those on the other side. As the skeleton of the wide tongue-bar is separated into two halves, the synapticula must necessarily likewise be separated.

By insisting on detailed differences, and more or less neglecting the broader distinctions to which they are subordinate, and which would in great measure account for the former, one can really arrive at any conclusion to which the individual mind is inclined.

To see the synapticula in P. flava the pharyngeal wall must be viewed from the inside, since, as pointed out by Spengel, these structures are placed towards the inner side of the gill-bars, and not on their outer face as in Amphioxus. I have found it a good method to kill in dilute formol, and having removed and opened out the pharynx to mount it in the same liquid. It is well to cut away portions of the genital pleura before preservation in formol, as they are otherwise liable to become glued together in the dorsal middle line.

The number of synapticula in a vertical or dorso-ventral series in P. flava is from ten to thirteen. In this species, as in the majority of Enteropneusta, the gill-bars are not straight as they are in Amphioxus approximately, but are markedly bowed, the convexity facing outwards.

Gonads of P. flava.

Another remarkable feature of the species under consideration, which it presents in common with P. erythræa, P.

bahamensis, and the post-branchial region of P. aurantiaca, is the diffuse arrangement of the gonads. They are not in the remotest degree arranged one after the other, in a manner resembling a paired metameric series, as they are more or less in most other Enteropneusta, but they are scattered in the most irregular way in the substance of the genital pleura (cf. figs. 4 and 5). In correspondence with this multiplication of the gonads, Spengel has shown in the above-named three species of Ptychodera, which he had at his disposal to examine by sections, that several gonaducts may be involved in a single transverse section, each gonad having its own duct, opening to the exterior on the inner surface of the genital pleura. This is also the case in Balanoglossus canadensis, Spengel, in which, however, there is a multiple series of gonads, both medial and lateral, of the gill-pores (cf. Spengel, loc. cit., Taf. 17, fig. 22). Spengel states that he has never found gonads mediad of the gill-pores, either in Ptychodera or Schizocardium.

It has been quite impossible for me, under the circumstances, to prepare a series of sections, and I have had to make the best of hand preparations and dissection. But the diffuse and irregular arrangement of the gonads in P. flava can perhaps be even better realised in in toto preparations than in sections.

Figs. 4 and 5 represent a few of the gonads in male and female individuals respectively, as seen under a low power in small detached portions of the genital pleura. The gonads, as shown in the figures, have in both sexes the most variable outline. Their appearance naturally varies somewhat with the state of contraction or extension of the animal or portion of the animal. Detached fragments of the genital pleura will creep from under the cover-glass like a Planarian.

The integument over the testes on the inner face of the genital pleura in P. flava is characterised by patches of dark brown pigment, and on this account it is possible to distinguish the males from the females (fig. 4*a*).

The female gonads (fig. 5) contain a variable number of ova, which do not take up the whole volume of the gonads, but

are surrounded by a mass of small refringent globules. As the ova in the individual from which the preparation represented by fig. 5 was taken appeared to be sub-mature, it seems not impossible that these globules are of the nature of mucous granules. Spengel says that in P. minuta, when the formation of sexual cells commences the fat-like substance begins to disappear, and is finally quite replaced by ova and spermatozoa. The conditions in P. flava appear to differ from this.

The ova (fig. 6), when obtained free by artificial rupture of the gonads, are seen to be surrounded at an interval by a hyaline double-contoured membrane, the follicular membrane. They are opaque, being filled with fine yolk granules. They measure, apart from the membrane, ·006 mm. in diameter.

With regard to the shape of the gonads in the branchial and post-branchial regions of the genital pleura, there is no difference whatever in P. flava (cf. fig. 4*a*). But in P. minuta and P. Sarniensis, Spengel states (loc. cit., p. 653) that in the branchial region the gonads are almost always simple unbranched sacs, while in the post-branchial region their form becomes more complicated.

In one instance of a male individual of P. flava I observed a much elongated gonad, as long as four or five of those on either side of it taken together.

Systematic Position of P. flava, Eschscholtz (char. emend. mihi).

As might be expected, the short description given by Eschscholtz, beyond indicating by the presence of the genital pleura that his species belonged to the genus Ptychodera in Spengel's system, fell far short of being a satisfactory specific diagnosis.

In consequence of this, Spengel has wrongly placed this species in his genus or sub-genus Tauroglossus. He does not, however, finally assume this, but puts a mark of interrogation against it.

Spengel has, as it seems with justice, subdivided the genus Ptychodera, suggesting the formation of the family Ptychoderidæ, with the three genera, Ptychodera, Tauroglossus, and

Chlamydothorax. But, to avoid confusion, it is more convenient at present to regard these as sub-genera of the genus Ptychodera.

Ptychodera (sensu stricto) has rudimentary genital pleura; to it belong P. minuta, Kowalevsky, and P. Sarniensis, Koehler.

Tauroglossus is distinguished by the dorsal origin of the genital pleura; and to it belong T. apertus, Spengel, T. claviger, Delle Chiaje, T. gigas, Fr. Müller, T. aurantiacus, Girard, and T. australiensis, Hill.

Finally, Chlamydothorax is characterised by the ventral origin of the genital pleura; and to it are assigned Ch. erythræus, Spengel, Ch. bahamensis, Spengel, and probably Ch. ceylonica, Spengel, although the last-named species is only referred to in Spengel's monograph, and not fully diagnosed.

From the account given above of P. flava, it is at once evident that its place is under the sub-genus Chlamydothorax.

The fact of its close affinity to P. bahamensis instead of to its neighbour, P. australiensis, of New South Wales, is interesting in connection with the fact that the Amphioxus (Asymmetron caudatum) which I obtained from the Louisiade Archipelago, and described in a previous communication, is likewise much more closely related to the Bahama species (A. lucayanum, Andrews) than to the Australian forms.

Conclusions.

My investigation of P. flava, necessarily somewhat superficial, has nevertheless sufficed to establish its systematic position, but would hardly allow me to engage in an extended morphological discussion. Still there are a few points upon which one might venture an opinion, especially since it is impossible to have once seen the free, erect, upstanding pharynx of P. flava without being deeply impressed.

Moreover the account, admirable enough, which Spengel

has given of the other two described species of the sub-genus Chlamydothorax was based in each case on a single specimen preserved in spirit, so that an Enteropneust with an eminently free pharynx has never been studied in the living condition before. And this makes a difference.

The conclusions arrived at by Spengel, based as they were upon such laborious and prolonged researches, are entitled to the profoundest respect. Still, with the best will in the world, I cannot follow him in his adverse criticism of the theory as to the relationship of the Enteropneusta to the Chordata. One might conceivably be able to relinquish the idea of the existence of a notochord or its representative in the Enteropneusta, but the gill-clefts are a perpetual fact, and it seems little less than perverse not to recognise it.

Indeed, in his remarks directed against the assumed Chordate affinities of Balanoglossus, it would almost appear that Spengel has carried the analytical method of argumentation to an extreme, and that he is unable to see a general correspondence or homology through the veil of differences in detail. The other extreme is to imagine correspondences where none exist. But it is certainly not necessary to force matters in any way in order to clearly recognise an affinity between the Enteropneusta and the Chordata.

Unfortunately we are here in the presence of one of those distressing instances, so common in the realm of morphology, in which two entirely opposed views can be more or less equally supported. This is due, as Spengel himself points out (loc. cit., p. 722), to the lack of a definite method or criterion in attempting to answer morphological questions.

There is, however, a principle which should be of service in this connection, namely, the principle of correlation between structural and topographical features on the one hand, and physiological or functional peculiarities on the other.

Spengel lays great stress upon the dorsal position of the gill-pores in the Enteropneusta and their ventral (sic) position in Amphioxus, this difference in position being especially indicated by the relations of the vascular system, the propelling

vessel being dorsal in the former and ventral in the latter.[1] This fact, according to Spengel, is in itself almost enough to prove that the gills of the Enteropneusta and of Amphioxus are essentially different structures, and that they do not correspond with one another morphologically. It may, indeed, be said to be Spengel's strongest point in his objection to the supposed Chordate affinities of the Enteropneusta. But, by applying the above-mentioned principle of correlation to the elucidation of this problem, these and other differences may be viewed from quite another aspect.

The question, with what other characteristic in the organisation and mode of life of the Enteropneusta the dorsal position of the gills and gill-pores may be correlated, is not considered by Spengel.

Balanoglossus (employing the name in the wide sense) is a creeping animal,[2] and the ventral surface, as in all creeping animals, is the locomotor surface. Some animals may swim on their backs and others on one side, but all who creep do so on their ventral surface. It is inconceivable that gills or gill-pores could occur on the locomotor surface.

On the contrary, Amphioxus, when active, is essentially a swimmer, and it can no more creep than Balanoglossus can swim. There is, therefore, no such locomotor surface in Amphioxus, and the dorsal region of the primitive alimentary canal is converted into a skeletal support for the body, viz. the notochord.

The gill-slits and gill-pores of the Enteropneusta are placed dorsally, therefore, in correlation with the locomotor function of the ventral surface, the latter not having such a function in Amphioxus; and the general homology between the pharyngeal apparatus in the two types is not thereby prejudiced.

[1] That such a difference in the direction of flow of the blood should not be overrated in the Protochordata is shown by that very well-known faculty of the Tunicate heart of reversing its action and consequently the direction of propulsion.

[2] The kind of burrowing undertaken by Balanoglossus is a variety of creeping, but it creeps too, apart from its burrowing propensities.

To return to Ptychodera flava, the formation of a temporary atrial cavity round the branchial sac by the mutual approximation of the genital pleura is a most striking fact. Spengel calls attention to this, and rightly urges that the peripharyngeal cavity so formed is more readily comparable to the atrium of Amphioxus than anything else that has been suggested. He then goes on to add, however, that in his opinion it is nothing but an entirely superficial resemblance.

Nevertheless it is a real resemblance. The branchial sac being dorsally placed in accordance with the principle above referred to, the peribranchial cavity must be also dorsal in Ptychodera. Presumably there can be no doubt that there is a general homology between the atrium of the Ascidians and that of Amphioxus; and yet in the former it is a dorsal structure (except in the free-swimming Appendiculariæ), and in the latter ventral.

With regard to the synapticula or cross-bars in the branchial skeleton, Spengel (loc. cit., p. 725) draws attention to the fact that they are not present in the genera Glandiceps and Balanoglossus, but are present in Ptychodera and Schizocardium, which, he says, are probably younger phylogenetically. But it is very much open to doubt whether Ptychodera is phylogenetically younger than the other genera of the Enteropneusta.

On page 357 of his beautiful monograph Spengel gives a list of what he regards as signs of a primitive organisation in the group. These are open to the criticism that they are, without exception, all negative properties,—the lack of this, that, and the other.

Then with regard to Ptychodera he says (p. 358), "Als die höchste Form erweist sich endlich Ptychodera." For my part, I deny this, and oppose the view on the following grounds.

In the first place, the positive fact of the diffuse arrangement of the gonads, which is a characteristic of P. flava and of the other species belonging to the sub-genus Chlamydothorax, bears all the marks of an archaic type.

Secondly, it seems only reasonable to suppose that the

elements which compose the branchial skeleton, namely, primary or septal rods, secondary or tongue-rods, and synapticula or cross-rods, were developed at a time and in a type in which their presence was absolutely necessary to prevent the collapse of the branchial sac. It is not so easy to see that their presence is directly necessary to those forms in which the septa between adjacent gill-slits are fused with the thick parenchymatous tissue of the body-wall, and the slits only open to the exterior by minute pores. But they are there notwithstanding, namely, because they are derived from forms in which the presence of skeletal supports for the much-perforated pharyngeal wall was a sine quâ non. Such a form is P. flava, with its free and otherwise unsupported pharynx.

If so much is admitted, then the presence of the genital pleura, covering over the unprotected pharynx, needs no special comment.

Thirdly, the fact that in Schizocardium and in Glandiceps Hacksi the anterior region of the alimentary canal is not subdivided into branchial and œsophageal portions militates strongly against these genera being regarded as more primitive than Ptychodera.

Finally, the habitat of Ptychodera in the littoral zone, often between the tide-marks, is another positive indication of the primitive character of the genus. The greatest depth recorded by Spengel for a species of Ptychodera is 20 feet for P. minuta in the Bay of Rio de Janeiro. Schizocardium (S. brasiliense, Spengel) descends to 18 to 20 fathoms; Glandiceps (G. talaboti, Marion) descends to 450 metres, while another species (G. abyssicola, Spengel) was obtained by the "Challenger" from 2500 fathoms; Balanoglossus Kupfferi, von Willemoes-Suhm, was obtained from 12 to 16 fathoms.

It is very possible that the forms which have migrated into deeper water may have retained some primitive features which are lost to the littoral or tidal forms, just as many of the Elasipoda among the Holothuroidea have retained the primitive connection of the stone-canal with the exterior,

which has been lost by all other recent Holothurids.[1] But it seems clear enough that the occurrence of diffuse gonads, and the free, open pharynx in the sub-genus Chlamydothorax, and particularly in P. flava, are facts which point conclusively to the archaic character of Ptychodera.

Summary of Principal Results.

1. This is the first time that an Enteropneust with a free pharynx has been studied in the living condition.

2. The Ptychodera flava of Eschscholtz (char. emend. mihi) is rightly assigned by Spengel to his amended genus Ptychodera, as shown by the presence of the genital pleura, of external liver saccules, and by the length of the collar region.

3. P. flava belongs to Spengel's sub-genus Chlamydothorax, as shown by the ventral origin of the genital pleura, the diffuse gonads, and the free pharynx.

4. In the fact of the gill-slits being open directly to the exterior throughout their entire length, P. flava is more closely related to P. bahamensis than to any other described species. This is also indicated by the simple rows of paired liver saccules as opposed to the irregular multiple arrangement met with in P. erythræa.

5. The genus Ptychodera (referring more especially to the sub-genus Chlamydothorax) probably represents an archaic type, as shown by the diffuse arrangement of the gonads, the free pharynx, and its littoral habitat; and it is probably not, as Spengel supposes it to be, phylogenetically younger than the other genera of Enteropneusta.

6. The gill-slits, branchial skeleton, and the temporary atrium formed by the apposition of the genital pleura in Ptychodera, offer a general homology to the corresponding structures in Amphioxus and the Ascidians, while presenting many differences in the details of their structure and relations.

7. Some of these differences are comparatively unimportant,

[1] Cf. Hjalmar Théel, "Report on the Holothuroidea," part ii, 'Chall. Rep. Zool.,' vol. xiv, 1886.

and such as might well be expected to occur in distantly related forms with such totally different habits of existence, while others are to be accounted for by a wide interpretation of the principle of correlation between structure and function.

8. Many differences of detailed structure in the pharyngeal wall and its skeletal supports between the Enteropneusta and Amphioxus are to be correlated with the fact that, in the former, the tongue-bars are larger (often, as in P. flava, very much larger) than the primary bars, while in the latter the reverse condition obtains.

Additional Note.

As the Marshall Islands are distant about two thousand miles from New Caledonia, and as the species figured by Eschscholtz renders possible the interpretation that its genital pleura had a more dorsal origin than in the species above described from New Caledonia, it is advisable provisionally to name the latter P. flava-caledoniensis, or simply P. caledoniensis, until the form from the Marshall Islands comes to be re-examined.

Isle of Pines;
August 2nd, 1896.

EXPLANATION OF PLATE 5,

Illustrating Mr. Arthur Willey's paper "On Ptychodera flava, Eschscholtz."

Fig. 1.—Dorsal view of Ptychodera flava. The annulations of the genital pleura are indicated anteriorly on the left side; those of the body proper behind the hepatic region. *pr.* Proboscis. *c.* Collar region. *b* Branchial region; the genital pleura are slightly divaricated, and the pharynx is visible. *p.b.* Post-branchial region. *h.* Hepatic region. *l.s.* Dorso lateral streaks marking the course of the ciliated grooves in the intestinal wall. *d.n.* Line marking the course of the dorsal nerve-cord. *a.* Anus. Drawn from the living object.

Fig. 2.—Anterior portion of another individual from the dorsal side, showing the tuberosity caused by a Copepod parasite. The cross-lines over the infested region represent continuations of the annulations of the genital pleuron.

Fig. 3.—Portion of the pharyngeal wall of P. flava, including the lower portions of three tongue-bars, to show the larger size of the latter as compared with the primary bars. The lower free extremities of the tongue-bars are differentiated from the rest of the bar, being marked off in each case by a pigmented line of demarcation. *t.b.* Tongue-bars. *p.b.* Primary or septal bars. *sy.* Synapticula. *g.s.* Gill-clefts. *œ.s.* Œsophageal ridge (= Grenzwulst of Spengel), forming the boundary between the branchial and œsophageal portions of the alimentary canal. Drawn from a preparation in formol. Zeiss cam. luc., oc. 3, obj. A.

Fig. 4.—Groups of male gonads of P. flava sketched from a detached piece of the genital pleura.

Fig. 4*a*.—A single testis from the branchial region with the pigment patches over its surface.

Fig. 5.—Groups of female gonads of P. flava with ova. The portions of the gonads not occupied by the ova are filled up with coarse mucous (?) granules.

Fig. 6.—An artificially liberated ovum of P. flava surrounded by the double-contoured follicular membrane. Zeiss cam. luc., oc. 3, obj. D.

On the Nephridia of the Polychæta.

Part I.—On Hesione, Tyrrhena, and Nephthys.

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With Plates 6—9.

The following observations on Hesione sicula, Dch. (Fallacia sicula), and Tyrrhena Claparedii, Quatref., were begun last year at Naples, and completed at Oxford; those on Nephthys scolopendroides, Dch., and Nephthys cæca, Fabr., were begun at Roscoff, continued at Naples, and finished here.[1] The material was examined in all cases both fresh and preserved.

Hesione.

The Ciliated Organ.—When full grown, Hesione sicula is of about the size and appearance of Peripatus capensis, and can be easily dissected. On opening up the worm dorsally, and turning back the cut edges of the body-wall, the segmental dorso-lateral blood-vessels are seen extending outwards from the intestine to large bundles of oblique vertical muscles attached to the body-wall between each pair of parapodia. On reaching these bundles the vessels pass downwards amongst the muscles, and reappear near the median

[1] I must express my sincere thanks to the directors and staff of the Zoological Stations at Naples and at Roscoff for the kind and helpful way in which they received me.

ventral line, entering the ventral vessel of that side above the nerve-cord. Dr. Eisig has accurately described and figured the vascular system of Hesione.[1] I can refer the reader to his paper for further details. It is at the point where the dorso-lateral vessel (A of Eisig) first touches the hinder edge of the oblique muscles that the "ciliated organ" is situated.

Fig. 1 is a careful drawing of an inner view of the left side of a portion of a worm hardened and cut in half. It shows the position and relative size of the ciliated organ, *c. o.*, in two segments about the mid-region of the body in front of the posterior extremity of the long pharynx, which has been removed, together with the longitudinal dorsal vessels. Below are seen the longitudinal ventral vessels (*v. v.* of Eisig), into which enter the ventro-lateral vessels, *vl. v.* (*v. vv.* of Eisig). The ciliated organ, of considerable size, is crescent-shaped, with two free horns or limbs, the internal one alone being visible in this drawing. The external horn curls round to the outer side of the bundle of muscles.

A microscopic examination shows that that surface of the organ which faces backwards and away from the muscle is furrowed with deep grooves, alternating with sharp-edged ridges, running transversely to its long axis (figs. 3, 14, and 20). The ridges (fig. 3, *c. r.*) extend up to the very edge along the dorsal free margin; ventrally, on the contrary, they stop short of the edge, leaving a narrow smooth strip beyond. The whole of this grooved surface is densely covered with fine cilia.

A comparison of three transverse sections (figs. 9, 10, and 11), taken from before backwards, of three sagittal sections (figs. 14, 15, and 16), taken from within outwards, of three frontal sections (figs. 20, 21, and 22), taken from above downwards, and of the reconstruction in fig. 2 will make clear the structure of the ciliated organ. It is free along its dorsal margin; but for about the middle third of its length the lower edge is attached to the muscles by a thin septum, formed by a double layer of peritoneum (figs. 3, 21, and 16, at *v. e.*). About half-

[1] H. Eisig, "Ueber das Vorkommen eines schwimmblasen ähnlichen Organs bei Anneliden," 'Mittheil. Zool. St. Neapel,' vol. ii, 1881.

way the ventral edge is produced downwards into a sort of hollow groove (figs. 2, 10, 13, 16, *v. prol.*), which becomes directly continuous with the lip of the nephridial funnel, *neph. f.* This point will be dealt with again later. The ventral prolongation of the ciliated organ (not ciliated at its lowest extremity) lies very near the epidermis lining the intersegmental groove, being attached to it by a fold of peritoneum (fig. 13).

The histology of the ciliated organ presents no striking peculiarity. Like that of Nereis,[1] it is similar throughout, and shows no trace of glandular structure. No cell walls are visible ; the cytoplasm is even, but stains more deeply towards the ciliated surface (fig. 5). Numerous small round or oval nuclei are situated near the outer surface, chiefly along the base of each ridge. The anterior surface of the organ, which is turned towards the muscle, is lined with flat cœlomic epithelium (fig. 5, *c. ep.*).

A pair of ciliated organs occurs in the anterior region of every segment of the body after the third parapodium. The organs are largest in the median segments, where they attain a length of 2 mm. from the end of one horn to that of the other, and possess from fifty to eighty transverse ridges.

The Nephridium.—The nephridium of Hesione opens internally into the cœlom by means of a simple funnel, provided with long, stiff, curved cilia (fig. 17). The lips are reflected all round the aperture, on one side curving round the dorso-ventral blood-vessel, to which the nephrostome is attached (figs. 2 and 13). A narrow neck of varying length leads from the funnel to a wide and somewhat twisted tube (figs. 2, 16, and 17, *neph. t.*), which in turn becomes narrower and more convoluted, forming a mass flattened dorso-ventrally, stretching backwards on the floor of the segment (fig. 2). The nephridial tube does not branch in its course. Finally it widens slightly, and runs into the body-wall, through which it opens by a small pore immediately below the base of the parapodium (fig. 2, *neph. p.*).

[1] "On a New Organ in the Lycoridea, &c.," 'Quart. Journ. Micr. Sci.,' vol. xxxiv, 1893.

Sections show that, as already mentioned, the lip of the nephrostome is directly continuous with the ventral prolongation of the ciliated organ. Since this connection is, I believe, of considerable morphological importance, I have figured it in detail. A transverse section of the two organs is shown in fig. 12 (an enlarged view of part of fig. 10), a frontal section in fig. 22, a sagittal section in fig. 16, and a transverse in fig. 13. From these it will be seen that the ventral prolongation of the ciliated organ is distinguishable from the lip of the nephridial funnel,—the cells of the former being small, deeply staining, and not ciliated; those of the latter being ciliated, less deeply staining, and larger. The convex outer surface of the large cells forming the funnel can be seen in the living tissue (fig. 17).

Passing downwards, a section through the wide tube (fig. 4) shows that the lumen is intra-cellular, and the cilia arise from various places round the inner surface; *cil.*, fig. 6, represents a section through the convoluted mass, where the lumen is cut through seven times. The whole organ is, of course, covered with cœlomic epithelium (*c. ep.*).

Such nephridia occur in all the segments of the body occupied by the ciliated organs.

Tyrrhena.

In Tyrrhena Claparedii the condition of the ciliated organ and of the nephridium is very much the same as in Hesione. As in the latter, the nephridial funnel is in direct continuity with the ventral prolongation of the ciliated organ. The nephridium is of simpler structure, the tube being very little convoluted. The ciliated organ itself is in the same position, but it is less elongated in shape, and its ciliated surface has fewer ridges.

Nephthys.

The Ciliated Organ.—When describing the ciliated organ of the Lycoridea[1] I stated that I had been unable to

[1] Loc. cit.

discover it in Nephthys. At that time I had only been able to study poorly preserved material. After the examination of a large number of living and well-preserved worms, I am able to say that what I had before mistaken for the nephrostome is really the "ciliated organ."

In fig. 7 is given a careful enlarged drawing of the inner view of the right side of some segments taken from the mid-region of the body of a large hardened specimen of Nephthys cæca,[1] and cut in half. The musculature is much more developed and complicated than in Hesione. Thin strands of muscle stretch from the dorsal surface of the intestine outwards to the body-wall, forming an incomplete longitudinal oblique septum, *obl. sept*. Two powerful strap-like longitudinal muscles lie beneath the intestine on either side of the ventral blood-vessel, *v. v.*, and give off transverse muscles at every segment. This ventral band overlies the thick muscular transverse septa, *sept.*, which extend upwards to the dorsal longitudinal muscles, *d. l. m.* Various bundles of vertical oblique muscles extend outside the main longitudinal muscles.

The dorsal vessel, *d. v.*, gives off a dorso-lateral vessel at each segment, *d. l. v.* (dorso-pedal of Jaquet[2]), which passes down to the large bundle of oblique muscles, *obl. m.*, corresponding to those described above in Hesione, and on which are situated the ciliated organs, *c. o.* Thence the dorso-ventral blood-vessel (branch *a* of Jaquet) runs downwards and inwards to join the ventral subintestinal vessel, *v. v.* An offshoot of the ventro-lateral vessel goes to the neural longitudinal vessel of that side.

The ciliated organ when dissected out resembles somewhat the shell of Pecten (fig. 19). It is smaller and more rounded in shape than that of Hesione. The ciliated surface of the organ faces outwards and forwards; it is raised into about twenty sharp ridges, alternating with deep grooves, which

[1] A specimen from St. Andrew kindly given to me by Dr. W. B. Benham.

[2] M. Jaquet, "Recherches sur le Système vasculaire des Annélides," 'Mittheil. Zool. St. Neapel,' vol. vi, 1886.

converge towards the ventral and posterior extremity. The edge of the ridges is extremely thin and jagged (figs. 19 and 26). The clear protoplasm of which it is formed contains a number of fine refringent granules.

Sections show that the organ is composed of the loose tissue characteristic of these worms, which has a fibrous appearance when preserved (figs. 23, 24, and 25, *c. o.*). The nuclei are scarce and scattered irregularly.

In Nephthys the position of the ciliated organ is essentially the same as in Hesione (figs. 7 and 8). Its upper expanded portion rests on the same dorso-lateral vessel, *d. l. v.*, and its lower end forms a sort of deep groove or ventral prolongation, *v. prol.*, running down the body-wall near the intersegmental groove. There appears to be no communication whatever with the lumen of the nephridium.

These ciliated organs occur in both sexes throughout the body in every segment except about the first ten. They are more fully developed in mature than in young specimens.

The Nephridium.—The nephridium of Nephthys is of very remarkable structure, representing, indeed, an entirely new type of Chætopod nephridium, unlike that of any member of that group hitherto described.

The small external aperture lies on the ventral surface of the body below the parapodium, and a little beyond the outer edge of the ventral longitudinal muscles. Leading from this nephridiopore (fig. 8, *neph. p.*) is a narrow canal running upwards, then obliquely forwards through the muscular septum, at which point it becomes narrower still (at all events in sections). Emerging from the septum the nephridial canal runs inwards and forwards, clinging closely to a blood-vessel from the body-wall which joins the ventro-lateral vessel. Ascending the dorso-ventral vessel, and increasing slightly in diameter, it passes along the inner and anterior edge of the ciliated organ on the posterior non-ciliated surface. Finally it emerges on the top of the ciliated organ, where it divides into free branches forming a sort of plume.

The structure of the minute nephridial tube is simple. The

wall is composed of loose fibrous-looking tissue (an appearance probably due to the presence of a large quantity of water in the living cells). A denser layer surrounds the lumen (figs. 24 and 25, *neph. t.*). Nuclei are seen in the wall here and there, but the lumen is probably intra-cellular. As in Nereis, the cilia are disposed along one side of the tube only (occasionally, however, in two opposite rows), and in a nephridium freshly dissected out, the characteristic undulation produced by such an arrangement of cilia is very marked.

The path of the canal as it passes up behind through the substance of the ciliated organ is shown in fig. 19 by the dotted line, *p. neph. t.*, and in the sections drawn in figs. 23, 24, and 25, *neph. t.* On reaching the upper edge of the ciliated organ the tube, with its lumen, divides into three, four, or five branches, more generally four, which float freely in the cœlom. One or more of the branches usually has a T-shaped extremity, and the multiplication of the branches (to the maximum number of five) would appear to take place by the splitting of such a T-shaped branch to its base. The lumen of the nephridial canal ends blindly at the tip of each branch (as far as I have been able to make out). But the chief interest lies in the minute structure of the branches themselves.

Roughly speaking, each branch may be said to consist of a double row of cells, with swollen bases containing the nuclei, enclosing the lumen of the canal (figs. 26, 27, and 28). Each cell tapers off into a long narrow neck, *ne.*, which stretches out at right angles to the axis of the branch on which the cells are set. At its distal extremity the neck-like process becomes slightly swollen, and sharply bent round towards the corresponding cells of the other side. This sort of crook bears at its extreme end a long narrow tube, *tu.*, which runs down parallel to the neck towards the nephridial canal at the base of the cell. Piercing the wall of the canal the delicate tube leads directly to its lumen, even projecting slightly into it. A very long and slender flagellum undulates freely in the tube. Attached by its base at the distal end of the tube, the flagellum passes downwards and out from the tube into the lumen of the

canal, where it is continued for some length, *fl*. Undulations swiftly pass along the flagellum from its base to the free end in the canal.

The protoplasm of the tube-bearing cells is very granular, many of the granules being probably of an excretory nature. At the distal curved end of the neck are generally seen delicate protoplasmic projections, often of great length, floating in the cœlomic fluid, *pr*. These are not cilia, but appear to be rather of the nature of amœboid processes. I have not observed them moving. The tube itself is rather narrower at its base than at the end which enters the canal. It is quite straight as a rule, and oval in section. The wall is composed of a clear refringent substance, apparently of a cuticular nature, which resists the action of caustic potash longer than the protoplasmic parts of the cell.

The large oval nuclei (figs. 26—29, *n*.) have in the fresh tissue a vacuolated appearance. They are remarkable for the extreme avidity with which they take up ordinary nuclear stains, such as carmine or hæmatoxylin. So pronounced is this tendency that in a preparation or section they become deeply stained when the other nuclei are hardly yet affected, and become intensely overstained by the time the other nuclei are sufficiently coloured (see fig. 25, *term. pl. neph*.). A stained preparation of a whole branch of the terminal plume, somewhat flattened out, is figured (fig. 29), showing the nuclei closely packed in an irregular double row.

So far as I have observed the tube-bearing cells are never placed singly, but are ranged in pairs along each side of the canal (fig. 28). The bases and necks of two adjacent cells are closely applied to each other along one side to near the distal extremity, where they diverge to form the terminal crooks. Although the cells are thus firmly fixed to each other, yet a clear line of demarcation can always be detected separating them along the middle line. Occasionally three cells are joined together, as shown in the middle of the branch in fig. 26. The nephridia occur throughout the body of the worm, excepting in the first and last few segments.

From the above description it appears that in Nephthys there is no internal opening to the nephridium, which ends in a bunch of short blind branches. The current in the lumen of the tube produced by the flagella of the tube-bearing cells and the cilia along the canal travels from the terminal branched organ towards the external pore. It seems obvious, then, that excretion must take place through the walls of the nephridium as it does in these organs in the Platyhelmia and Nemertina. Possibly the thin-walled tubes in which the flagella work act as osmotic filters, allowing liquid to pass through from the cœlom. Solid excretory products are more probably conveyed to the lumen of the canal by the cells themselves. The wall of the nephridial canal often contains such a number of granules as to appear of a distinctly brown or greenish hue.

A discussion of the bearing of the facts described above on the question of the morphology of the nephridium and ciliated organ is reserved for a second paper, in which it will be shown that the nephridium of the Glycerids is built on essentially the same plan as that of Nephthys, with flagellated "tube-bearing" cells.

EXPLANATION OF PLATES 6—9,

Illustrating Mr. Edwin S. Goodrich's paper "On the Nephridia of the Polychæta."

List of Reference Letters.

ac. Aciculum. *at. v. e.* Attached ventral edge of the ciliated organ. *b. w.* Body-wall. *c. ep.* Cœlomic epithelium. *c. f.* Ciliated furrow. *cil.* Cilia. *c. o.* Ciliated organ. *cœl.* Cœlom. *cœl. corp.* Cœlomic corpuscles. *c. r.* Ciliated ridge. *cut.* Cuticle. *c. w. int.* Cut wall of the intestine. *d. l. m.* Dorsal longitudinal muscles. *dl. v.* Dorso-lateral vessel. *dp. v.* Dorso-pedal vessel. *d. v.* Dorsal vessel. *dv. v.* Dorso-ventral vessel. *epid.* Epidermis. *f. d. e.* Free dorsal edge of the ciliated organ. *fl.* Flagellum. *go.* Gonads. *int.* Intestine. *irr. m.* Iridescent muscle. *l. v. v.* Left ventral vessel. *lu.* Lumen. *m.* Muscle. *n.* Nucleus. *n. c.* Nerve-cord. *ne.* Neck-like pro-

cess of the cell. *neph.f.* Nephridial funnel. *neph.p.* Nephridial pore. *neph.t.* Nephridial tube. *obl.m.* Oblique muscles. *obl.sept.* Oblique muscular septum. *par.* Parapodium. *ph.* Pharynx. *p.neph.t.* Path of the nephridial tube. *pr.* Protoplasmic process. *r.v.v.* Right ventral vessel. *sept.* Septum. *subint.m.* Subintestinal muscular band. *term.pl.neph.* Terminal plume of the nephridium. *tu.* Tube. *v.l.m.* Ventral longitudinal muscle. *v.l.v.* Ventro-lateral vessel. *v.prol.* Ventral prolongation of the ciliated organ. *v.v.* Ventral vessel.

PLATE 6.

Figs. 1—6.—Hesione sicula.

Fig. 1.—Enlarged inner view of the right half of two segments from the mid-region of the body. Drawn from fresh and hardened specimens.

Fig. 2.—Diagrammatic reconstruction of the ciliated organ, the nephridium, and accompanying blood-vessels, as seen from in front. The cilia are not represented.

Fig. 3.—Portion of the ciliated organ much enlarged. From the fresh. × 95, cam.

Fig. 4.—Section through the wide part of the nephridial tube. × 400, cam.

Fig. 5.—Section through the ciliated organ, across the ridges, and showing an accumulation of cœlomic corpuscles. × 400, cam.

Fig. 6.—Section through the narrow and convoluted region of the nephridial tube, cutting the lumen seven times. × 400, cam.

Fig. 7.—Enlarged inner view of the right half of four segments from the mid-region of Nephthys cæca. A portion of the intestine is represented in front. Drawn from a hardened specimen.

Fig. 8.—Diagrammatic reconstruction of the ciliated organ, nephridium, and accompanying blood-vessels of Nephthys scolopendroides, as seen from in front. The cilia are not represented.

PLATE 7.

Figs. 9—17.—Hesione sicula.

Figs. 9, 10, 11.—Portions of three transverse sections, taken from before backwards, showing the relation between the ciliated organ and the nephridium. × 20, cam.

Fig. 12.—More enlarged view of portion of Fig. 10, showing the connection between the ciliated organ and the nephridial funnel. × 130, cam.

Fig. 13.—Similar figure of a portion of a transverse section from another series, showing the continuity between the ventral prolongation of the ciliated organ and the lip of the nephridial funnel. × 400, cam.

FIGS. 14, 15, 16.—Portions of three sagittal sections, taken from within outwards, illustrating the relation of the nephridial funnel to the ciliated organ. × 250, cam.

FIG. 17.—Enlarged view of the nephridial funnel freshly dissected out.

FIG. 18.—Piece of the dentated edge of a ridge of the ciliated organ of Nephthys scolopendroides, much enlarged.

FIG. 19.—Enlarged view of the ciliated organ of Nephthys scolopendroides, freshly dissected out. The cilia are not represented.

PLATE 8.

Figs. 20—22, Hesione sicula. Figs. 23—25, Nephthys scolopendroides.

FIGS. 20, 21, 22.—Portions of three frontal sections, taken from above downwards, showing the ciliated organ and its ventral connection with the nephridium. × 40, cam.

FIG. 23.—Portion of a frontal section of a female, showing the ciliated organs and nephridial tubes. × 100, cam.

FIG. 24.—More enlarged view of the ciliated organ and nephridial tube, from the same series as Fig. 23. × 400, cam.

FIG. 25.—Portion of a transverse section, showing the nephridial tube cut twice, the ciliated organ, and a piece of the terminal branch of the nephridium. × 130, cam.

PLATE 9.

Figs. all of Nephthys scolopendroides.

FIG. 26.—Enlarged view from a fresh specimen of a small piece of the ciliated organ and a branch of the nephridial plume, showing the tube-bearing cells.

FIG. 27.—Semi-diagrammatic transverse section of a branch of the terminal nephridial plume.

FIG. 28.—Semi-diagrammatic longitudinal section of the same.

FIG. 29.—Branch of the nephridial plume stained and somewhat flattened out. × 500, cam.

The Pre-ocular and Post-ocular Tentacles and Osphradia of Nautilus.

By

Arthur Willey, D.Sc.

With Plate 10.

The occurrence of a special tentacle in front of the eye and another behind the eye in Nautilus is well known. These tentacles resemble the large number of remaining tentacular appendages in being ringed, and also in being retractile within sheaths, but differ from them in almost every other respect. In the first place, most of the tentacular appendages of Nautilus have essentially an adhesive function, to which is related a prehensile function. They are employed for seizing hold of food and for attachment to surfaces.

Attachment is effected by the definite suctorial ridges on their lower and inner sides (fig. 3). When attached by its tentacles, Nautilus holds on with considerable tenacity;[1] and sometimes in forcibly detaching it some of the tentacles break off, and remain fixed to the surface of attachment. The shape of a section of the tentacular processes of Nautilus is that of a spherical triangle, the base of which corresponds to the suctorial ridges, while the apex and sides are directed

[1] In the next number of this 'Journal' Dr. Willey's figure of a pearly Nautilus crawling on a glass surface by means of its tentacles will be published, as also some notes on the cœlom and pericardium of Nautilus. His discovery of the deposited eggs of the Nautilus has been published with figures of the eggs in the 'Proc. Roy. Soc.'

outwards, and are distinguished by a deep brown pigment. The suctorial ridges have a pale neutral tint. We may, therefore, speak of those tentacles which are used for prehension and attachment as the adhesive tentacles. Their function speaks for the pedal nature of the tentacular processes of Nautilus, as does also their innervation; and further, their function allows them to be compared with the arms of the Dibranchiata. If the comparison is carried into detail the suctorial ridges of the former would correspond with the definite suckers of the latter.

That prehension as well as adhesion is a function of the Molluscan foot is well illustrated by the method of capture of a species of Oliva at Lifu. This species can be obtained in large numbers by employing a line baited with the animal of a land-shell (Placostylus). The Oliva wraps its foot round the bait, and so can be lifted out of the water and landed.

Recently arguments have been brought forward by Kerr[1] against the supposed pedal nature of the Cephalopod arms in general and the tentacular processes of Nautilus in particular. If embryological data are not to be trusted on account of the large quantity of food-yolk in Cephalopod ova, we are obliged to consider, among other things, function and innervation. With regard to the latter, Kerr throws doubt on the generally accepted identification of the sub-œsophageal ganglionic masses of Cephalopods. It is well, however, to remember that in Dentalium the ganglionic centres have the same topographical relations as in Nautilus, the undoubted pedal ganglion being placed far in front of the pleural and visceral ganglia.

Returning to the tentacular appendages of Nautilus, it will not be surprising to learn that the adhesive tentacles are not ciliated; but it is necessary to mention this negative fact, because the pre-ocular and post-ocular tentacles are ciliated. On the side corresponding to the suctorial ridges of the adhesive tentacles the annulations of the pre- and post-ocular

[1] J. Graham Kerr, "On some Points in the Anatomy of Nautilus pompilius," 'Proc. Zool. Soc.,' 1895.

tentacles form deep grooves, between which the ridges project as prominent lamellæ. The upper and lower surfaces of the lamellæ and the bases of the grooves are covered with vibratile cilia (fig. 2). There can be little doubt that the pre-ocular and post-ocular tentacles of Nautilus represent tentacular processes, homologous with the adhesive tentacles, which have been modified to serve an accessory olfactory function. We will therefore speak of them as the olfactory tentacles, in contrast to the adhesive tentacles. As is well known, there is a rhinophore in Nautilus, placed directly below the eye, corresponding to the rhinophore or olfactory groove of the Dibranchiata. In Nautilus there is a small tentacle as well as a fossa in connection with the rhinophore, but it is not annulated and not retractile.

The olfactory tentacles (apart from the rhinophore) when extended stand out from the body nearly at a right angle, the pre-ocular tentacle being directed slightly forwards, and the post-ocular tentacle usually tending backwards (fig. 1). The ciliated olfactory lamellæ are directed strictly forwards.

In the living Nautilus the olfactory tentacles otherwise offer a strong contrast to the adhesive tentacles by their almost uniform white colour. When examined under the microscope there is found to be a little brown pigment in the annulations and at the edges of the lamellæ, but when viewed in toto under water the general colour effect is white.

Moreover the adhesive tentacles can be touched without necessarily being retracted, but at the slightest contact with a foreign body the olfactory tentacles are instantly retracted within their sheaths.

The presence of accessory olfactory tentacles in Nautilus can, I think, be related to an essential bionomical difference between the existing Tetrabranchiata and the Dibranchiata.

Nautilus finds its food chiefly by the sense of smell, while it is a matter of more or less common observation that the Dibranchiata with their remarkably perfect eyes pursue their quarry by the sense of sight. This difference, which is to a certain extent evident from the facts of organisation, is further

emphasised by the different modes adopted by the natives for trapping these animals.

One of the surest ways of obtaining Nautilus, and, in fact, the method by which I have obtained most of my specimens at Lifu, is to bait the fish-basket with the cooked and bruised exoskeleton of Palinurus or an allied form. The strongly scented "potage" so produced is then wrapped up in cocoa-nut fibre like a small parcel, and placed in the fish-trap overnight. There is therefore nothing to be seen, but on the other hand there is something to be smelt, and by this means I have obtained as many as ten Nautilus at one time.

For taking Octopus the natives of Lifu employ a very different method. A rounded oval piece of stone backed by a well-fitting piece of the shell of a species of Cypræa, to which are added pieces of leaf to simulate legs and tail, is dangled along the surface of the water at the end of a line. The natives say that the Octopus mistakes this for a rat, against which it has a special grudge; but whatever the reason may be, the fact remains that Octopus attacks this singular non-scented contrivance, and so is captured.

The Osphradia of Nautilus.

In an article published in 'Natural Science' for June, 1895 (vol. vi, pp. 405—414), I suggested that the post-anal papilla represented a pair of osphradia—namely, the inner osphradia, in addition to the outer osphradia which were originally described by Lankester and Bourne. The nerve to the outer osphradium on each side is bound up together with the nerves to the branchiæ into a common trunk, the respective nerves separating out from the trunk towards the base of the branchiæ. The nerve supplying the inner osphradium has a generally independent course close beside the above-mentioned common nerve-trunk. I cannot believe that this slight difference in the behaviour of the osphradial nerves constitutes an obstacle

to the identification of the post-anal papilla as a pair of osphradia, as has been recently suggested.[1]

However, by means of macroscopic sections of fresh material the presence of vibratile cilia on the sensory epithelium of both the inner and outer osphradia can be demonstrated, and this I regard as the final proof of the osphradial character of the so-called post-anal papilla (figs. 4, 5). The sensory epithelium of both osphradia is distinguished from the surrounding ectoderm both by the presence of the cilia and by the general absence of goblet-cells.

The olfactory lamellæ of the accessory olfactory tentacles and the sensory epithelium of the osphradia are the only places where I have observed vibrating cilia in Nautilus hitherto.

EXPLANATION OF PLATE 10,

Illustrating Dr. Arthur Willey's paper on "The Pre-ocular and Post-ocular Tentacles and Osphradia of Nautilus."

Fig. 1.—View from above of the "hood" and tentacles of Nautilus during life.

Fig. 2.—To show the ciliated ridge of the olfactory tentacles.

Fig. 3.—To show the suctorial ridges of the ordinary tentacles.

Fig. 4.—The ciliated structure of the surface of one of the anterior osphradia.

Fig. 5.—Ditto of the posterior.

Fig. 6.—Outline sketch of the Gastropod Aplustrum to show "laminate organ" comparable to the olfactory tentacles of Nautilus.

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[1] W. Garstang, "The Morphology of the Mollusca," in 'Science Progress,' vol. v, March, 1896.

On Heteroplana, a New Genus of Planarians.

By

Arthur Willey, D.Sc.

Since I have been in Lifu I have obtained one specimen only of a very remarkable flat-worm which must form the type of a new genus and a new family. The quite unexpected relations of symmetry presented by this form, which I will call Heteroplana, speak for the accuracy of the observations, although I should be more satisfied if I could obtain more examples of it.

Heteroplana presents externally a sub-symmetrical appearance, but in its structure it is characterised by what may be roundly described as the atrophy of the left half of the body. The left intestinal diverticula are aborted. The mouth is placed at the middle of the length of the body, but approximated to the left margin. The cerebral ganglion is similarly approximated to the left margin. Through the whole body, and especially prominent in the anterior and posterior regions, is a close reticulum formed by the anastomosis of fine moss-like tubules which probably constitute the genital apparatus.

I had the specimen under observation for a comparatively long time, and made several drawings from it, one of which I enclose. On account of its remarkable relations of symmetry I should place this genus in the order Archiplanoidea, established by me for the reception of Cœloplana and Ctenoplana, because, although it is very different from the two latter forms, yet it would appear to be more nearly related to a biradial type than to a bilateral type like the Planarians. This seems to follow from a consideration of such a form as

Ctenoplana. I have proved, as I think conclusively, that the tentacle axis of Ctenoplana corresponds to the longitudinal axis of the flat-worms. But when Ctenoplana creeps, it does so, not with one of its pinnate tentacles directed forwards, but with one side foremost.

In Heteroplana as indicated in the accompanying figure, the locomotion is usually conducted in a somewhat one-sided fashion, and the number of marginal eyes on the forwardly directed lobe is more than twice that on the corresponding lobe on the opposed side of the frontal region. There are no tentacles in Heteroplana.

Apart from the presence or absence of pinnate tentacles I think it is fairly evident that Heteroplana is almost directly derivable from a biradial type of the same grade of organisation as Ctenoplana.

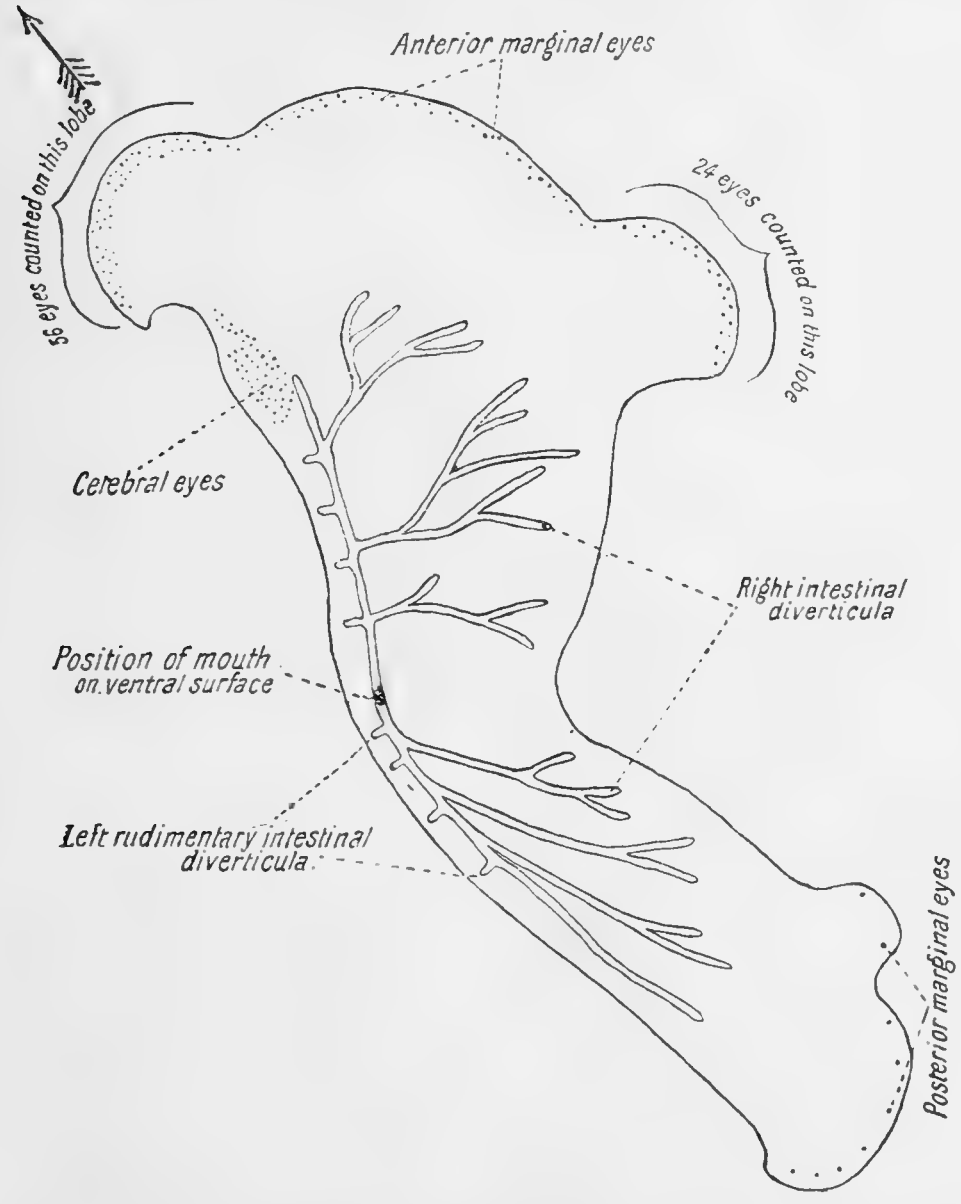
But in Heteroplana the direction of locomotion (creeping) has been definitely localised, and the side (namely, the right side) to which the preference has been given has for that very reason predominated over the other (left) side. In other words, in Heteroplana there is hypertrophy of the right side and atrophy of the left.

Heteroplana is perhaps the completest novelty that I have obtained. I trust that my contributions to a knowledge of the primary relations of symmetry in animals in respect of this form and of Ctenoplana (see 'Quart. Journ. of Micr. Sci.,' vol. xxxix) will prove of value. There can be no doubt that some form of symmetry is primarily present in all animals from the Protozoa (amœboid animals excepted) to the Vertebrata; amorphous groups like the Gastræidæ (Physemariidæ) and the Porifera being of a secondary or derived nature. Radial symmetry of every description seems to have been preceded by what may be called polar symmetry or directive polarity, as seen in the ciliate Infusoria, the Dicyemidæ, and the larvæ of Actinians, &c.

Heteroplana Newtoni was found by me on the lower side of a Madrepora-stock in Sandal Bay, Lifu, on August 12th, 1896. The general colour, especially of the mid-region,

is orange-yellow. The expanded anterior region and the less expanded posterior region are transparent and almost colourless, whilst the moss-like, dendritic, anastomosing gonadial tubules are to be plainly seen.

These transparent extremities of the body (especially the anterior region) possess great adhesive power. There are marginal eyes in front and behind, but not at the sides of the body. The shape of the anterior portion of the body is constant and highly characteristic. The two posterior lobes, one smaller and one larger, are also constant.



Heteroplana Newtoni, nov. gen. et sp. (The arrow indicates the usual direction of locomotion.)

The Adhesive Tentacles of Nautilus, with some Notes on its Pericardium and Spermatophores.

By

Arthur Willey, D.Sc.

With Plate 11.

Adhesive Tentacles.—In the plate (Pl. 11) accompanying this note I have given a sketch (fig. 1) taken from a living nautilus showing the way in which the ordinary tentacles are applied to a foreign object lying in the water (in this case a glass vessel) so as to adhere very firmly to it; whilst the pre-orbital and post-orbital tentacles have no such adhesive power, and remain erect on either side of the ocular bulb. As I mentioned in a previous note, the ordinary tentacles sometimes adhere so tightly to a foreign body as to become torn away from the animal when the foreign body is forcibly removed.

Spermatophore.—In Plate 11, fig. 3, is given a figure of Nautilus pompilius, showing the spermatophore sac in situ at the dorsal base of the buccal cone. This position was originally discovered by Van der Hoeven; but it has not been seen since, or at least not described, and Van der Hoeven's figures are a little wanting in clearness.

Pericardium. — The structure of the vena cava of Nautilus, discovered by Owen, was again very exactly described and figured by Keferstein in 1865. This was apparently overlooked by Mr. Kerr in his work "On some Points in the Anatomy of Nautilus pompilius" ('Proc.

Zool. Soc.,' 1895). This important paper, and one by Béla Haller ("Beiträge zur Kenntniss der Morphologie von Nautilus pompilius") in Semon's 'Forschungsreisen in Australien, &c.,' 1895, have appeared since I left England. In a former paper I dealt with the question of the genital arteries of Nautilus.

There is another matter concerning Mr. Kerr's work to which I wish to refer. Mr. Kerr (op. cit.) says, "The true cœlom (viscero-pericardial sac, Owen) has received comparatively little attention from previous investigators, Grobben and Lankester being the only authors who devote to it more than a few passing words."

Haller (op. cit.), referring to the orifices in the pallio-visceral ligament leading from the pericardium into the visceral portion of the cœlom, says, "doch so viel ich aus der Literatur ersehe, wurden diese Oeffnungen zwischen den beiden Cölomtheilen weder ihrer Zahl noch ihrer Grösse und Lage nach, beschrieben."

What I wish to point out is that both these authors have evidently overlooked Huxley's brief but pregnant memoir "On some Points in the Anatomy of Nautilus pompilius" ('Journ. Linn. Soc.,' "Zool.," vol. iii, 1859, pp. 36—44), in which the delimitations of the cœlom and the position and features of the three openings leading from the pericardium into the visceral portion of the cœlom are described with perfect accuracy and with marvellous clearness. It was not necessary for Huxley to have illustrated his article, so lucid is his account, and as a matter of fact the figures he does give do not help much. But I might simply transcribe Huxley's description as an explanation of the figure (Pl. 11, fig. 2) which I have drawn from a dissection showing the three openings in question. I have rarely read an account of somewhat complicated anatomical relations so perfectly and immediately intelligible as the one above referred to by Huxley, and it is undesirable that such work should be ignored.

EXPLANATION OF PLATE 11,

Illustrating Dr. Willey's note on "Adhesive Tentacles, Spermatophore, and Pericardium of Nautilus."

Fig. 1.—Nautilus macromphalus attached to the side of a vessel by its cephalic (pedal) tentacles; not by their extremities, but by their recurved sides. Lifu, September 9th, 1896.

Fig. 2.—Nautilus pompilius dissected to show the pericardium by throwing back the ventral integument. June, 1896. *p. v. l.* Pallio-visceral ligament (Huxley). *c. l.* Cardiac ligament. *o. p. l.* Outer or posterior pericardial ligament. *i. p. l.* Inner or anterior pericardial ligament. *o. p. g.* Pericardial follicles of outer or posterior renal organ. *o. r.* Outer or posterior renal sac. *i. p. g.* Pericardial follicles of the inner or anterior renal organ. *i. r.* Inner or anterior renal sac. *r. m.* Reflected flaps of median ventral integument. *m.* Mantle.

A, B, C. "The three apertures of communication between the two divisions of the fifth chamber" of Huxley—that is to say, between the pericardial cœlom and the perivisceral cœlom (the four other chambers treated of by Huxley being the two pairs of renal sacs).

H. Systemic heart (ventricle).

Fig. 3.—Male Nautilus pompilius, to show spermatophore sac in position. Blanche Bay, New Britain, May 24th, 1895.

CORRIGENDA.

(To be bound opposite to Plate 11.)

The following corrections should be noted in the lettering of fig. 2 of the plate (Pl. 11) accompanying Dr. Willey's note on "The Adhesive Tentacles of Nautilus, &c. &c.," in Part 2 of Vol. 40 of this Journal:

1. For "ovarian artery" read anterior pallial artery.
2. For "siphonic artery" read posterior pallial artery.
3. The bristle is passed through the left external viscero-pericardial orifice.

On some Modifications of Structure subservient to Respiration in Decapod Crustacea which burrow in Sand; with some remarks on the Utility of Specific Characters in the genus Calappa, and the description of a new species of Albunea.

By

Walter Garstang, M.A.,
Fellow and Lecturer of Lincoln College, Oxford.

With Plates 12—14.

A good deal of scepticism has been expressed in recent years by various writers as to the utility of the more trivial features which distinguish the genera and species of animals from one another. I do not think that such scepticism can excite much surprise if one remembers that the vast majority of "biologists" are almost exclusively engaged in the study of comparative anatomy and embryology. The amount of attention paid to these branches of biology has long been utterly out of proportion to the scant attention devoted to the scientific study of the habits of animals and of the function of the organs and parts composing their bodies. With isolated and noteworthy exceptions, the only naturalists who seriously add to our knowledge of the latter subjects are those who travel in distant countries, and who are thus thrown into close relations with animals in their native haunts. Yet all the time there are thousands of forms living on our own coasts and almost at our very doors of whose detailed habits and life-conditions we know practically nothing. I venture to think that the time has come for consideration whether the

subject of bionomics (in Professor Lankester's sense of the word) should not receive more adequate recognition than it does at present in the biological curriculum of our universities. That such recognition would almost immediately produce effects in a rapid extension of our knowledge is certain; and the subject is invested with so much intrinsic interest, as well as with such important bearings on the problems of evolution, that I believe such recognition would also have the effect of attracting many students to the pursuit of morphology who at present avoid it as a region of mere comparative anatomy.

The present paper contains an account of some modifications of form in certain exotic Crustacea upon which a new light appears to be thrown by my recent researches upon the habits of certain less specialised forms which inhabit British seas (1896, 1897). My thanks are tendered at the outset to Professors Lankester and Poulton for the facilities which they have kindly placed at my disposal during my study of these and numerous other forms of Decapod Crustacea. I am particularly indebted to Professor Lankester for the services of his artist, Mr. Bayzand, from whose beautiful drawings my figures are copied.

1. Calappa granulata, Linn.

The figure (Plate 12, fig. 1) represents a front view of a specimen of this crab as seen when lying flat upon a plane surface.

The genus Calappa is distinguished, among other points, by the extraordinary size and shape of the chelipeds, which in flexion are pressed tightly against the inferior surface of the carapace, and by interlocking with one another form a sort of buckler, the anterior and upper margins of which exactly coincide with the anterior and lateral margins of the crab's carapace. If the "hands" (propodites) of the chelipeds were of the simple form usual among crabs, the anterior part of the buccal apparatus would be visible even when the chelipeds were pressed against the carapace, as is the case, for example, in the species of Atelecyclus or in the species of Matuta

figured in this paper (Plate 12, fig. 1). But in Calappa the buccal apparatus is completely covered by the chelipeds when flexed, owing to the fact that these appendages are here provided with a pair of remarkable cockscomb-like crests on the upper (anterior) margin of the hands (fig. 1, *d*). The margins of these crests are serrated, but otherwise coincide with the outline of the anterior region of the carapace during flexion of the chelipeds.

What is the meaning of this extraordinary opercular apparatus furnished by the chelipeds in this genus, and why do the chelipeds fit so nicely to the carapace during flexion?

I have found no satisfactory explanation from a study of the literature dealing with the habits of these crabs. Risso (1816, p. 18) states that crabs of this species "inhabit holes in steep rocks 20 to 30 mètres deep. When they are obliged to abandon their usual retreats they withdraw their feet under the carapace, draw their chelipeds together, and let themselves fall like balls to the bottom of the water." He implies, in fact, that the arrangement is an adaptation for defence, similar to the ball-forming habits of the common wood-louse (Oniscus), or of its marine relative Armadillo.

But it is now known that crabs of this genus do not usually live upon rocky, but upon sandy shores, and that they possess markedly fossorial habits (Macgillivray, 1852, p. 102; Henderson, 1893; Schmidtlein, 1879, pp. 24, 25). I understand that the sand-burrowing habits of this particular species have been frequently observed in the tanks of the Naples aquarium.

The only other suggestion which I have met with as to the function of the chelipeds is contained in Schmidtlein's interesting observations on specimens in the Naples aquarium (l. c., pp. 24, 25). Schmidtlein states that the chelipeds serve "zum Einwühlen und zum Schutze." It would appear from his observations that the crab, if placed in a tank containing a number of hungry fishes, protects from their thievish attacks any morsels of food which it may be engaged in devouring, by hiding them beneath its tightly closing chelipeds. Neither this use, however, nor the act of burrowing, provides any

explanation of the wonderful exactitude with which the chelipeds fit the carapace, nor of certain peculiar relations which they bear to the respiratory channels to be now described.

It is well known that in most, if not in all, of the Oxystome crabs, the exhalant orifice of the respiratory canal is carried to the tip of the snout by a prolongation of the endopodites of the first maxillipeds, either alone as in Calappa (fig. 1, *a*, *b*), or with the co-operation of the endopodites of the third maxillipeds, as in Matuta (fig. 2, *a*, *b*).

The inhalant orifice has been shown, on the other hand, by Milne-Edwards to vary considerably in position in the same group of crabs. In most forms it is situated at the base of the chelipeds, between these appendages and the adjacent edge of the branchiostegite ; but in Dorippe,[1] as already shown by Milne-Edwards (1834, i, p. 89), it is situated further forwards, and has the form of a deep emargination of the edge of the pterygostomial portion of the branchiostegite. In Ebalia and the Leucosiidæ in general, the inhalant apertures occupy a position which has hitherto been regarded as unique, being situated beneath the orbits on the outer sides of the exhalant orifice. They lead into a pair of deep afferent gutters excavated in the external wall of the pterygostomial[2] plates. The gutters are converted into closed canals by opercular expansions of the exopodites of the third maxillipeds. In the

[1] Examination of a specimen of this crab has convinced me that in this form also the chelipeds furnish an operculum for the peculiar afferent orifice, but in a way quite different from that described below for Calappa and Matuta.

[2] Not in the prelabial plate, as stated by Milne-Edwards (1839, p. 135) and Dana (1852, p. 62). The language used by the former naturalist is somewhat vague, but Dana at any rate has completely misinterpreted the relations of the afferent gutter to the carapace in this family. I may here draw attention to the fact that in the crab Myctiris platycheles of Australian seas, the edge of the pterygostomial plate also exhibits a deep gutter, which extends from the infra-orbital region to the afferent aperture at the base of the cheliped, and thus closely resembles the afferent canal of the Leucosiidæ. The gutter is clearly subservient to the respiratory function, and interesting results will undoubtedly reward the first careful investigator of the respiratory processes in this aberrant representative of the Catometopa.

remaining Oxystomata the exhalant orifices have been regarded as situated at the base of the chelipeds, i. e. as occupying the normal position.

It is perfectly true that the proximal aperture by which water enters the branchial chamber does occupy this position; but an examination of the crabs Calappa and Matuta has revealed to me that before the water enters the branchial chamber by this aperture, it has in all probability previously traversed certain accessory channels formed by the apposition of the chelipeds to the inferior surface of the carapace.

In the case of Calappa granulata it may be observed that on each side of the median exhalant orifices there exists a well-marked slit-like aperture between the infra-orbital margin of the carapace, and the serrated crests of the apposed chelipeds (fig. 1, *e*). If one of the chelipeds be now withdrawn from its flexed position, it may be noticed that this slit-like aperture leads downwards and backwards as a well-marked channel to the inhalant aperture at the base of the cheliped. The channel is bounded internally by the ridge-like outer edge of the third maxilliped; its other boundaries are furnished by the approximated surfaces of the inner face of the chelipeds, and the pterygostomial region of the carapace. It is in fact an accessory channel formed by the cheliped; and the inhalant aperture is carried by its means to the tip of the snout in precisely the same way that in the Leucosiidæ the corresponding aperture is carried forward by means of the exopodite of the third maxilliped.

I propose to call this accessory water-channel the "exostegal canal." In its situation on the external side of the pterygostomial portion of the branchiostegite it contrasts with the more primitive branchial canals, which are endostegal in position. It differs from the characteristic afferent canal of the Leucosiidæ in requiring the participation of the chelipeds to complete its external wall, whereas in the Leucosiidæ the third maxillipeds are alone concerned in bringing about the same result.

Attention may now be directed again to fig. 1, which shows

that in Calappa granulata of the Mediterranean, the antero-lateral margins of the carapace are free from denticulations, while the propodial crests of the chelipeds are conspicuously serrated.

On the other hand, it will be remembered that the presence of teeth along the antero-lateral margins of the carapace is a conspicuous feature of a great number of the less specialised types of crab (Cyclometopa), as, for example, in Carcinus and the Portunidæ in general.

Why are these marginal teeth so commonly found among other crabs, while they are absent in Calappa granulata?

I have recently determined (1897) by experiments upon forms such as Atelecyclus and Bathynectes, which possess well-developed marginal teeth, and which adopt sand-burrowing habits, that when these crabs are partially or wholly buried in sand, the chelipeds are approximated to the branchial regions of the carapace, as in Calappa and Matuta, but in such a manner that the marginal teeth of the antero-lateral regions of the carapace exactly overhang the elongated slit-like orifice between chelipeds and carapace. Moreover, during life a current of water can be demonstrated incessantly pouring into this orifice between the marginal teeth of the carapace, whence it traverses the accessory channel between chelipeds and carapace, in order to reach the afferent branchial aperture at the base of the chelipeds.

These types, therefore, possess a pair of functional exostegal canals, which differ from those which I have described in Calappa merely in their greater extent and in their less specialised form. I have, moreover, shown that the marginal teeth which overhang the orifices of these canals act as a "coarse sieve or grating" which prevents the accidental intrusion of foreign bodies, such as grains of sand, into the respiratory canal.

It seems to me to be accordingly probable that the absence of spines and teeth from the antero-lateral margins of the carapace in Calappa granulata is functionally correlated with the restriction of the exostegal orifice in this form to the

infra-orbital region of the carapace, where the teeth on the margin of the propodial crests of the chelipeds have taken over the sieve-function which in more primitive types is discharged by the marginal spines of the carapace.

A similar argument may be employed to explain the absence of marginal teeth on the carapace in Ebalia and the Leucosiidæ in general. In these forms, also, there is a very highly specialised exostegal afferent canal, the aperture of which is restricted to a very narrow area beneath the orbit of the crab. Since the canal is completed in this group by the exopodite of the external maxilliped alone, the respiratory process is independent of chelipeds and carapace margins alike, and there is consequently no necessity for sieve-forming teeth on either of these parts of the body. Whether, however, this independence was maintained throughout the whole ancestral history of the Leucosiidæ is another matter; the considerations advanced in the present paper seem to me to render it probable that the peculiar respiratory adaptations of these forms have also been derived from the more generalised type of adaptation found in the Cyclometopa. In that case the Leucosiidæ have lost the spines on the carapace margins pari passu with the restriction of the area occupied by the exostegal afferent current of water;[1] and the chelipeds have re-acquired their independence simultaneously with the expansion of the third maxillipeds to form an opercular floor to the exostegal gutter. In Calappa, both the chelipeds and the third maxillipeds are concerned in forming the walls of the gutter. It is quite conceivable how the maxillipeds could gradually usurp the whole opercular function to the exclusion of the chelipeds, especially as the specialisation of the Leucosiidæ has clearly been accompanied by a gradual diminution of size, rendering possible

[1] It is interesting to note that the larger types of Leucosiid appear to have acquired a new set of denticulations at the anterior (infra-orbital) extremity of the narrow afferent gutter. I have observed the presence of such denticulations in species of Ilia, Iphis, and Philyra. Their function is probably the same as that of marginal spines in the Portunids, and of the teeth on the crests of the chelipeds in Calappa.

a continuous relative reduction of the water-supply for the branchiæ, and consequently of the area occupied by the afferent current.

Utility of Specific Characters in the Genus Calappa.

The most recent revision of the genus Calappa, that of Alcock (1896, pp. 140—148), although confined to the nine species found on the Indian coasts, shows that the characters which are employed to discriminate the different species are principally the following:

1. Proportion of length to breadth of carapace.
2. Extent of postero-lateral clypeiform expansions of carapace.
3. Serrations of carapace margins:
    i. Antero-lateral margins.
    ii. Margins of clypeiform expansions.
4. Hairiness of pterygostomial regions.

I make no pretence to be able to explain the possible utility of the varied combinations of these features which the different species of Calappa present, when my only material is the literature upon the genus and some spirit specimens of several species. Nevertheless I venture to point out certain correlations which are not without their significance in this connection.

1. On the whole the more elongated species are restricted to deeper water than the broader species.

2. The clypeiform expansions are largest in the shallow-water species and smallest in those which inhabit deep water; cf. small size of expansions in C. pustulosa (25 fms.), C. Woodmasoni (34 fms.), and C. exanthematosa (100 fms.).

This correlation is confirmed by the fact that the species of the allied genus Mursia, "which is practically Calappa without the wings to the carapace," are found exclusively in the deep sea (e.g. M. bicristimana, 150—400 fms.).

3. The denticulations of the antero-lateral margins I have shown to subserve a sieve-like function in British crabs with an elongated orifice to the exostegal canal. It is probable that

the ancestors of Calappa had a similarly elongated orifice to the exostegal canal, and that the serrations of the antero-lateral margins in the modern species of Calappa are the last relics of the marginal spines which covered the afferent orifices in their ancestors. In C. granulata they have completely disappeared. In support of this contention I may point out that the antero-lateral border of the carapace, which in C. gallus is merely "crenulate" in the adult, is "sharply serrate" in the young (Alcock, 1896, p. 147).

The denticulations of the clypeiform expansions are also known to present a similar process of alteration from youth to maturity (Alcock, l. c.; Henderson, 1893). No adequate explanation, however, of the function of the clypeiform expansions has been yet put forward. In view of Henderson's remarks concerning the prevalence of protective tints among the arenicolous crabs of the Madras shores (l. c., 1893), the further suggestion may be hazarded that the flattening and expansion of the carapace in the shallow-water species of Calappa may possibly indicate a process of selective assimilation towards the appearance of empty bivalve shells. I am inclined, however, to think that the explanation when found will probably involve something more than mere protective resemblance.

4. Upon the hairiness of the pterygostomial regions I can, I think, throw some positive light. Just as the marginal spines serve as a sieve for fragments of sand and shell, so the pterygostomial hairs serve as a sieve for the finer particles of mud. In Calappa granulata the pterygostomial region forms a triangular area, bounded on all three sides by a dense row of fine hairs—a submarginal series beneath the antero-lateral margin of the carapace, an internal series along the exopodite of the third maxilliped, and a posterior transverse series along the interior edge of the meropodite (arm) of the cheliped. The submarginal series is supplemented by a carpet of fine hairs on the outer side of the branchiostegite, the afferent channel being alone free. In my specimens these hairs are full of mud, indicating that they have efficiently discharged their sieve function during life.

The same remarks apply to my specimens of C. hepatica from Honolulu and the Sulu Sea. On the other hand, Alcock states that the pterygostomial region of several species (C. exanthematosa, gallus, pustulosa, and Woodmasoni) is characterised by possessing but "few scanty hairs." When the study of habits is considered worthy of the attention of men of science, we shall perhaps learn whether or not it is true, as I venture to believe, that the species with few pterygostomial hairs live in cleaner ground than those having the outer part of the pterygostomial regions "densely hairy." That Calappæ do inhabit mud, as well as sand, is certain from Macgillivray's remark (1852, vol. i, p. 102) that on a coral reef off the coast of Queensland containing all varieties of coral, mud and sand, "smooth Calappæ seek refuge . . . . in the shallow muddy pools . . . . by burrowing beneath the surface."

2. Matuta picta,[1] Miers.

The figure (Plate 13, fig. 2) which I give of this species has been carefully drawn by Mr. Bayzand from a specimen (an adult male) brought back by Mr. G. C. Bourne from Diego Garcia in 1892. It represents the crab lying in a somewhat inclined position, the anterior part of the body being elevated so as to display more effectually the infra-orbital and pterygostomial regions of the carapace.

The genus Matuta is closely related to Calappa, but in the form of the carapace and chelipeds is less specialised than the latter type. In the broad swimming plates of the hindmost pair of thoracic legs, in the obsolescent teeth on the antero-lateral margins of the carapace, and in the great pair of epibranchial spines, Matuta betrays obvious signs of derivation from an early progenitor of the Portunid type, such as

[1] My specimen is identical with M. picta of Miers (1877) and the synonymous M. lunaris of Leach (1817). It would be referable to the more comprehensive M. Banksii of Alcock's recent revision (1896), were it not for the fact that the "posterior granulated ridge" is prolonged as far as the epibranchial spine, on the posterior border of which it dies away. Alcock's distinctions under this head are far from satisfactory.

Lupa or Callinectes. All the species of this genus are remarkably specialised for burrowing in sand, as indicated by the great compression of the four hindmost thoracic pairs of legs, and by the spade-like modification of their terminal joints (cf. Rumphius, 1705, and Krauss, 1843).

The chelipeds are curved and moulded to fit the sides of the carapace during flexion, exactly as in the genus Atelecyclus. They are destitute of the cockscomb-like crests which furnish such a characteristic feature of the genus Calappa. The anterior part of the buccal apparatus is consequently exposed even during flexion of the chelipeds. The protection of the mouth parts is, however, ensured by the forward prolongation of the external maxillipeds (fig. 2, *b*), a feature which is not to be observed in the genus Calappa, but which is a marked characteristic of the allied Leucosiidæ.

If I am right in my interpretations, the exostegal canal in this crab has a most extraordinary course. The orbits, in which the eye-stalks are retractile, have the form of a pair of stony cups. The outer and inferior angle of the orbit is, however, incomplete, and its cavity is continued downwards and outwards over the pterygostomial regions by a deep semicylindrical gutter (fig. 2, *e*). This gutter is converted into a tube by two dense rows of hairs which arise from the inner and outer edges of the gutter, and by their interdigitation beneath the cavity of the gutter furnish it with a complete hairy floor. So closely are the hairs set to one another, and so intimately do they interlock, that upon a cursory examination the hairy nature of the floor of the orbital gutter is not at first suspected, and Hilgendorf actually figures the gutter (1869, fig. 2) as a completely tubular passage excavated in the substance of the thick calcareous wall of the carapace.

The orbital gutter, as soon as it loses its tubular appearance, turns obliquely outwards and backwards (fig. 2, *f*) until it is lost in a thick carpet of hairs, which is especially well developed in front of and to the outer side of the afferent aperture at the base of the chelipeds, but which is also continued backwards along the whole inferior edge of the branchiostegite.

The chelipeds are smooth and concave on their inner face, and are capable of being closely apposed to the inferior wall of the carapace, so that they cover the afferent aperture and the whole carpet of hairs in this region, as is well shown in the figure here provided. Their upper margins do not coincide with the edge of the carapace, as they do in Calappa, but during flexion appear to come as far forwards as the posterior aperture of the orbital gutter.

Water clearly seems to enter the orbits, travelling backwards through the orbital gutter into the carpet of hairs (which, when the chelipeds are flexed, must furnish a most efficient sieve for the finer particles of mud and sand), through which it no doubt eventually makes its way to the afferent aperture at the base of the chelipeds.

This aperture is also furnished with a special hair-sieve of its own, since the edge of the branchiostegite which forms its anterior wall is fringed with a special line of stiff hairs, and there is a corresponding series of hairs on the opposing surface of the basal joint of the cheliped. When the cheliped is apposed to the carapace, the two sets of hairs interdigitate and constitute a sieve completely covering the aperture. A similar arrangement exists in Calappa also, but in the latter form the basal portion of the epipodite of the third maxilliped furnishes a much more obvious operculum to the aperture than is the case in Matuta.

The remarkable course of the exostegal canal in Matuta, with the restriction of its principal aperture to the cavity of the orbit, appears explicable to me only on the view which I have set forth in the case of Calappa and the Leucosiidæ, viz. that the common ancestor of all three types possessed a continuous waterway along the whole extent of the antero-lateral margins of the carapace to the base of the chelipeds; that the antero-lateral margins were denticulated; and that a process of restriction of the inhalant orifice began, by which the closure of the whole inhalant gap between chelipeds and carapace became gradually effected, except in the infra-orbital region. This process of restriction was effected as a con-

tinuous process of adaptation to a sand-burrowing existence The marginal denticulations becoming useless, gradually lost their sharp and prominent form, until they assumed the blunt, irregular, variable and obsolescent character which they exhibit in the modern species of Matuta. The great epibranchial spine is in itself evidence of the validity of this view, for it clearly represents the posterior spine of the antero-lateral series in such genera as Bathynectes, Callinectes, and Lupa. The same spine is again met with in the allied but less specialised genus Mursia; and a comparison of M. armata (De Haan, 1833, pl. xix, fig. 2) with M. cristata (Milne-Edwards, 'Règne Animal,' pl. xiii, fig. 1) confirms the views I have put forward. In M. armata the epibranchial spine is longer, while the antero-lateral teeth are absent; in M. cristata both are present, but the epibranchial spine is less elaborately specialised and still forms part of the marginal series.

The reason for the great elaboration of this epibranchial spine in Mursia armata and Matuta is less clear, and can scarcely be found without special study of the living animal. In its initial stage, however, as presented in Bathynectes longipes, I have every reason to believe it functions as a stay or barrier to the cheliped during apposition to the carapace, thus mechanically maintaining the arm of the cheliped in the right position for the closure of the exostegal canal (1897, p. 400). It seems to discharge this function also in Matuta picta, but I am doubtful whether this function is the only one which it discharges in cases where it is so highly developed.[1]

I make no suggestions as to the utility of specific characters in this genus, owing to the fact that the species of Matuta,

[1] Krauss (1843) remarks on the frequency of similar spines in arenicolous animals of various groups, e.g. fishes and molluscs, as well as crabs. One function may be to protect the crab from the danger of forcible dislodgment from the sand by wave-currents, as ably maintained by my friend Mr. Hunt in the case of the spiny species of Cardium ('Proc. Linn. Soc.,' xviii, "Zool.," p. 269).

and the range of variability in the different species, are as yet very inadequately determined. I would only remark that the beaded ridge which bounds the posterior and postero-lateral borders of the carapace, and which frequently bears a pair of tubercles in its course (see fig. 2), is clearly the homologue of the posterior dentated ridges of Calappa and Hepatus, and is probably degenerate in character. If this is true, the variations presented by this ridge during the stages of its disappearance are little likely to furnish the satisfactory characters for specific discrimination which some systematists have ascribed to them.

3. Albunea symnista, microps, and scutelloides, n. sp.

The problem of a pure water-supply in the case of sand-burrowing crabs has been solved in certain instances, as I have elsewhere shown (1896, 1897) in a manner even more original than that which I have illustrated for Calappa and Matuta. In the forms to which I refer (Corystes cassivelaunus, Portumnus nasutus, and Atelecyclus heterodon of the British coasts) the normal respiratory current of water—the constancy of whose direction has been an accepted maxim among naturalists since Milne-Edwards' classical elucidation of the process nearly sixty years ago,—the normal current is actually reversed in direction, and flows through the branchial chamber from before backwards, instead of from behind forwards. In Corystes cassivelaunus I have shown that it enters the chamber through a long tube formed by the apposition of the second antennæ, whose double rows of hairs interdigitate with one another in a most effective manner (Plate 14, fig. 3).

I now show that the structure of the first antennæ in the genus Albunea is strikingly similar to that of the second antennæ in Corystes, and that the tube formed by their apposition has the same relations to the branchial chamber as in Corystes. The species of Albunea are known to have sand-burrowing habits of life, so that in all probability a reversal of the branchial current takes place in this genus as in Corystes.

Figs. 3 *a* and 3 *b* on Plate 13 illustrate the arrangement of parts in Corystes cassivelaunus, for a detailed description of which I refer to my paper on that animal (1896). The only point that I need emphasise here is that the tube is formed by the outer or second antennæ, the first antennæ being situated in the interior of the tube.

Figs. 4 *a* and 4 *b* illustrate the structure of a Madras specimen of Albunea symnista, Fabr., which belongs to the Hippidea. The systematic position of this group of Crustacea is discussed by Miers (1879). The various naturalists who have previously examined specimens of this genus have all failed to recognise the fact that the hairs on the antennules are arranged along two longitudinal lines, and that they are directed towards the axial line of the body. The figures which have been published are all ludicrously conventional in this respect, and represent the irregularly hairy antennæ of a Palinurus less incorrectly than they do the antennules of an Albunea (see Milne-Edwards, 'Règne Animal,' pl. 42, fig. 3; 'Crustacés,' pl. 21, fig. 9; Miers, 1879, pl. 5; Henderson, 1893, pl. xxxviii). The converging double rows of hairs interdigitate naturally to form a tube, as I have recognised in A. symnista, Fabr. (Pl. 14, fig. 4), Albunea microps, Miers, and another Albunoid form (Pl. 14, fig. 5) which I have not been able to identify with any described species, and which I here name Albunea scutelloides, n. sp.

The antennular tube expands at its base into a prostomial chamber, as does the antennal tube of Corystes. In the latter case the floor of this chamber is formed by the third maxillipeds (fig. 3 *b*); but in Albunea it is formed by the broadly ovate terminal lobes of the endopodites of the first maxillipeds (fig. 4 *b*)—the homologues of the organs which in Calappa form the opercular floor of the exhalant passages (fig. 1 *b*). The prostomial chamber communicates on each side by a wide aperture with the branchial chamber. The channels of communication are bounded externally and ventrally by large lamellate expansions of the basal joints of the first and

second antennæ. The scaphognathite is of unusual size; both in A. symnista and in A. microps its anterior edge touches the basal joint of the antennules, while its posterior extremity is level with the base of the second thoracic leg. It is, in fact, half as long as the body (excluding the antennules) in the attitude represented in fig. 4 *a*.

In Corystes the roof of the prostomial chamber is largely furnished by the projecting frontal area of that animal (fig. 3 *a*). In Albunea, however, the frontal area is emarginated (fig. 4 *a*), and the roof of the prostomial chamber is furnished by the eye-peduncles, whose flattened scale-like form, varying in shape in the different species, is one of the most characteristic features of the genus (figs. 4 *a* and 5).

In Corystes the three stout basal joints of each antenna are disposed at right angles to one another in the vertical plane, bringing about a characteristic bend in the basal part of the antenna (figs. 3 *a* and 3 *b*), a feature which is functionally correlated with the reversal of the branchial current and its course through the antennal tube.

A precisely similar arrangement is recognisable in the species of Albunea, but in connection with the antennules instead of the antennæ (figs. 4 *a* and 5). In A. symnista (fig. 4 *a*) the joints are disposed at right angles to one another, as in Corystes cassivelaunus; but in A. scutelloides (fig. 5) the distal joint is pressed much further back than in either of these forms, thereby greatly reducing the angles of inclination. This difference may be readily seen to be correlated with the fact that in the latter form the part played by the frontal region in covering the prostomial chamber is very much less than in Corystes cassivelaunus or A. symnista. In Corystes the roof is provided by the prominent frontal area (fig. 3 *a*); in A. symnista by the apposed plate-like optic peduncles (fig. 4 *a*), but in A. scutelloides the optic plates are so small and short that they scarcely project from the orbital emarginations. The increased backward bend of the antennules in the latter species compensates for this deficiency.

In the figure of A. scutelloides (fig. 5) the antennules

are represented after being pulled forwards to some extent, in order to show the cavity of the prostomial chamber beneath and behind them.

Enough has, I think, been said to justify my view that many of the characters which distinguish the species of Albunea, both from one another and from their allies, are correlated with the function of respiration under arenicolous conditions of life. The verification of this inference must rest with those who have the opportunity of examining these animals alive under the proper conditions.

It must in any event, however, remain clear that the great problems which Darwin left us as his heritage, after so greatly illuminating them, are not to be solved by the exclusively morphographical researches which occupy the time and zeal of the great majority of naturalists to-day. Even in the best of hands such researches are capable, as I have shown from the history of the forms discussed in this paper, of obscuring even the simple facts of structure which they profess to elucidate; while the study of the functional relations of parts, side by side with the anatomical elucidation of the parts themselves, provides not only the data for generalisations of intrinsic importance, but assistance of an invaluable character in the field of morphological criticism.

APPENDIX.

Description of the New Species of Albunea (A. scutelloides, n. sp.) mentioned in the foregoing Paper.

By Walter Garstang, M.A.

With Plate 14, fig. 5.

This new species of Albunea closely resembles Albunea microps, Miers, in size, colour (in spirit) and shape. I found a single male individual among a number (placed at my dis-

posal by Professor Lankester) of Albunea microps (both ♂ and ♀) in the Oxford Museum, labelled "Sulu Sea, H.M.S. Nassau, 1871–2," and the accompanying description is derived from an examination of this single specimen.[1]

Length of carapace, 9 mm. Sculpture on back closely resembling that in A. microps, but readily distinguishable by the following points:—The principal M-shaped transverse line across the middle of the carapace is relatively more conspicuous in A. scutelloides, and the remaining transverse interrupted step-like ridges are relatively much more numerous (quite twice as numerous). They are consequently more closely set and give the carapace a still rougher appearance than in A. microps. Under a lens the ridges are seen to have a minutely tuberculate or beaded character, which is not seen in specimens of A. microps.

Mid-frontal area emarginated, broadly concave, but the emargination is wider than in A. microps, and therefore appears less deep; provided with a median tooth, as in A. microps, and a pair of small admedian teeth, as in the same species. Antero-lateral margin (from the admedian tooth to the antero-external angle of the carapace) divided into two approximately equal halves by a sublateral prominence; each half presents a slightly concave curvature. The inner and outer halves of the antero-lateral margin correspond with the bases of the antennules and antennæ respectively, and may therefore be termed the antennular and antennal curves. Antero-lateral margin without teeth, but with twelve or thirteen minute close-set tubercles distributed along the antennular curve and around the border of the sublateral prominence (thus differing from A. microps).

Antennules long; each provided with two rows of hairs which interdigitate with those of the other. Antennules presenting a marked double bend at their basal joints.

Antennæ provided with an accessory joint (aciculus), as long as the joint of the flagellum to which it is approximated,

[1] The type specimen of this species will be deposited by Professor Lankester in the British Museum.

i. e. of the same relative length as in A. symnista and A. microps.

Third maxillipeds. The carpal lobe is not produced beyond half the length of the propodite (in this agreeing with A. microps).

Optic peduncles scale-like, elliptical, broader than long, presenting a deep emargination at their antero-internal angles which lodges the cornea. The peduncles occupy the lateral compartments of the median emargination of the frontal area, which may accordingly be termed the orbital emargination.

Telson in the ♂ somewhat like that of A. microps, but more elongated and slender, the broadest part being a little nearer the base of the telson, and the sides more distinctly concave. No transverse rows of hairs on back of telson like those in A. microps and A. Guérinii; but a double admedian longitudinal series, and a group at each of the basal angles.

This new species approaches in certain features the species Albunea scutellata, described by Milne-Edwards (1834, ii, p. 204, pl. 21, figs. 9—13), which, with two other forms (venusta and myops), has been referred by Stimpson to a new genus, Lepidops (1858, p. 230; Miers, 1879, p. 231). These features are—(1) the absence of frontal denticulations; (2) the broad, scale-like optic plates. In fact, were it not for his remark concerning the truncation of the optic plates, Milne-Edwards' short description would be perfectly applicable to the present species. His figures, however, are not applicable. In his figure the carapace breadth is greatest in front; in my specimen it is greatest across the middle (as in A. microps). He figures no accessory joint to the antenna; and if the truncated plates in front of the carapace represent the optic plates, as his account implies, the possible identity of the two forms is altogether precluded. Moreover, there is no projecting lobe at the base of the sickle-shaped dactylopodite of the third pair of thoracic legs in my specimen, while such a lobe is clearly indicated in his figure.

Dana's description of A. scutellata (1852, i, p. 406) is

also inapplicable in regard to the form of the frontal margin. My figure (Pl. 14, fig. 5) does not indicate the median orbital emargination as deep as is actually the case.

Stimpson's descriptions (1858, p. 230) of the genera Albunæa (sic) and Lepidopa (sic) render the identity of the two forms still more improbable, owing to the characters of the antennal aciculum and of the carpal lobe of the third maxillipeds which I have described for my specimen. These characters are such as also exist in the species of the restricted genus Albunea, from which Stimpson removes the species scutellata. On these grounds, therefore, I refer my specimen to a new species of Albunea, distinguishable from the other known species by the character of the carapace-sculpture, frontal margin, optic plates, and telson.

On the other hand, Milne-Edwards' figure is clearly bad (note the absence of any distinction between the abdominal tergites and their lateral lamellate expansions); and it is possible that if his (or Desmarest's) specimens could be re-examined, some, if not all, the distinctions upon which I have relied would vanish. If the form of the ocular plates is determined in this genus, as I have rendered probable in the preceding paper, by their opercular relations to the prostomial chamber; and if the emarginations of the frontal area are functionally correlated with the play of the optic peduncles, antennules, and antennæ, as appears to me to be the case after examination of three species of Albunea; then it is perfectly clear that Milne-Edwards' fig. 9 does not correctly represent his specimen in these respects.

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EXPLANATION OF PLATES 12—14,

Illustrating Mr. W. Garstang's paper on "Some Modifications of Structure subservient to Respiration in Decapod Crustacea."

PLATE 12.

Fig. 1.—Calappa granulata, Fabr., from the Mediterranean. Front view, showing the closure of the chelipeds beneath the carapace and the respiratory orifices above them. *a*. Exhalant orifice. *b*. Branch of endopodite of first maxilliped, forming opercular floor of exhalant canal. *c*. Antero-lateral margin of carapace. *d*. Dentated crest on propodite of cheliped. *e*. Inhalant orifice of exostegal canal. *f*. Second antenna, forming part of orbital wall.

PLATE 13.

Fig. 2.—Matuta picta, Miers, from Diego Garcia. View from above, the anterior part of the crab's body being elevated to display the buccal region. *a*. Exhalant orifice. *b*. Third maxilliped. *c*. Carpet of hairs on pterygostomial plate. *d*. Propodite of cheliped. *e*. Orbital gutter, having a ventral floor of interlocking hairs. *f*. Postero-lateral extension of orbital gutter on pterygostomial plate.

PLATE 14.

Fig. 3 *a*.—Corystes cassivelaunus from Plymouth. Frontal area, showing tube formed by second antennæ (dorsal view).

Fig. 3 *b*.—Corystes cassivelaunus from Plymouth. Ventral view, showing floor of prostomial chamber.

Fig. 4 *a*.—Albunea symnista, Fabr., ♀, from Madras. Dorsal view, showing tube formed by first antennæ, and the ocular plates which form the roof of the prostomial chamber.

Fig. 4 *b*.—Albunea symnista, ♀, from Madras. Ventral view, showing basal part of antennular tube, lamellate terminal expansions of first maxillipeds, and the pediform second and third maxillipeds.

Fig. 5.—Albunea scutelloides, n. sp., ♂, from Sulu Sea (Oxford Museum). Dorsal view. The antennules are pulled slightly forwards, showing the prostomial chamber beneath. Also showing the double bend of the basal joints of antennules and the broad elliptical optic plates, and eye-spots.

N.B.—The figure does not represent the median emargination as deep as is actually the case. The small admedian teeth, situated on the outer sides of the optic plates, are also inadvertently omitted.

Notes on the Anatomy of Sternaspis.

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With Plates 15 and 16.

In the beautiful works both of Professor Vejdovsky (5) and of M. Rietsch (3) on Sternaspis we find certain statements which, if correct, would place that worm in a very exceptional position. These authors describe the excretory organ as a lobulated sac with neither internal nor external opening, and the genital organ as a somewhat similar sac entirely shut off from the cœlom, but opening to the exterior by two long ducts with which the sac is directly continuous. Thus Sternaspis, in having a completely closed excretory organ (nephridium?), and in having the ovary or testis situated in a special cavity without communication with the cœlom, would differ from all known Polychæta, of which group it is no doubt a highly modified member.

It was, therefore, with a view to either confirm or correct these descriptions that I began a study of Sternaspis thalassemoides, Otto, during a recent visit to Naples. I may say at once that they both proved to be erroneous.

To these observations have been added some notes on the cuticle and muscular system.

The Genital Organs.—Fig. 1 represents, somewhat diagrammatically, a ventral view of the ovary, or ovisac, as it

would be more correct to call it,[1] removed from a female Sternaspis, the oviducts having been cut through near their attachment to the body-wall. These ducts pass forward and open to the exterior at the end of the processes seen in fig. 16, in front of segment 8.

When viewed from the dorsal surface the two ducts appear to be simple tubes, uniting and passing into, or expanding to form, the lobulated genital sac.

When viewed from the ventral surface, however, we see that on either side a small blood-vessel, *lat. v.*, fig. 1, comes off from the large ventral vessel, *v. v.*, passes along the inner surface of the wall of the sac for a short distance, and then emerges on the outer surface of the duct down which it runs to the body-wall. Now this blood-vessel passes out from the genital sac by an open canal formed by the folding of a ciliated membrane, *op.* The edge of this membrane forms a sort of ciliated funnel, *cil. mb.*, opening into the cœlom, and is produced as a ciliated ridge down the outer and ventral side of the duct for about two thirds of its length.

The exact conformation of the cœlomic opening of the genital sac will be better understood on looking at the series of sections represented in figs. 2—8. Fig. 2 is taken through the narrow region connecting the sac with the ducts; fig. 3 is through the same region, but nearer their point of origin. Although the blood-vessels lie in the wall of the sac, the ciliated epithelium never covers them. In fig. 4, a section immediately after the bifurcation, the beginning of a ridge is visible projecting into the lumen of each duct. In fig. 5 this ridge is seen to project far inwards in the upper section, whilst in the lower section it has cut the lumen completely into two. The blood-vessel, *lat. v.*, has passed into the ventral and smaller lumen. The sections represented in fig. 6 show the blood-vessel lying near the edge of the ciliated membrane, so as to close the aperture of the funnel, which is seen to be widely

[1] The ova and spermatozoa are developed on the walls of the branches of the ventral vessel, which enter the sacs near the point of bifurcation of the genital ducts.

open farther forward in figs. 7 and 8. More forward still, the membrane, which is for a considerable distance attached to the tube by one edge, becomes quite free. The membrane and the wall of the duct are ciliated on one surface only, and this is continuous from one to the other. This description applies to both sexes.

As for the structure of the wall of the lobulated sac itself, previous observers do not seem to have noticed that it also is ciliated internally,—if not over its entire surface, at all events along extensive tracts reaching up the lobes. Like the ducts, it is covered on its outer surface with flat cœlomic epithelium, shown in fig. 10, a drawing of a fragment of the wall treated with silver chloride and stained. In certain regions, separated from the outer epithelium by a very thin connective-tissue layer with a few muscular cells, is the ciliated internal lining, *cil. epith.*, formed of elongated cells with oval nuclei. The cilia are short and closely set along narrow longitudinal tracts directed towards the base of the organ where the ducts come off (fig. 9). The direction of the ciliary current is from the tip of the lobes to the base, and down the ducts to the external openings. The cilia on the membranous funnel, and on the wall of the narrow canal leading from the cœlom into the genital sac, produce a current running inwards towards the cavity of the sac. In this way, although the ducts and the sac may be full of ova or spermatozoa, none are allowed to stray into the body-cavity.

The Nephridia.—These are two lobed sacs of a yellowish-brown colour, situated in front of the genital organ (fig. 11). Their general form and relations have been well described and figured by Rietsch (3)[1]. The main mass of each organ lies closely applied to the œsophagus, and is connected with the body-wall at the level of the intersegmental groove between segments 6 and 7 (fig. 20) by a rapidly diminishing stalk, with a narrow lumen, apparently ending blindly; for, like Vejdovsky and Rietsch, I failed to find any external opening.

[1] "J'ai vainement cherché à leur surface des entonnoirs vibratiles, les faisant communiquer avec la cavité générale."—Reitsch (3).

After a careful search I found a small ciliated funnel opening into the cœlom, and situated on the narrow stalk a little way above its point of attachment (figs. 11, 12, 13, *cil. fun.*). The blood-vessel which accompanies the stalk (figs. 12 and 13, *bl.v.*) sends a branch into the lip of the funnel, running immediately below the ciliated epithelium, as in many Polychæte nephridia. Fig. 13 is a view of a funnel in a still living condition, whilst fig. 14 shows the edge of the lip of another funnel. The cells of which it is composed are vacuolated and slightly granular, bear numerous cilia, and occasionally irregular processes. Flat cœlomic epithelium covers the outside of the whole nephridial organ. The internal epithelium is entirely composed of large cells, protruding far into the lumen, and loaded with "granules" of peculiar structure, to be described farther on. In fig. 21 is represented a fragment of the wall of the nephridium, showing the outline of the cells with the nuclei situated at their base.

Vejdovsky denies the presence of an internal cavity and of cilia:—"Ein innerer Hohlraum, sowie die Bewimperung fehlen hier gänzlich." There can be no doubt that a cavity of considerable size really exists inside the organ. As to the presence of cilia, which is also denied by Rietsch, I must confess that I feel by no means convinced of their absence even in the main sac. When these soft-walled organs are placed under the microscope the cells of the internal surface, which are so full of granules, and bulge inwards into the reduced lumen, become inevitably pressed against each other, and being very thin-walled, they have a great tendency to burst, so that a few scattered cilia would be very difficult to detect. When teased up the cells break off, and present the appearance shown in fig. 23 *a*. On the other hand, the cells which line the narrow duct leading towards the funnel are less bulky, and preserve their shape better. In this region I have seen cilia producing a current from the funnel towards the main cavity of the organ.

The "granules" filling the cells of the internal epithelium of the nephridium are of curiously complex and quite con-

stant structure (figs. 23 *a* and *b*). They consist of an outer transparent sphere filled with clear liquid, in which some minute granules are occasionally seen floating. In the centre of the sphere is situated a highly refringent yellowish body, which I shall call the concretion. This body is composed of two halves, one of which is slightly larger and darker than the other. In each cell spheres may be found of varying sizes, from a maximum diameter of about 15 μ to a mere speck. But even when quite minute they always contain, as far as I have been able to see, a central concretion of structure similar to that described above. When teased out and brought into some foreign medium the spheres always burst and disappear; no fixative, as far as I am aware, will preserve them. The concretions, on the other hand, are much more resistent. Treatment with divers reagents reveals the fact that the two halves have different properties, and, moreover, that they are cup-shaped, enclosing a third element, which may be called the central granule. Distilled water, ammonia, alcohol, and ether have no effect on the concretion. Caustic potash (5 per cent.) dissolves the small half, and apparently the central granule, but not the large half, which resists even when heated. Acetic acid, on the other hand, dissolves the large half, and the central granule after prolonged action; the small half remains unaffected. Weak hydrochloric acid dissolves the large half first, and then the smaller, whilst the central granule remains. Strong mineral acids destroy the whole concretion. Neither osmic acid nor iodine stains the concretion to any marked extent.

Fig. 22 shows the smaller half and central granule (the larger half having been dissolved) of concretions in cells preserved with Hermann's fluid, and stained with alum carmine, a stain which the concretions readily take up.

Vejdovsky (5) briefly mentions the refringent granules in the cells of the nephridium of Sternaspis; but Rietsch curiously enough seems to have mistaken them for nuclei.[1]

[1] "La couche épithéliale interne se compose de cellules très inégales (fig. 52): les unes, volumineuses, presentent de nombreux noyaux dont la plupart

It will be seen from the description here given that these are no ordinary excretory granules.[1] On comparing them with the granules found in the nephridia of other Polychætes, we find in some forms, such as Trophonia and Pectinaria, transparent spheres containing concretions; but the latter are often numerous, and are composed of irregularly aggregated smaller concretions without constant shape.

Of course, there is no direct evidence that these granules in Sternaspis are of an excretory nature; they certainly do not appear to be got rid of by the animal, since the nephridium is not known to open to the exterior. It is, therefore, not impossible that they may be stored up in the organ to serve some further purpose in the economy of the worm.

In the foregoing account the anterior paired brown sacs have, for convenience' sake, been called nephridia. It is quite possible, however, that they are not true nephridia, but peritoneal funnels peculiarly modified, and retaining but a small ciliated opening into the general body-cavity. The question of their exact homology can only be answered by the help of embryological data which we do not yet possess.

The same may be said concerning the homology of the genital organs. Yet in this case the anatomical evidence seems to point more clearly to the ducts and lobed sac being formed by the modification of a pair of ciliated peritoneal funnels. Cases of the overgrowth of the gonad by the funnel of the genital duct (or a fold of the peritoneum), so as to almost or entirely close it off from the general cœlomic cavity, are by no means rare amongst the Cœlomata. The male organs of Lumbricus offer a familiar example in which the testis and genital funnel become enclosed in a sac surrounded by, yet separate from, the general cœlom. The peritoneal

sont en voie de division. . . . À première vue on pourrait prendre pour des vésicules adipeuses les nombreux noyaux très réfringents de ces cellules; mais ils ne se colorent nullement par l'acide osmique, et il est facile aussi de constater que la plupart d'entre eux sont en voie de segmentation" (3).

[1] The "chloragogen" granules in the cells of the intestine do not resemble them.

sacs of the Eudrilidæ described by Beddard (1) are an instance of the same thing occurring in connection with the female organs. Throughout the higher Vertebrata the male genital cells are shed directly into the ducts; whilst in certain cases—as, for instance, many Teleostean fish and the mouse—the ovary is likewise shut off from the body-cavity, being enclosed in a sac in direct continuity with the duct to the exterior. It is, indeed, amongst the Mammalia that we find perhaps the closest parallel with the state of things described above in Sternaspis. In the female racoon (Procyon) or badger (Meles) the ovary is overgrown by a fold of the peritoneum ("broad ligament") and the funnel of the Fallopian tube, leaving only a narrow aperture communicating with the general cœlom (cf. A. Robinson, 4). The steps intermediate between the ordinary condition in Polychætes, in which the gonad is situated close to the wide-mouthed peritoneal funnel, and the condition in Sternaspis, in which the gonad is enclosed by the funnel, are easy to conceive.

The Cuticle, Ventral Shield, and Chætæ.—The cuticle of Sternaspis has been well described by Vejdovsky and Rietsch. It is very thick, and consists mainly of intercrossing fibres. On the outer surface, however, is a thin layer of somewhat different nature, to which are fixed numerous sandy (chiefly siliceous) particles (fig. 24). In this respect Sternaspis resembles the Chlorhæmids, in which the cuticle is also covered with sand. These foreign particles are chiefly grouped round the base of the numberless little papillæ which cover the surface of Sternaspis, and are especially numerous and coarse behind the seventh segment. On the anterior retractile segments the particles are finer and fewer in number.

Both inner and outer layers of the cuticle are insoluble in alcohol and ether. The thin outer layer is insoluble in hydrochloric acid, but soluble in strong solution of caustic potash. On the other hand, the thick inner layer is soluble not only in potash and hydrochloric acid, but also in boiling distilled water. It therefore resembles the cuticle of the earthworm (2).

The thick brown ventral shield is insoluble in boiling water, caustic potash, alcohol, and ether. Placed in cold concentrated hydrochloric acid, it gives an orange-yellow solution; a transparent colourless portion remains, which dissolves with difficulty on boiling.

The chætæ are insoluble in water, caustic potash, alcohol, and ether. As in the case of the earthworm (2), an outer shell and distal cap remain undissolved when they are boiled in hydrochloric acid.

The shield, then, is probably formed of the same substance as the chætæ—not of true chitin.

The Muscular System.—Since the musculature of Sternaspis has been only very slightly dealt with by previous observers, a somewhat detailed account of the highly modified muscular system of this Polychæte is here given. It is well known that, when irritated, Sternaspis can rapidly withdraw the first seven segments into its hind body; this introversion is brought about by a complicated system of muscles, derived chiefly from the longitudinal layer.

A side view of an expanded specimen is shown in fig. 16, and of a retracted specimen in fig. 15. In the latter the whole of the anterior region has been withdrawn to the level of the genital papillæ (*gen. p.*), which remain projecting from the front edge of the body. The branchiæ at the hind end are not withdrawn, but merely contract into close spiral coils. Their contraction is, however, quite independent of that of the body.

Externally we notice the first segment, bearing the mouth (*m.*) and the rounded prostomium (*prost.*), followed by segments 2, 3, and 4, armed with strong chætæ. Each row of chætæ is set on a crescentic and slightly elevated area on each side at the hinder margin of the segment. As has been shown by previous observers, the bundles of chætæ in segments 8 to 14 are sunk in the body-wall, and completely hidden from view. The grooves separating the first seven segments are shallow, and completely surround the animal; those separating the posterior segments are deeper (in the expanded worm), and do

not reach right round, but leave a narrow smooth strip on the mid-dorsal and mid-ventral regions. The posterior bundles of chætæ are set round the lateral and posterior edges of the ventral shield; the lateral bundles are held by small conical parapodia.

The anterior end of the body is extruded (pleurecbolically) by the contraction of the circular layer of muscles in the hind body. These muscles are disposed in a thin layer, interrupted dorsally and ventrally at the smooth strips already mentioned (fig. 16, *d. a.* and *v. a.*). The inner layer of longitudinal muscles, passing from one intersegmental groove to another, lines the body-wall within (figs. 17—19, *long. m.*). The muscles which serve to move the large anterior chætæ are disposed as follows (figs. 17—20) :—The chætæ are bound together at their inner ends with connective tissue into three bundles on each side. A short thick muscular band joins these three bundles together, and a similar band attaches them to the posterior end of the pharynx. A larger strand of muscles runs forwards to the body-wall near the base of the prostomium, passing along the side of the pharynx (fig. 20). A slender band of muscle, starting from the inner end of the outer bundle of chætæ, passes almost horizontally outwards to the body-wall, where it is attached at the hinder limit of the fifth segment (*lat. m.*). This muscle draws the ends of the chætæ inwards and towards the body-wall, whilst the other bundles just described push them outwards and towards the pharynx. Each bundle of chætæ is, moreover, provided with proper protractor muscles (*protr.*). The retractor muscles are joined together in two large bundles (*retr.*) (not three, as figured by Vejdovsky and Rietsch), which pass backwards to their point of attachment on the anterior edge of the ventral shield on either side. Not only the chætæ, but also the whole anterior region of the body, are withdrawn by these powerful muscles.

Large strands of longitudinal muscles extend dorsally from behind the prostomium to the median dorsal region, from the anterior seven segments to segments 8 to 14 (figs. 17 and 18, *d. retr.*). Similar strands extend ventrally from beneath the

pharynx to segments 8 to 14, and the front edge of the shield (*v. retr.*) on either side of the nerve-cord. A retracted specimen cut in half vertically (fig. 18) shows these muscles plainly; dorsally and ventrally they are attached to the smooth areas interrupting the intersegmental grooves on the outer surface of the worm. They are the chief retractor muscles of the anterior region of the body; the sharp bend in the dorsal retractors (fig. 18) is due to the pressure of the viscera. This figure also shows the position of the prostomium and pharynx in a retracted specimen, and the nerve-cord bent back at a sharp angle. It will be noticed that owing to the length of the nerves running to the body-wall from the ventral surface of the nerve-cord, the latter organ does not closely follow the curve and folds of the body-wall, and thus is possibly saved from injury during the rapid process of retraction.

The fourth set of retractor muscles are seen in fig. 17, and in fig. 19 a retracted Sternaspis cut horizontally above the introvert. These are narrow muscular ribbons running from the posterior margin of the fifth segment to the grooves between the 7th, 8th, 9th, 10th, 11th, and 12th segments (*lat. retr.*).

It is obvious that all these muscles, by co-ordinate and successive action, form a very perfect apparatus for the acrembolic introversion of the first seven segments.

Posteriorly the rectum is provided with paired dorsal retractors (figs. 17 and 18, *d. rect. m.*), and with paired ventral retractors (figs. 17 and 19, *v. rect. m.*), attached to the ventral shield.

One of the functions of the ventral shield seems to be to act as a sort of fulcrum for the attachment of the main retractor muscles.

The ten large lateral bundles of chætæ set round the sides of the shield are provided with protractor muscles, and with peculiar slender retractors attached above the nerve-cord (*retr. lat. ch.*, fig. 19). No such muscles belong to the small posterior bundles of chætæ as figured by Vejdovsky. They have small retractors attached to the shield.

Summary.

It has been shown in the foregoing account that the cavity of the genital sac of Sternaspis communicates with the body-cavity; that the nephridium is provided with a small ciliated funnel, possesses a lumen, and in one region, at all events, is ciliated internally. The complex granules of the nephridial cells have been described in detail; experiments have been recorded as to the solubility in certain reagents of the granules, the cuticle, the ventral shield, and the chætæ. A detailed account of the muscular system has also been given.

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EXPLANATION OF PLATES 15 & 16,

Illustrating Mr. Edwin S. Goodrich's paper "Notes on the Anatomy of Sternaspis."

List of Reference Letters.

a. Anus. *bl.v.* Blood-vessel. *br.* Branchiæ. *ch.* Chætæ. *cil.* Cilia. *cil. epith.* Ciliated epithelium. *cil. fun.* Ciliated funnel. *cil. mb.* Ciliated membrane. *cil. ri.* Ciliated ridge. *cœl. epith.* Cœlomic epithelium. *d. a.* Dorsal area. *d. rect. m.* Dorsal rectal muscles. *d. retr.* Dorsal retractors. *extr.* Extremity of the nephridium attached to the body-wall. *gen. d.* Genital duct. *gen. p.* Genital papilla. *gr.* Granule. *int. gr.* Intersegmental groove. *lat. retr.* Lateral retractors. *lat. m.* Lateral muscle. *lat. par.* Lateral parapodium. *lat. v.* Lateral vessel. *l. gen. s.* Lobes of the genital sac. *long. m.* Longitudinal muscles. *lu. can.* Lumen of the canal leading from the body-cavity to the cavity of the genital sac. *m.* Mouth. *n.* Nucleus. *n.c.* Nerve-cord. *neph.* Nephridium. *o. cut.* Outer layer of cuticle. *op.* Opening leading into the genital sac. *ov.* Ovum. *ph.* Pharynx. *post. ch.* Posterior chætæ. *prost.* Prostomium. *protr.* protractors. *rect.* Rectum. *retr.* Retractors. *retr. lat. ch.* Retractors of lateral chætæ. *sil. p.* Siliceous particles. *sph.* Transparent sphere. *st.* Stalk of nephridium. *v. a.* Ventral area. *v. rect. m.* Ventral rectal muscles. *v. retr.* Ventral retractors. *v. v.* Ventral vessel. *w. gen. d.* Wall of genital duct. *w. gen. s.* Wall of genital sac.

PLATE 15.

Fig. 1.—Somewhat diagrammatic enlarged view of the genital sac and ducts of Sternaspis dissected out, and viewed from the ventral surface. showing the openings leading from the body-cavity into the genital sac. On the right side the blood-vessel which accompanies the duct is not represented.

Figs. 2—8.—Series of transverse sections through the base of the genital sac and origin of the ducts, showing the communication with the cœlom by means of the narrow canal formed by the folding of the ciliated membrane. × 400, cam.

Fig. 9.—Inner surface of a piece of the ciliated epithelium lining of the genital sac, drawn from the fresh and stained tissue. × about 400.

Fig. 10.—Outer surface of a piece of the wall of the genital sac, stained with silver nitrate and carmine. The cœlomic epithelium has been partially torn off, exposing the inner ciliated epithelium. × about 400.

Fig. 11.—General enlarged dorsal view of the nephridia, showing the slender stalk inserted into the body-wall and the small ciliated funnel.

FIG. 12.—Extremity of the stalk of a nephridium, with its accompanying blood-vessels, and the ciliated funnel. From a stained preparation. × 400, cam.

FIG. 13.—Enlarged side view of a ciliated nephridial funnel, from the fresh.

FIG. 14.—Enlarged view of the edge of a nephridial funnel, from the fresh.

PLATE 16.

FIG. 15.—Retracted Sternaspis, enlarged side view of a living specimen.

FIG. 16.—Expanded Sternaspis; enlarged left side view from living and preserved specimens.

FIG. 17.—Enlarged inner view of the right half of a hardened expanded specimen. The viscera have been removed, and the branchiæ are not represented.

FIG. 18.—Similar view of a retracted specimen.

FIG. 19.—Enlarged inner view of the ventral portion of a retracted specimen, cut through horizontally.

FIG. 20.—Similar view of the anterior end of an expanded specimen.

FIG. 21.—Wall of the nephridium in optical section, showing the base of the granule-bearing cells with their nuclei. From a stained preparation.

FIG. 22.—Front and side view of nephridial granules, from a preparation preserved in Hermann's fluid and stained with alum carmine. Cam. × 400.

FIG. 23.—*a*. A portion of a nephridial cell teased out, showing the contained granules. From the fresh. *b*. Isolated granules, more enlarged.

FIG. 24.—Portion of the outer layer of the cuticle, with the foreign particles fixed on to it. Cam. × 400.

On the Relation of the Arthropod Head to the Annelid Prostomium.

By

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The question of the segmentation of the Arthropod head, and of the homology of the preoral region in Arthropods and Annelids, has for long excited the interest of naturalists, giving rise to much discussion, and leading investigators to the discovery of many important facts. The present paper, written at the suggestion of Professor E. Ray Lankester, does not claim to be a contribution to our knowledge of the problems involved, nor an exhaustive history of the subject; it aims neither at originality nor completeness, but is merely an attempt to give a plain account of the questions at issue, and the advance that has been made towards answering them, for the benefit of those who have not devoted special attention to the subject.

If we wish to compare the preoral region of an Arthropod with that of an Annelid, it is necessary first of all clearly to understand the relation of the prostomium and the peristomium, or buccal segment, to each other, and to the other segments of the body of an Annelid worm.

It was Professor Huxley who first introduced the word prostomium in his 'Lectures on General Natural History,' published in 1856 (5). "The body of the Polynoë," says Huxley, "is composed of a series of twenty-six 'somites,' terminated anteriorly by a 'segment,' the prestomium[1] (Kopf-lappen, Grube), and posteriorly by another, the pygi-

[1] The modified form prostomium was introduced by Lankester (8).

dium, which may or may not represent single somites." The first somite with parapodia, chætæ, and acicula he calls the "peristomium."

Fig. 1.

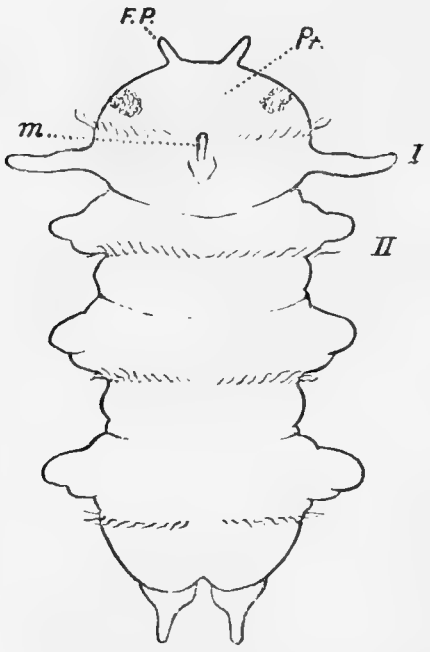


Fig. 2.

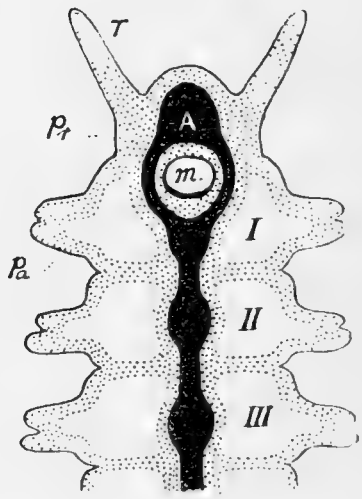


Fig. 1.—Ventral view of a young Nereis.

Fig. 2.—Diagrammatic plan of the anterior segments of the Polychæta. The nervous system is represented in black, the mesoblastic tissues are dotted, the cœlomic cavities are left white. *F. P.* Frontal process. *m.* Mouth. *Pa.* Parapodium. *Pr.* Prostomium. *T.* Tentacle. A. Archicerebrum. The Roman numbers refer to the segments.

The prostomium (Kopflappen, lobe céphalique), then, is a median anterior process lying above and in front of the mouth. It may be small and insignificant, as in most Oligochætes, or it may be large and of great physiological importance, as in many Polychætes. The prostomium may be long and produced into a moveable process (Stylaria), or distinctly annulated (Glycera). In the Polychæta it contains the brain or supra-œsophageal ganglion, and often bears specialised organs of sense, such as dorsal tentacles and eyes, and ventral palps. In the Oligochæta, on the other hand, the brain recedes from the prostomium (except Æolosoma) into

the first, second, or third segment; whilst in some forms the prostomium is quite rudimentary (Diacheta), in others it extends backwards dorsally (as marked by a groove) to the hinder limit of the peristomium (Lumbricus).

In the Amphinomids it grows backwards over several segments.[1]

The peristomial segment, to which the prostomium is attached, is almost always considerably modified in connection with the mouth, and may even be sharply marked off from the posterior segments. It is often called the cephalic segment (Kopf segment, segment céphalique, buccal segment, head segment, &c.). The peristomium and prostomium together constitute the head in Oligochætes and some Polychætes. In many Polychætes, however, several anterior segments may become so modified as to contribute to the formation of the head.

Having thus briefly reviewed the structure and relations of the prostomium in the Chætopoda, we must proceed to a more accurate study of its homology.

The prostomium can be one of three things: (1) a modified or reduced segment; (2) an incipient segment, growing on the anterior surface of the peristomium; (3) not a segment at all, but a structure of different and special nature.

Before attempting to prove that the last interpretation is the true one, it must be clearly established what we mean by a true segment or metamere, and then how such a metamere differs radically from the prostomium.

It is comparatively easy to give a serviceable definition of a typical segment: it is a region more or less distinctly marked off from the rest of the body by transverse grooves, surrounding the alimentary canal, containing a special cœlomic cavity (more or less completely separated off from the cœlom of adjoining segments by means of transverse septa), a pair of nephridia and of peritoneal funnels communicating with the exterior, a pair of ganglionic enlargements of the ventral

[1] An excellent discussion of the structure and morphology of the prostomium and brain of the Polychæta has lately been given by M. Racovitza (14).

longitudinal nerve-cords, and (in Polychætes and Arthropods) a pair of appendages. But we know very well that such fully equipped segments are rarely found in nature. Intersegmental grooves frequently disappear (head and thorax of Arthropods); there are segments without cœlomic cavity (the thoracic segments of insects, for instance); and again, there are segments with neither peritoneal funnels nor nephridia (most Arthropod and Chætopod anterior segments). Some metameres have no ventral nerve-cord (the first two segments of Lumbricus, the posterior abdominal segments of many insects); appendages are often absent.

It is clear, then, that the examination of adult structure will help us little in deciding whether a debatable region represents a true segment or not, though a careful comparison with allied types may often be of use. Embryology is our best guide in these cases, and generally furnishes a decisive answer. We find, as a matter of fact, that the segments, which lack in the adult those structures most essential, possess them at some time during early development, and lose them at a later period. Yet here, again, it must be admitted that undoubted metameres may have lost even during development one or more of the structures characteristic of true segments (for instance, no distinct cœlomic cavity is known to occur in some of the anterior segments of many Crustacea). We cannot, therefore, assert that a given region is not a metamere because it does not possess this or that character. The only dogmatic statement we are justified in making is, that when a region exhibits during development a sufficient number of the essential structures of a typical segment, it may be assumed to be a true metamere.[1] What is "sufficient" has to be decided in each case. It should be added that one positive fact outweighs many negative ones; the known presence of a certain characteristic of a segment in a certain region of an Arthropod, for instance, is of far greater importance than its ascertained absence in numerous other cases. A good example of this

[1] Theories as to the origin of metameric segmentation do not concern us here; at any rate, I do not propose to include them in this discussion.

kind is offered to us in the case of the loss of the limbs in snakes. The argument which might be urged—that the ancestors of the Ophidia were legless, since no obvious vestiges of limbs are seen in by far the greater number of snakes at any time in their development—is entirely disproved by the few instances, such as the Python and Tortrix, in which such vestigial hind limbs are known to occur.

Segments may be suppressed, either temporarily in the young, as in the zoœa larva; or, on the contrary, in the adult,—as, for instance, the first abdominal segment in Arachnida. On the other hand, neglecting the special cases of reproduction by fission, new segments are never intercalated between old ones, except in the normal process of growth at the tail end of the animal. This brings us indeed to one of the most important characters of the segmentation of Annelids and Arthropods, namely, that new segments, during the development of an individual, are invariably added between the last segment or telson, and the one immediately in front of it. All apparent exceptions to this rule, often called the law of Milne-Edwards, seem to be due to retardation in development, as in the case of the zoœa already mentioned.

At the risk of wearying the reader, it has been necessary to indulge in these commonplace and well-known remarks for the sake of clearing the ground. We may now return to the discussion of the morphology of the peristomium in Annelids.

Careful modern researches (Vejdovsky, Wilson, &c.) have shown that in Oligochætes the peristomium exhibits the essential characters of a true segment. It develops as a region surrounding the mouth, in which are formed a pair of mesoblastic somites which become hollowed out to form the cœlom; a ganglionic thickening is produced ventrally, which soon fuses with that of the succeeding segment; a nephridium (head kidney) is developed. In the Polychætes, on the other hand, where the head in the larva is so often enlarged to a disc-like shape, it is generally more difficult to trace the origin of the cœlom in the peristomium, as indeed also in the segments behind it. In some cases, at all events, it has been

shown that a pair of somites are formed in the peristomium, become hollowed out, and even give rise to peritoneal funnels (E. Meyer, 11). Nephridia are almost invariably developed in this segment. In Polychætes, moreover, a pair of lateral appendages are often developed, although they generally

Fig. 3.

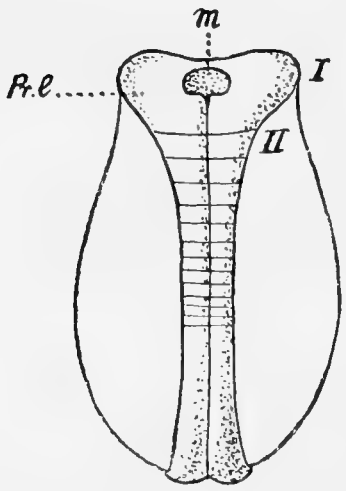


Fig. 4.

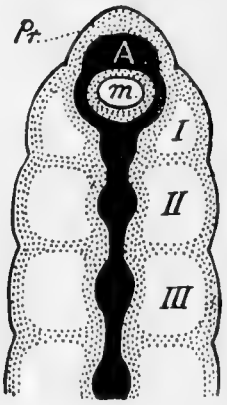


Fig. 3.—Ventral view of an embryo of Allolobophora putra (after Vejdovsky).

Fig. 4.—Diagrammatic plan of the anterior segments of the Oligochæta. *Pr. l.* Procephalic lobe; other letters as in Figs. 1 and 2.

become highly modified. In fact, it becomes evident, when we examine the development and the adult structure of the peristomium in the various groups of the Annelids, that it is really a metamere strictly comparable to the posterior segments, even when much modified owing to its position at the anterior end of the animal.

The prostomium, on the contrary, presents none of the characters essential to a segment. It never surrounds the alimentary canal; it never possesses a pair of mesoblastic somites.[1] The cavity which it contains is primitively of the

[1] Considerable confusion has been introduced into this question, apparently by the misunderstanding of Kleinenberg's results (6). The terms "cephalic germinal streak," "head segment," "head cavity," "cephalic zoonite," used by that author all seem to refer to the peristomium (not prostomium). Describing the development of the first pair of somites, he says, "The

nature of a blood-space, most clearly seen in trochosphere larvæ, where it is much enlarged. Although later in development in both Oligochæta and Polychæta, the prostomial cavity becomes confluent with the cœlom of the peristomium. It is only cœlomic by virtue of this connection.[1] No nephridia are developed in the prostomium, and on its upper surface is formed the primitive brain or supra-œsophageal ganglion. This brain may develop from the first as a single median structure, or may originate from several centres connected with the organs of sense, which subsequently become fused (Kleinenberg, Racovitza). We see that not only does the prostomium in Annelids differ from a metamere in size and shape, but it never at any time during its development exhibits the characters of a true segment. It therefore cannot be considered as a reduced or vestigial metamere. Can it be considered as an incipient segment? Obviously not, except on the very strongest evidence, since such a fact as the growth of a new segment in front of the first metamere would be opposed to the rule which is known to hold good for Annelid segmentation (Milne-Edwards' law). Such evidence is entirely wanting.

The only other opinion that can be held is that the pro-

splanchnic layer of the cephalic ring, which at first covers only the upper side of the buccal fossa and œsophagus with a thick layer of mesoderm, extends gradually its lateral parts towards the central [ventral ?] surface, and embraces the ingestive aperture completely." And again, "The anterior end of the head segment becomes more and more prominent, and is transformed into a cylindrical process, the upper lip, a kind of proboscis " = prostomium ?

[1] As Vejdovsky says (p. 320, 16), "die Kopfhöhle [peristomial cœlom] sowohl von Rhynchelmis als der Lumbriciden weicht also genetisch nicht von Leibeshöhle der nachfolgenden Segmente als. Sie wächst erst nachträglich zum sog. Præstomium aus, welches letztere daher nicht als Kopf, sondern als ein Kopf-fortsatz oder Kopflappen aufzufassen ist. Dafür sprechen zuerst die embryologischen Thatsachen bei Lumbriciden, wo der Mund am vorderen Körperende terminal nach aussen mündet, und erst nachträglich durch den sich verlängernden Kopflappen von der Rückenseite verdeckt wird. Dies allerdings erst sehr spät, nachdem das Kopfganglion längst angelegt ist und sich somit nicht im Praestomium bilden kann, wie unlängst von einer Seite behauptet wurde." With regard to the latter fact, the brain nevertheless arises from the prostomial region, although the prostomium may be retarded in development.

stomium, being neither a reduced nor an incipient segment, is a special region not of segmental value. Further, we may take two views of this question: the first, and the one generally held, is that the prostomium represents the region lying in front of the mouth of the primitive unsegmented ancestor; the second is that the prostomium is a new growth from the first segment, or region surrounding a terminal mouth in the primitive ancestor.[1] According to the first, the prostomium is a region of great morphological and phylogenic importance. According to the second, a more recent addition of relatively little significance. A comparison of the merits of these two views would land us in the midst of theories into which there is no need to enter here; it is sufficient for our present purpose to have shown that the prostomium is not a true segment.

In the Arthropoda we find a region in front of the mouth of varying size, bearing as a rule antennæ and well-developed sense-organs, and containing the brain. A certain number of segments behind bear appendages connected with and modified in relation to the mouth. These, together with the preoral region, constitute the "head." Long ago the evidence of comparative anatomy and embryology convinced naturalists that the head of Arthropods, both in front and behind the mouth, is composed of several metameres, more or less fused together. It is with the preoral region that we are most directly concerned in our comparison with the Annelid.

How do true segments come to lie in a preoral position? is one of the first questions we have to answer. If the first

[1] Such a supposition would lead us, perhaps, to somewhat modify our conception of the peristomium (first segment) as being merely a metamere, since it would have the property of producing an anterior prostomial outgrowth (and brain). It must be remembered, however, that, in the cases of reproduction by fission, other and posterior segments possess the same power. Lankester has assumed that theoretically every segment should develop a prostomium, and is only as it were withheld from this completion of itself by a "longitudinal cohesion or integration." "In most Annulosa this longitudinal cohesion counteracts entirely the opposing tendency to produce a head and to separate" (8).

segment behind the mouth in Arthropods represent the peristomium in Annelids, then those between it and the anterior extremity must be new metameres—a supposition which, if true, would contradict the general law of segmentation and the evidence of ontogeny.[1] Professor Lankester in 1873 argued that these preoral segments must originally have been post-oral, and that they have since moved forwards in front of the mouth—or, in other words, that the mouth has shifted backwards.[2] This fertile suggestion is supported by the facts observed in the ontogeny of Arthropods generally; and even in the Polychæta there is often a tendency for the primitively post-oral segments to shift forwards in front of the mouth, as in many Amphinomids and in Aphrodite. Lankester's explanation has been generally adopted, the only difference of opinion being as to how many of the "head segments" are true metameres, and therefore of post-oral origin.

Of the highest importance in connection with this problem is the study of the structure and development of the brain. It is well known that the Arthropod brain presents the appearance, either in the embryonic or adult state, of being formed of several segments. Here again the suggestion made

[1] Segments develop from before backwards. Although the sequence of the differentiation of the anterior segments of Arthropods may be somewhat obscured by what is almost certainly secondary modification and retardation (cheliceral segment in Arachnids), yet we never find the germ bands, after the first segment has been formed, growing forwards beyond it to give rise to new segments in front.

[2] "The segmentation of the prostomial axis in Arthropoda and some Annelids, which has an appearance of being a zooid segmentation comparable to that of the metastomial axis, on account of the identity in the character of the appendages with those of the metastomial axis, has yet to be explained. It may be suggested that it is due to a distinct breaking up of this axis like the posterior one into zooid segments or zoonites; there is much against this supposition (see 'Trans. Linn. Soc.,' 1869, "On Chætogaster and Æolosoma"). Much more likely, it seems, is the explanation that the oral aperture shifts position, and that the ophthalmic segment alone in Arthropoda represents the prostomium, the antennary and antennular segments being aboriginally metastomial, and only prostomial by later adaptational shifting of the oral aperture" (9).

by Lankester in 1881, that the ganglia of primitively post-oral metameres have shifted forwards, to fuse with the primitive Annelidan brain, the archicerebrum, to form a syncerebrum of compound structure, has been amply supported by the facts of comparative anatomy and embryology.[1] But this suggestion must not be pushed too far; not every lobe, not every epidermic invagination or centre of proliferation in the embryo must be taken for a metameric ganglionic mass. We know, as already mentioned, that the unsegmented archicerebrum of Annelids may be much lobed and differentiated, and may even arise from several separate centres in the prostomium itself. An apparent "neuromere" can only be accepted as of metameric value when the interpretation is supported by evidence derived from other parts, such as the mesoblastic somites and the appendages.

Let us now examine the various groups of Arthropods.

The Peripatoida (figs. 5 and 6).

In Peripatus, the most worm-like Arthropod, we find a head bearing a pair of antennæ and eyes, and two pairs of more posterior appendages modified in relation to the mouth—the mandibles and oral papillæ.

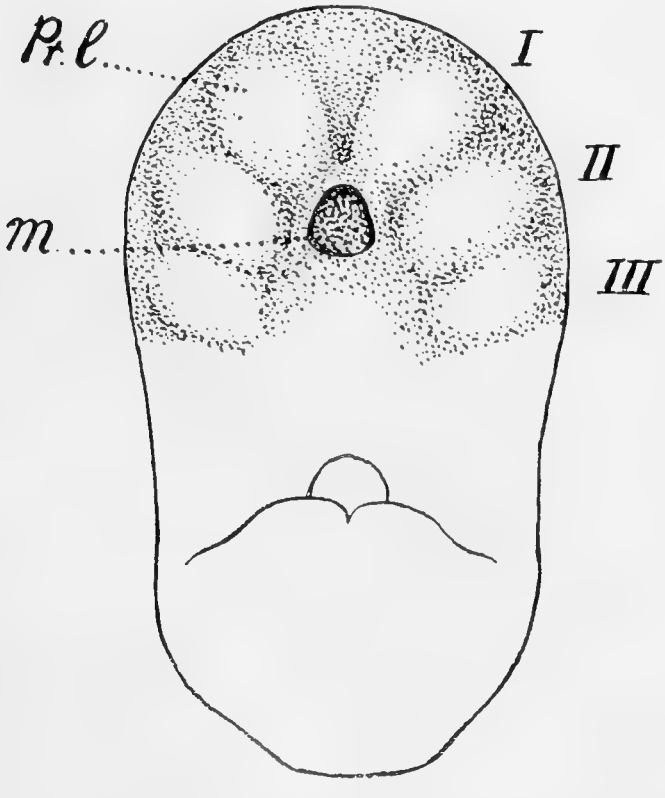
A study of the development has shown that the head of Peripatus is formed of three segments. All observers are agreed that the posterior two, to which the oral papillæ and mandibles belong, are genuine metameres; but some doubt exists as to the nature of the preoral segment bearing the eyes and antennæ, many writers having compared the antennæ to the prostomial tentacles of Annelids. Von Kennel and Sedg-

[1] "In the Chætopoda, the pre-œsophageal ganglion appears always to remain a pure archicerebrum. But in Crustacea (and possibly also all other Arthropoda, though there is a case to be considered for Peripatus and for the Hexapoda and Myriapoda, on the supposition that their antennæ are not the equivalents of Crustacean antennæ, but of the processes of the cephalic lobe of Chætopoda) the pre-œsophageal ganglion is a syncerebrum, consisting of the archicerebrum and of the ganglion masses appropriate to the first and second pair of appendages, which were originally post-oral, but have assumed a preoral position whilst carrying their ganglion masses up to the archicerebrnm to fuse with it."—E. Ray Lankester (10).

wick do not express a definite opinion on this point, but they show most conclusively that in its development the preoral segment resembles a true metamere. It has paired mesoblastic somites, developed post-orally as the first of the series which shift forwards in front of the mouth. As in the posterior segments, each of these somites becomes hollowed out to form the cœlom, from the wall of which is developed a rudimentary "nephridium" (peritoneal funnel). "It presents exactly the same relations as do the nephridia of posterior somites," says Mr. Sedgwick (**15**), and adds, "The first somite, therefore, behaves exactly as do the posterior somites."

As pointed out by Professors Korschelt and Heider, in their excellent text-book of embryology (**7**), it can now hardly be

Fig. 5.

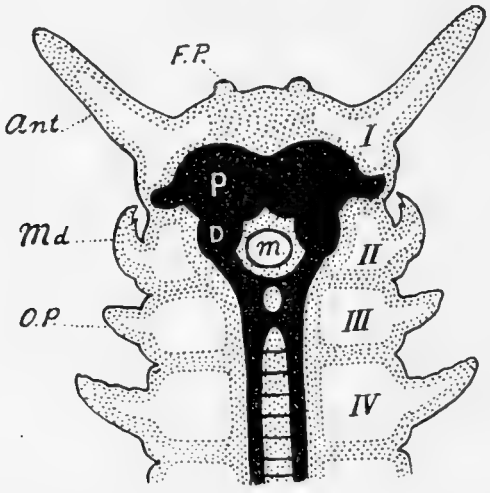


Ventral view of an embryo of Peripatus capensis (after Balfour). *Pr. l.* Procephalic lobe. *m.* Mouth.

doubted that the antennæ of Peripatus were primitively post-oral, and that the segment to which they belong is therefore

homologous with the first segment or peristomium of Annelids. Heider has further suggested that the two small processes found in front of the head, near the median line in the embryo, represent the prostomial tentacles.

Fig. 6.



Diagrammatic plan of the anterior segments of the Peripatoidea. *Ant.* Antenna. *Md.* Mandible. *O.P.* Oral papilla. P. Protocerebrum. D. Deutocerebrum. Other letters as in Figs. 1 and 2.

The study of the development of the brain in Peripatus confirms the conclusions derived from that of the mesoblastic structures. It is formed by the fusion of two pairs of ganglionic masses derived from the first two segments. Whether the archicerebrum is still to be distinguished, perhaps in the median anterior region, in connection with the pair of small processes mentioned above, is a question which remains to be solved, and requires a renewed investigation.

The first and largest segment of the brain, which supplies the eyes and antennæ, is developed from the ectoderm of the large procephalic lobes (first metamere). The process is aided by the formation of a crescentic pit, a fold of the surface on either side. The second segment of the brain supplying the mandibles is smaller. The oral papillæ are innervated from the ventral nerve-cord.

We conclude, then, that in Peripatus the first segment has become much enlarged in development, and that the mouth

has shifted behind it. The first pair of ganglia have attained a great size, and differentiation in connection with their anterior position and relation to the sense-organs. The prostomium is insignificant, and the archicerebrum no longer clearly distinguishable. The antennæ are not prostomial tentacles, but outgrowths of the first metamere.

The Myriapoda.

Unfortunately the development of the Myriapods is very imperfectly known; but, according to the account we have of Julus (Heathcote, 1), it resembles exactly that of Peripatus as regards the segmentation of the head. In the adult there are three pairs of cephalic appendages—the antennæ, the mandibles, and the labial plate (fused maxillæ). The last two pairs belong to undoubted metameres. The first pair, the preoral antennæ, are developed on the large procephalic lobes, which give rise to the main segment of the brain (cerebral grooves are formed here also). As in Peripatus, the antennary segment contains the first pair of mesoblastic somites.

The Hexapoda (figs. 7 and 8).

Four pairs of appendages are borne on the adult insect's head. A study of its development shows that, in reality, it is composed of six regions. Of these the three posterior, belonging to the labium, maxillæ, and mandibles, are universally considered to represent true metameres. The next, counting from behind forwards, the recently discovered premandibular segment (Wheeler, 18), although possessing in the earlier stages a distinct pair of cœlomic somites and cavities, and in some cases rudimentary appendages, becomes reduced, and disappears in the adult. The next anterior segment, bearing the antennæ, was for long considered to be not only preoral in position, but prostomial in origin. Here, again, embryology shows that, like the posterior segments, it has a special pair of mesoblastic somites, with well-developed cœlomic cavities (as a rule).[1] Moreover, since in the early

[1] "The deutocerebrum [antennary segment] in all the Orthoptera which I

stages of development the antennary segment, together with its appendages, is distinctly post-oral in position, most authors are now agreed that it is a true metamere of primitively post-oral origin.

Fig. 7.

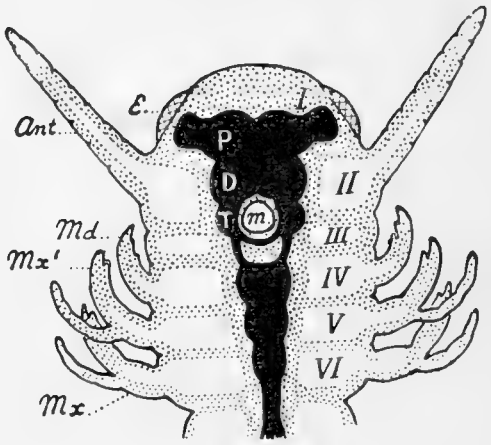


Fig. 8.

Fig. 7.—Ventral view of an embryo of Anurida maritima (after Wheeler).

Fig. 8.—Diagrammatic plan of the anterior segments of the Hexapoda (the numbering of the segments is doubtful, as explained in the text). *Ant.* Antennæ. *E.* Eye. *Lb.* Labrum. *Md.* Mandible. *Mx.* Maxilla. *Pr. l.* Procephalic lobe. P. Protocerebrum. D. Deutocerebrum. T. Tritocerebrum. Other letters as in Figs. 1 and 2.

We now come to the first serious difficulty in the interpretation of the Arthropod head. In front of the antennary segment, in the embryo Hexapod, extend the large procephalic

have examined is provided with a pair of true mesodermic somites and with a pair of appendages, the antennæ. Each mesodermic somite sends a hollow diverticulum into an antenna."—Wheeler (**18**).

"Das Cœlomsäckchenpaar des Antennensegments wor bei den von mir untersuchten Embryonen stets in typischer Weise ausgebildet."—R. Heymons (**3**).

lobes, from which region are developed the anterior segment of the brain, the optic ganglia, and the eyes. Three views may be maintained with regard to the homology of the procephalic lobes: (1) they represent the prostomium of Annelids; (2) they are merely the specialised anterior region of the antennary segment, due to its secondary subdivision; (3) they represent a true metamere, and the first.

In answer to the first suggestion, it may be said that they would be a strangely large rudiment (anlage) for a prostomium;[1] that they are differentiated before the posterior segments; that, although in the Insecta no special cœlomic cavities have been found as yet to develop in this region, they are known to occur in the similar procephalic lobes of some Myriapods and Arachnids; that in their development they strikingly resemble in general shape, position, and in their markedly bilobed character the first segment of Peripatus (antennary); and finally, that they give rise to the same and largest segment of the brain, which includes the optic centres.

The same arguments may be used to refute the second view, though perhaps not quite so convincingly. On the other hand, it must be remembered that embryologists are all agreed in considering the antennary segment of insects as complete in itself, and therefore as not including the procephalic lobes.

The third view, that the lobes represent the first metamere, remains as the most probable. It has recently been held, if I understand him rightly, by Heymons (3).

Viallanes[2] has shown, by his very careful researches on the structure of the adult brain (17), that it consists in insects of three segments. This conclusion is thoroughly supported by embryological evidence. The first or protocerebrum, includ-

[1] The procephalic lobes are also distinctly paired. The rudiment of an Annelid prostomium is unpaired (except Rhynchelmis, Vejdovsky).

[2] Although Viallanes speaks of these segments as belonging to three "zoonites," he draws a distinction between the first as preoral, and the second and third as originally post-oral. Such a view is difficult to reconcile with what we know of Peripatus.

ing the optic centres, corresponds to the first segment in Peripatus. The second or deutocerebrum, supplying the antennæ, corresponds to the mandibular segment; whilst the third, or tritocerebrum, represents the segment in Peripatus which supplies the oral papillæ.

It would appear, then, that in the Hexapoda the prostomium and archicerebrum have not been plainly distinguished;[1] that the large ophthalmic segment represents the primitive peristomium, or first metamere, which has shifted in front of the mouth together with the antennary or second metamere.

The Arachnida (figs. 9 and 10).

In the Arachnids the head appears to be formed of two segments—the anterior represented by the procephalic lobes in the embryo, and the posterior by the segment bearing the chelicerae. The syncerebrum is formed by ganglionic masses from these two regions.

Concerning the metameric nature of the cheliceral segment there can be no doubt. It is primitively distinctly post-oral in position, and contains a pair of mesoblastic somites with cœlomic cavities extending into the appendages. These are innervated by a pair of primitively ventral post-oral ganglia, which subsequently move forward and dorsally to form the second and posterior segment of the syncerebrum.

The procephalic lobes, on the other hand, offer almost the same difficulties of interpretation as in Hexapoda. As a rule, they are separated from the cheliceral segment only after the appearance of several more posterior segments. No distinct appendages are produced. This large "procephalic" region,

[1] Mr. Wheeler says "it is extremely improbable that so highly important a structure as the Annelid brain should have completely disappeared in the Arthropods" (18). This no doubt is quite true; yet it must not be taken for granted that in the remote Annelidan ancestor of the Arthropoda the brain was as important and fully differentiated an organ as in certain modern Polychætes; and anyhow it must be admitted as a fact that some of the functions of such an archicerebrum have come to be shared, if not usurped, by the ganglia of posterior metameres.

which in certain forms occupies a peristomial position in the embryo, bears the same relation to the brain as the first seg-

Fig. 9.

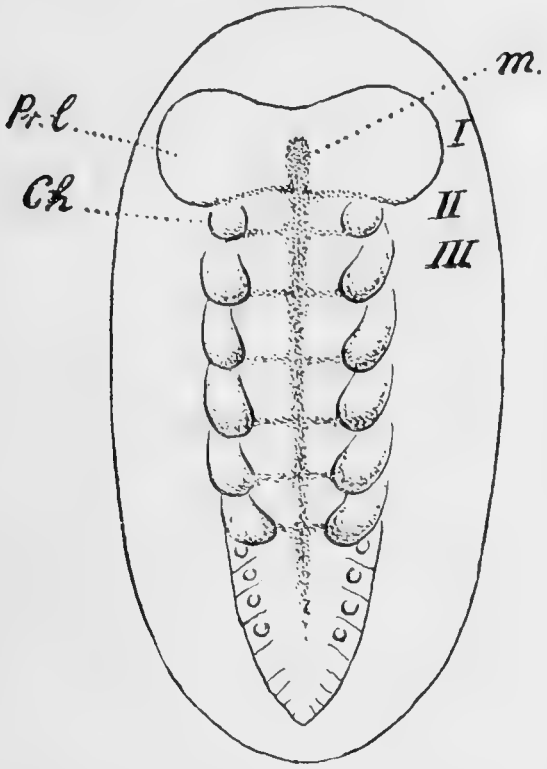


Fig. 10.

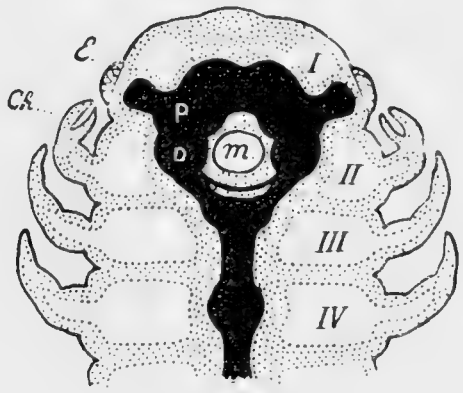


Fig. 9.—Ventral view of an embryo of Agelena labyrinthica (after Balfour).

Fig. 10.—Diagrammatic plan of the anterior segments of the Arachnida. *Ch.* Chelicera. *E.* Eye. *Pr. l.* Procephalic lobe. P. Protocerebrum. D. Deutocerebrum. Other letters as in Figs. 1 and 2.

ment in Peripatus and insects, giving rise to the large protocerebrum including the optic centres. Mesoblastic somites are present, containing in scorpions and spiders (according to Balfour, Metschnikoff, Kowalevsky and Schulgin, Laurie, and Schimkewitsch) a pair of distinct cœlomic cavities. In Limulus and some scorpions (according to Kingsley, Kishinouie, and Brauer) the cœlom of the procephalic region is formed by the forward extension of the cavities of the cheliceral segment. It is unnecessary to repeat the arguments concerning the homology of the procephalic lobes already used in the case of the Hexapoda; one may add that the presence of a cœlomic

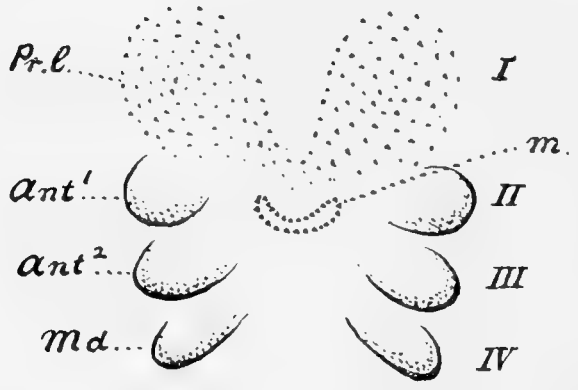
cavity in the case of the Arachnida somewhat strengthens the evidence in favour of this region representing the primitive peristomial metamere.

The Crustacea (figs. 11 and 12).

The Crustacean head is composed of six regions. The last three are obviously true metameres, post-oral in position, and innervated from ganglia on the ventral nerve-cord; they bear the two pairs of maxillæ and the mandibles.

The next two regions, as we go forwards, are preoral in position, carrying the two pairs of antennæ, but are now almost universally considered to be metameres of primitively post-oral origin which have shifted in front of the mouth. In the lower Crustacea (Apus, &c.) the second pair of an-

Fig. 11.



Ventral view of an embryo of Astacus fluviatilis (after Reichenbach). *Ant.*¹ and *Ant.*² First and second antennæ. *Md.* Mandibles. *Pr. l.* Procephalic lobe.

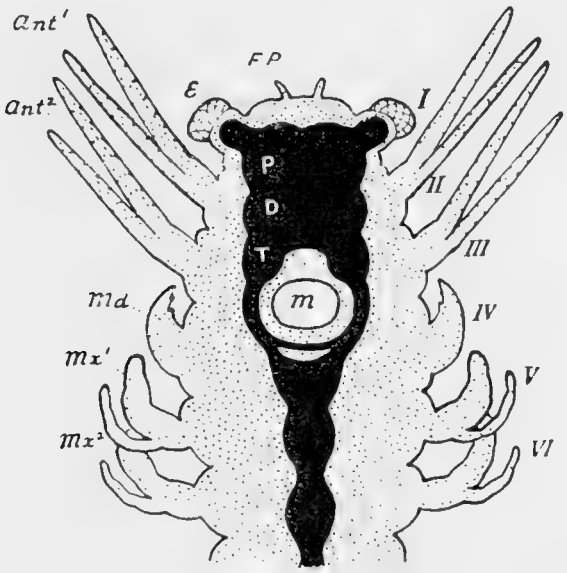
tennæ are still innervated from the œsophageal commissures (Pelseneer, 13). In the higher forms the brain supplies both pairs. These two segments assume the preoral position during development, and their ganglia fuse with those of the most anterior region to form the deuto- and trito-cerebrum of the adult brain.

There remains in front the sixth region, which bears the large paired compound eyes. Ever since Milne-Edwards, in

1834, put forth the view that the stalked eyes of Crustacea represented a pair of metameric appendages, observers have been divided into two camps; some supporting this theory (Huxley, Reichenbach, Nussbaum, &c.), others opposing it. The latter maintain that the eyes, whether stalked or sessile, are merely prostomial sense-organs.

In the embryo we find, in this region, two large procephalic lobes from which are developed the eyes and optic centres and the anterior segment of the brain, corresponding to the pro-

Fig. 12.



Diagrammatic plan of the anterior segments of the Crustacea. *Ant.*[1] and *Ant.*[2] First and second antennæ. *E.* Eye. *F. P.* Frontal process. *Md.* Mandible. *Mx.*[1] and *Mx.*[2] First and second maxillæ. *Pr. l.* Procephalic lobe. P. Protocerebrum. D. Deutocerebrum. T. Tritocerebrum. Other letters as in Figs. 1 and 2.

tocerebrum of other Arthropods (Viallanes). Unfortunately, no definite evidence has been obtained with regard to the metameric nature of this region from the study of the mesoblast, since no distinct somites or cœlomic cavities have been traced with certainty in the anterior segments of most Crustacea. It is evident, however, that although the procephalic lobes may represent the first metamere, the stalked eyes need not necessarily be its true metameric appendages. Neverthe-

less some evidence for this interpretation is afforded by the cases brought forward by A. Milne-Edwards of a Palinurus (12), and by Hofer (4) of an Astacus, in which the eye-stalk on one side was produced into a jointed flagellum; also by some recent experiments of Dr. Herbst, who, having cut off the eyes of Palæmon, finds that jointed antenna-like appendages are regenerated in their stead (2).

The prostomium itself may have to be sought for in the median anterior region in front of the procephalic lobes. It has been suggested that the median eye and little frontal processes of the Nauplius larva represent prostomial sense-organs, and it is possible that the anterior region of the brain in connection with these represents the archicerebrum.

In the foregoing pages the view that the procephalic lobes are homologous throughout the Arthropoda, and represent the peristomial segment of Annelids, has been consistently favoured, not because this interpretation can be considered as firmly established, but from a conviction that the best way of presenting the problem is to uphold a definite theory. Thus both the weakness and the strength of the position become clearer. It is quite plain, however, that much evidence is still needed bearing especially on the presence of distinct mesoblastic somites in the procephalic region in several groups, and on the possibility of distinguishing the true prostomium and archicerebrum in the Arthropoda.

ANNELIDA.	PERIPATOIDEA.	INSECTA.	ARACHNIDA.	CRUSTACEA.
Prostomium with or without tentacles. Archicerebrum.	? Frontal processes?	?	?	? Frontal processes?
Segment 1 or Peristomium.	Procephalic lobes, antennæ, protocerebrum.	Procephalic lobes, protocerebrum.	Procephalic lobes, protocerebrum.	Procephalic lobes, protocerebrum.
Segment 2.	Mandibles, deutocerebrum.	Antennæ, deutocerebrum.	Cheliceræ, deutocerebrum.	First antennæ, deutocerebrum.
Segment 3.	Oral papilla.	Rudimentary appendage, tritocerebrum.	Trunk segment.	Second antennæ, tritocerebrum.
Segment 4.	Trunk segment.	Mandibles.	Ditto.	Mandibles.
Segment 5.	Ditto.	First maxillæ.	Ditto.	First maxillæ.
Segment 6.	Ditto.	Second maxillæ.	Ditto.	Second maxillæ.

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On the Development of the Californian Hag-fish, Bdellostoma Stouti, Lockington.

By

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(Preliminary Note.)

With Plate 17.

Dr. Howard Ayers, in a lecture before the Wood's Holl Biological Laboratory,[1] has given a detailed account of the occurrence of the Pacific Hag-fish, Bdellostoma Dombeyi (= Stouti), in the Bay of Monterey. Its great abundance in this region gave promise of material for the study of the development of a Myxinoid; or, at all events, the chances to obtain these valuable stages seemed far more favorable here than in the European localities, mainly in the North Sea, where the eggs of Myxine had long been sought. Among the zoologists who endeavoured to secure hag-fish embryos in California Professor G. C. Price, of the Leland Stanford, Junior, University, was the first to succeed, and he has already published two accounts of his studies.[2] During the past summer additional developmental stages, including a number of the earlier ones, were collected by the present writer during a visit (July, August, September) at the Hopkins Marine Laboratory.

[1] 1893, Ginn and Co., Boston.

[2] "Zur Ontogenie eines Myxinoiden," 'Sitzungsberichten der mathematisch-physikalischen der K. Bayer. Akad. d. Wiss.,' Bd. xxvi, 1896, Heft 1, S. 67—74.

"On some Points in the Development of a Myxinoid," 'Verhandlungen der Anat. Gesellschaft.'

At the request of Mr. J. T. Cunningham, well known for his interest in the study of the Myxinoids, the following paper has been prepared, with a view of presenting in outline the main facts relating to the external development of this remarkable Chordate type.

The hag-fish finds a natural spawning ground in the Bay of Monterey. Here the spawning appears to take place over a wide area, since eggs have been secured at many and distant points. The embryos in the writer's collection, however, were taken from one particular neighbourhood (about a mile off shore, in twelve fathoms of water, sp. gr. 1·020, temp. 50°—60° F.), which represented doubtless a favorable spawning ground. About one fifth of the eggs here collected were found to yield embryos, while eggs from other regions rarely contained them, the worthless eggs averaging nearly 98 per cent. In view of the present method of collecting, success in obtaining embryos is to a large degree a matter of accident; for the eggs can very rarely be dredged, probably because they have been deposited among rock fragments or deep in the mud, as Plate suggests.[1] It is upon trawl-line fishing, as Price has noted, that the collector must finally depend. By this means many scores and even hundreds of hag-fishes may be caught during a day's fishing; but among these there will only rarely be one which has entrapped an egg-string in its encasing slime. Such an instance is shown in the accompanying figure (fig. 1), from a water-colour sketch by the writer. The hag, reddish purple in colour, has twisted itself into a knot around the trawl-line; the slime mass enclosing it is translucent, whitish, here and there blood-stained; the eggs are tangled securely in the meshes of the slime, suggesting a string of yellow beans. The eggs, as has often been noted, are fastened together at either end by clumps of wiry filaments, whose tips interlock. The tips, shown in fig. 2, vary notably in size and form (con-

[1] "Ueber die Eier von Bdellostoma Bischoffii, Schneider," 'Sitzungsberichten der Gesellschaft naturforschender Freunde zu Berlin,' Jahrg. 1896, No. 1, S. 17—21.

The writer's material was collected almost entirely from rocky bottom.

trast Plate's fig. 3); they are typically flower-shaped, their broadly everted petal-like margins enabling them to interlock.

Time and Mode of Oviposition.

Both Ayers and Price have agreed that in the Bay of Monterey the spawning season of Bdellostoma is an extended one. These conclusions agree fully with the writer's observations. He has obtained embryos of many ages from the same locality at the same time; he notes, however, that during the present season the embryos were most abundant of a particular size, a fact which suggests that a time of maximum spawning had occurred. As to actual oviposition, the following note is suggestive:—A fisherman brought in (August 25th) twenty-three eggs, which he said had been spawned in the boat shortly after the mother, which he also brought, had been taken. Each egg was encased in a transparent glistening outer capsule, delicate but elastic. One would be inclined to believe that the fisherman had observed the normal method of spawning, i. e. that the eggs were discharged at the same time, extruded from the abdominal pores the more readily on account of the sheathing capsule. The rupture of the capsule at or before fertilisation would set free the fastening processes, and thus enable the eggs to become attached, either together or to other eggs in the neighbourhood. This view, as opposed to that of Ayers, is supported by the fact that in a number of cases embryos of widely different ages have been found in the same egg-string.

General Mode of Development.

The material collected by the present writer includes an almost complete series of embryos from a stage in which the blastopore is closing, to one about the time of hatching; together with these are several possible stages of segmentation. A few of the most typical of these embryos are shown in the accompanying figures (figs. 3—8), and afford a convenient basis for an outline of the mode of Myxinoid development.

As Price has shown, and as others have indicated, the development of a Myxinoid is typically meroblastic ;[1] it might reasonably be surmised that the large amount of yolk-material would enable the embryo to attain an advanced stage before the period of hatching, and that the duration of development in the egg should be a long one. These conjectures are clearly confirmed by the writer's observations upon embryos reared for a time in aquaria ; he believes that the eggs do not hatch within two months, and thinks it possible that several more months may be taken. By this time the young Bdellostoma has attained a length of over 6 cm., and has outwardly the characters of the adult. Price notes that a distinctly larval period is absent.

There can be little doubt, as Price has noted, that Bdellostoma is not protandric in the sense demonstrated by Cunningham in Myxine.[2] The eggs are in all probability fertilised after deposition. An egg nucleus is then present immmediately below the single large micropyle at the operculated (animal) pole of the egg.

Segmentation. — Until the writer's material of the youngest stages shall have been sectioned, he can refer but doubtfully to a single egg. Of this transverse sections near the animal pole show on one side a number of nucleus-like structures embedded in a clear peripheral germinal area. There are no cell outlines, although the stage is probably early. One cannot doubt, however, that a condition of asymmetry with reference to the longitudinal axis of the egg appears in very early stages. And from the conditions of the following embryo it will be inferred that the dorsal lip of the blastopore appears on one side of the egg, not far from the rim of the opercule, and that from this region it closes fissure-wise backward.

Very Early Embryo (fig. 3).—At this stage the embryo is thread-like. The very narrow neural cord, N, represents

[1] Cunningham, Retzius (1889), 'Biol. Foren. Forhdgr.,' Bd. i, pp. 22—28.

[2] Cunningham, J. T., "Reproductive Elements in Myxine," 'Quart. Journ. Micros. Sci.,' vol. xxvii, 1886.

the fused lips of the blastopore, whose fissure is still to be made out behind the tail region, BP. Anteriorly the long narrow brain mass, B, is indicated, scarcely deeper and wider than the myelon into which it merges. No canalis centralis can be determined in surface view. Notochord and mesoblastic somites have not yet arisen. A mesoblastic thickening is apparent in surface view only at *.

Early Embryo (fig. 4).—This embryo is scarcely longer than the earlier one, but it is notably broader, and it has made marked advances in organogeny. Anteriorly the neural cord has acquired a lumen; its walls, thickened and folded asymmetrically, mark out vaguely the divisions of the brain. Auditory vesicles, AU, are prominent; optic vesicles present but very indistinct. Mesoblastic somites, PS, about ninety in number, appear close to the neural cord, and extend sideways but a very short distance. They are here differentiated in situ, but surface view cannot demonstrate definitely that gut cavities are present.

Interesting is the condition of the fore-gut, which now dilates under the definite head region, and is already, as far as one can judge from surface view, pierced with several gill-slits. The heart, H, is vaguely indicated in front of the head, extending directly forward.

Moderately Early Embryo (fig. 5).—The trunk has become outlined in this stage; on each side it is separated from the yolk region by a marginal artery which passes backward as it breaks into branches in the neighbourhood of the tail. Body length has somewhat increased, due apparently to growth in both directions, the head now extending under the operculum, the tail somewhat nearer the opposite pole of the egg. Advances in the development of the neural cord include anteriorly the enlargement and definite outlining of the brain parts which have now grown under the auditory vesicles and over the eyes, and the now prominent and fused nasal pouches, and posteriorly the extension of the canalis centralis into the region of the tail. The asymmetry of the foldings of the neural tube is now visible only in the region of the medulla.

Somites are prominent, PS. Pronephric tubules are apparent in connection with all the mesoblastic somites, PN, each narrowing out laterally into a delicate contorted tube, but as yet there has appeared no pronephric duct. In the gill region about the normal number of gill-slits have now been formed. The number increases from behind, but remarkably enough those latest formed (i. e. hindmost) are largest. The gill region is marked out clearly on either side of the embryo as a conspicuous lappet-shaped outgrowth.

Late Embryo (figs. 6, 7).—Continued growth has carried the head of the embryo around the animal pole as far on the ventral side as past the rim of the operculum; the tail, now narrow and tapering, has elongated until it has almost reached the hind end of the egg. Head, neck, and tail are separated below from the yolk-sac, although they lie in grooves, and are deeply sunken into it. Again, a marginal artery, DA, separates the trunk from the yolk-sac. Somites are now clearly marked; pronephric structures are prominent, PN, and a segmental duct, SD, is found. In the head region the principal advances include the enlarged size of the nasal pouch, N, the appearance of supporting tissue and of mouth, the latter originating as a single invagination. This is very definitely shown in one of the writer's preparations, who thus differs from the conclusion of Price as to its probable origin as a paired structure. The gill-slits at this stage, GS, may be seen through the back when this region is viewed as a transparent object. Since the last stage these have been drawn forward, and at the same time inward toward the ventro-median line by the growth of the head. The heart is at this stage a well-marked tube, passing straight toward the head from the hinder pole of the egg; on either side it receives asymmetrically the vessels from the yolk region, carrying the venous blood to the gills. The vessels are very conspicuous in the living object, the crimson threads in brilliant contrast to the rich yellow of the underlying yolk.

Latest Embryo (fig. 8).—The figure illustrates the latest stage in the writer's material. The young Bdellostoma

measures 6 cm. in length, and will (probably) shortly hatch. It exhibits occasional writhing, and its mode of growth suggests that in the process of hatching the operculum will be detached by movements of the tail. The colour at this stage is purplish, save in the region of the yolk-sac. This, as before, is bright yellow, closely traversed by vitelline blood-vessels. The yolk-sac is now attached to the embryo only in the middle portion of its trunk; both the anterior and posterior trunk regions seen in the figure are separate, therefore, from the yolk-sac, although pressed tightly against it, and embedded in deep grooves. The tail lies on its right side, and is growing over the head toward the opercular end of the egg. The head, lying somewhat on its left side, has now grown backward till it has nearly reached the posterior pole of the egg. Structurally this late embryo closely resembles the adult; cirri are present around the mouth as in the adult condition, although they cannot be seen in the figure, the mouth being pressed far backward on the ventral side. The anus is indicated at A, segmental mucous pits at M. Dermal-fin rays are well developed. The gills, GS, are now the characteristic pouches with infolded walls and with external tubular ducts. Their final position will be seen to be far backward of the head. It is evident, accordingly, when figs. 4, 5, 6, and 8 are contrasted, that the gills have not been growing forward at an equal rate with the head region. They early arise near the first slit even in front of the auditory sacs (fig. 4); they are seen later (fig. 5) in the process of being drawn forward under the head, but are now well behind the ear capsules; still later (fig. 6) they are seen in their normal position on the ventral side of the throat, but further back behind the ear sacs, separated from them by a distance of several somites; and finally (fig. 8) they appear in their adult conditions, still further caudad, now separated from the ear sacs by ten or more somites. By the process of unequal growth, therefore, the change in the position of the gills is explained.[1] As at no

[1] As has also been shown to be the case in Amphioxus by Lankester and Willey. (Editor.)

stage in the writer's material has he found any traces of either anterior or posterior gill-slits to increase the normal number, he is unable to accept Price's suggestion, based upon the number of spinal ganglia and the change in the position of the gills, that the embryos of Bdellostoma have as many as thirty-five pairs of gill-slits.

Conclusions.

The Myxinoid differs widely from all other Chordates in its developmental type. Its sharply marked differences from the mode of development of the lamprey emphasise the wide divergence of these branches of the Marsipobranchian stem; and this alone forms a strong ground for belief in the antiquity of the Cyclostomes, and for rejecting even a most remote Teleostean ancestry. Developmentally the two branches certainly differ as widely as sharks and Ganoids. There can be no doubt, furthermore, that the embryonic conditions of Bdellostoma are not to be derived directly from those of the lamprey. To what degree the converse is to be accepted must remain for further study. We may now reasonably believe that the ontogeny of a Myxinoid, when fully studied, will enable the interrelationships of the Marsipobranchs to be broadly outlined: and it is possible that many valuable suggestions will follow as to the general relations of these to Protochordates on the one hand, and on the other to the ancestral Gnathostome.

Some of the more striking features in the development of Bdellostoma, as above described, may finally be summarised.

I. The neural tract is laid down, nearly in its entire length, before the appearance of somites, and without any indication of neuromeres.

II. The neural tract, as in Petromyzon, acquires a lumen by dissociation of cells (as shown by sections), proceeding antero-posteriorly.

III. The brain is distinctly a tubular structure, and differs little in calibre from the spinal cord up to a relatively late period in development, i. e. to the time of the appearance of

paired sense-organs, of gill-slits, and of nearly the adult number of somites.

IV. The brain portion of the early neural tube is of very great length, one fifth the entire length of the nerve-tube (one quarter if the foldings in the brain wall be taken into account), at a stage when nearly the adult number of somites are present. It is to be inferred, therefore, that the craniote brain is composed of a far longer region of the anterior neural tract than has hitherto been supposed. It is thus probably homologous with the branchial region of the cord, as well as with the so-called brain of Amphioxus.

V. The numerous foldings (asymmetrical) of the brain wall, by which the regions come to appear, indicate a primitive condition of the craniote brain, i. e. that the latter was originally a tube of many vesicles[1] (eight of these at least between cerebellum and thalamus), which in the ontogeny of higher forms have become merged into fewer and larger ones.

VI. There is an almost entire absence of cranial flexure.

VII. Although the developmental type of Bdellostoma is distinctly meroblastic, it is noteworthy that there appears no trace of a germ ring; except in the immediate region of the tail, the somites arise in *situ* at the sides of the neural axis.

VIII. The early appearance in each segment of pronephric tubules, similar to each other in essential characters, demonstrates, as Price has shown, that the entire excretory system of Myxinoids must, from the standpoint of ontogeny, be regarded as univalent. If we accept, therefore, Spengel's criticisms of the results of Semon on the morphology of the excretory system in Myxine, we must nevertheless admit Semon's *a priori* view as to its homology as a pronephros.

Note on the above by J. T. Cunningham, M.A.—It is, I think, of some interest that the method of fishing by which alone eggs of a Myxinoid have been obtained in California is

[1] The writer believes that these may be distinguished from the neuromeres of Locy and recent authors, which are not distinctly vesicular, and which occur in cord as well as in brain.

commonly practised off the mouth of the Firth of Forth, and elsewhere along the east coasts of Scotland and England, although eggs of Myxine have never been obtained by that method. Some years ago I made strenuous endeavours to obtain fertilised eggs of Myxine, and received grants from the Royal Society to defray the expenses of the investigation. I frequently made excursions on fishing-boats engaged in fishing for haddocks with long lines, which the Americans call trawl-lines. On these lines large numbers of Myxine were always taken on the hooks, just as in the fishing described in the Bay of Monterey. But no extruded or fertilised eggs were ever seen by me entangled in the slime of the Myxine. The only explanation of this which seems possible is that off the English coast the fishing-lines have not been shot on a ground where Myxine spawn, all endeavours to obtain the eggs from the lines or from the fishermen having hitherto failed. I cannot agree with Mr. Bashford Dean in his remarks on the mode of oviposition. The sheathing capsules to which he refers as enclosing the spawned eggs observed by a fisherman must be, I think, the follicular capsules belonging to the ovary, in which the eggs are developed. I have frequently seen the eggs of Myxine enclosed in these capsules pressed from the fish, but I showed in my paper on the generative organs that the eggs normally escaped from the ovary by the rupture of these capsules, and the separation of the capsules without rupture is due to some violence or pressure in the capture or handling of the female. In Myxine it is certain that the eggs when extruded are enclosed only in the egg membrane provided with threads at the poles, this membrane being of the nature of a chorion produced in the follicular capsule. It is probable that in this respect the eggs of Bdellostoma Stouti are quite similar to those of Myxine glutinosa.

DESCRIPTION OF PLATE 17,

Illustrating Dr. Bashford Dean's paper "On the Development of the Californian Hag-fish, Bdellostoma Stouti, Lockington."

FIG. 1.—Bdellostoma taken on trawl-line, showing egg-string entangled in encasing slime. × $\frac{5}{8}$.

FIG. 2.—Tips of horn-like filaments of eggs. × about 30.

FIGS. 3, 4, 5.—Stages of early embryos. × $4\frac{1}{4}$. AB. Anterior end of brain. AU. Auditory vesicle. B. Brain. BP. Blastopore. GS. Gill-slits. H. Heart. N. Spinal cord. O. Rim of operculum. OP. Optic vesicle. PN. Pronephric tubules. *PS.* Somites. *UDT.* Undifferentiated tail mass. * Appearance of mesoblast.

FIGS. 6, 7, 8.—Two stages of late embryos. × $4\frac{1}{4}$. A. Anus. AU. Auditory vesicle. DA. Dorsal aorta. GS. Gill-sacs. H. Heart. M. Mucous pouch. OP. Eye. N. Nasal sac. PN. Pronephric tubule. SD. Pronephric duct.

On the Diplochorda.

1. The Structure of Actinotrocha.
2. The Structure of Cephalodiscus.

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With Plates 18—26.

Introduction.

Amongst the many and diverse types of animal structure which are familiar to the zoologist there are some, both simple and complex in character, which have never offered any serious difficulty with regard to their systematic position in the natural classification; and there are others which ever since their first discovery have provided material for discussion concerning their systematic relationships, and whose true genetic connection has not yet been clearly elucidated. From the nature of the case these latter animals are usually generalised types, and a certain proportion of the difference of opinion has arisen by different observers laying greater stress upon special structural features.

On the other hand, possibly on account of the doubtful nature of their genetic relationships, these animals are commonly either left out of zoological text-books altogether, or are merely referred to casually as incertæ sedes, although the study of their structure and development always gives the promise of renewed light upon the many morphological problems of the day. In other words, these animals form the anomalies or exceptions which in other sciences, as well as

that of biology, have always been the keys with which to unlock the secrets of the natural system.

Amongst these types the genus Phoronis holds pre-eminently the position here referred to; for though its first discovery only dates back some forty years, or, including its extraordinary larval form, a little over fifty years, and the genus only comprises some half-dozen known species, yet a long series of investigators have devoted their energies at one time and another upon it, and the divergence of opinion resulting from this and the attempt to point out its affinities has been truly remarkable.

The larval form, Actinotrocha, first discovered by Müller in the North Sea, was conjecturally assigned by sundry zoologists to the Turbellaria, Rotatoria, Polyzoa, Mollusca, Sipunculoidea, Annelida, and so on; and since its transformation into the adult Phoronis was proved it has been variously enligned with the Polyzoa, Sipunculoidea (Gephyrea), Chætopoda, Brachiopoda, and so on.

The whole history and literature of the Phoronidea have been so exhaustively dealt with, firstly by Professor McIntosh (15), and secondly, so recently as 1892, by Dr. Cori (7), that it is scarcely justifiable to repeat the account here, especially as the memoir by Dr. Cori is the latest publication till within the last year dealing with the genus. Suffice it to say that the generally accepted opinions of zoologists appear to be either in favour of regarding Phoronis as an aberrant Sipunculid[1] or as an aberrant Polyzoan.[2] An intermediate position between these two groups, or between one of them and some other distinct groups, such as Mollusca or Brachiopoda, has also been held by many.[3]

It was thus felt that the last word had not been said upon Phoronis, and that a renewed investigation of the group, especially with the assistance of modern methods, might be attended by profitable results.

[1] Krohn, Schneider, Wilson, Metschnikoff.

[2] Ostroumoff, Barrois, Gegenbaur, McIntosh, Lankester, Cori.

[3] Kowalevski, Claparède, Caldwell.

The contents of the following pages are the result of more or less intermittent work since 1894, which has led me to hold that, although Phoronis has some features in common with both Gephyrea and Polyzoa, its more immediate genetic allies are not usually found in either of these groups, but are commonly placed together under the title Hemichorda.

Thus whilst the more pronounced community of structure holds between Phoronis and Cephalodiscus, yet the likeness of the former to Balanoglossus in certain other characters is so marked that it rather tends to strengthen the alliance between the two latter.

No one can doubt that Phoronis is, in the adult stage, greatly specialised for a sedentary life, and that this specialisation is, as in the case of the Tunicata, a degeneration involving a loss of certain organs, degradation of others to a lower structure, and yet again hypertrophy of others which are not so prominent a feature in the archaic type. Thus a study of the adult alone, with a view to phyletic conclusions, would be embarrassed by these attendant complications; and were Phoronis to reproduce itself by a fœtal method, these could not be surmounted. Fortunately, in Actinotrocha we have a free larval form, which not only must be construed, with certain reservations, as exemplifying the structure of the undegenerate free-swimming ancestor of Phoronis, but which has a very well-marked culminating point in differentiation, with a sudden decline in morphological status to the adult condition. This being so, an investigation of the fully developed Actinotrocha should offer the readiest solution of the problem before us.

In Part I, I have gone over the whole structure of the Actinotrocha larva, as can be discerned by an examination of preserved specimens, both whole and in section. The method of examination by serial sections has enabled me to correct some misconceptions with regard to the structure of this unique larval form. It is purposed to show that Actinotrocha has so close a similarity in structure to the three members of the Hemichorda that the assumption of a

genetic connection appears to be the only conclusion. Nevertheless, for reasons stated below, I would suggest that Phoronis be considered as constituting a distinct subdivision of the Chordata, for which the name Diplochorda is proposed.

In this group the leading anatomical characters of the highest Chordata have their rudimentary homologues, and the distinctive features of the Vertebrata are reduced to so general a type that the transition to the true Invertebrata is but a small step.

Whatever value this research may have as an addition to our knowledge cannot therefore be summed up by the looked-for result that the Chordate affinities of Phoronis will be definitely acknowledged, but will lie in the fact that the recognition of this relationship will bring the base of the Chordate tree into contact with certain Invertebrata which have always been more or less connected with Phoronis. My suggestion of the group Archicœlomata is merely the embodiment of this conception, and the apology for its creation will be forthcoming if it may lead specialists in the groups of Sipunculids, Polyzoa, Brachiopoda, and Echinodermata (our great sedentary groups) to hold in view the possible occurrence of pre-chordal characters in the ontogeny of their investigated types. The discovery of the close connection of the Tunicata with Amphioxus served rather to remove them from their former alliance, and cannot be said to have pointed to any link between the Chordata and the Invertebrata.

The removal of Balanoglossus, and later the association with it of Cephalodiscus and Rhabdopleura, allowed the Chordate ancestry to be traced back to pre-chordal (Lankester) times; and the study of Phoronis, and especially its development, takes us back to the definite meeting-place between the two great types of segmented animals, the segmented Invertebrates and the higher Chordata.

During the progress of this work a detailed comparison was made between the several organs of Phoronis and Acti-

notrocha with the Hemichorda (24 and 25). In this comparison it was assumed that the structure of Balanoglossus had been modified by its peculiar habits in such a way that the notochord and other organs had become secondarily shifted into the pre-oral lobe. This led to the difficulty that the "notochord" of Cephalodiscus (Harmer, 10) was in the same position, and yet the burrowing habit is not in this case indulged in.

The discovery of the "subneural gland" (see below) in Actinotrocha, however, seemed to complicate the difficulty; for this organ, although epiblastic, occupies the same position and has the same relationships to other organs as the "notochord" of Cephalodiscus. The natural conclusion was that the "notochord" of Cephalodiscus was a subneural gland, and the notochord itself was yet to be found. With such an end in view I examined Cephalodiscus by sections, and there can be no doubt that this animal possesses a paired notochord on the dorso-lateral part of the pharynx. In the light of this and other facts given below, granting that the structural and phyletic relationships of animals are to be expressed in our classification, it seems reasonable, and indeed necessary, that Phoronis and Cephalodiscus be included in one group, Diplochorda; whilst their relationship to Balanoglossus (or Hemichorda) may be expressed by including the two groups under the name Archichorda.

The present paper will therefore be confined to an account of the structure of Actinotrocha, and a note upon the structure of Cephalodiscus. Part I.—The Structure of Actinotrocha. Part II.—The Anatomy of Cephalodiscus.

PART I.

The Structure of Actinotrocha.

Literature.

As is well known, the larva, Actinotrocha, was first described by J. Müller (16) as occurring in the North Sea. He gave an account of the general characters, the form of body, number of tentacles, &c. This was followed a year later by a research of Wagener, who, assuming the animal to be an adult, gave a description of its anatomical features. His work was conducted, as far as can be ascertained, without the assistance of sections, and it will be referred to in the following pages. It is evident that some of his statements are erroneous, more especially in the section referring to the nervous system.

In 1850, Siebold (19) contended for the larval nature of Actinotrocha, drawing a comparison with Bipinnaria.

In 1854, C. Gegenbaur (9) notified the occurrence of a similar form from Messina, in winter, of a small size (·35 mm.), and remarks on the differences from Müller's. He traced its growth and differentiation to ·4 mm., with an accompanying marked elongation of the hind region.

In 1858, Krohn (11), examining the same species as Gegenbaur, came to the conclusion that it had specific distinctions from that of J. Müller, mainly in the perianal ciliated band and the pigmentation. He also discovered the fact that its metamorphosis resulted in a gephyrean worm.

In 1862, A. Schneider (18) described and figured a new species, A. pallida, and gave an account of the metamorphosis into the worm-like "Sipunculid" afterwards identified as Phoronis. His description of the larval anatomy confirmed in most particulars that of his predecessors, but he corrected Wagener on some points, such as the blood-system. His description and that of Claparède (5) of the "notochord" are of special interest. His observations on the adult worm led him to find its nearest allies in Phascolosoma, Sipunculus, and Aspidosiphon.

In 1867 Actinotrocha again came under detailed notice, this time at the hands of Kowalevski (12), who gave an account of the developmental changes and metamorphosis. He identified the adult worm with Phoronis (syn. Crepina, van Beneden). Written in Russian, this memoir would be a sealed book to most morphologists were it not for a German epitome. Yet again, in 1871, E. Metschnikoff (14) obtained a number of early stages from the Mediterranean Sea, which he described and figured, and also gave further particulars upon the metamorphosis. Like his predecessors, he did not adopt the method of sections in his work, or it is probable that some further important facts on the structure of his young stages would be to hand. After a period of some ten years the Actinotrocha of Chesapeake Bay was investigated by E. Wilson (22), who discriminated two species, and followed out the metamorphosis. Disagreeing in minor points with Metschnikoff, he confirmed in the main this naturalist's account. A large part of his paper is devoted to a discussion of the significance of the metamorphosis and of the systematic position of Phoronis.

In 1883 a preliminary note upon the development of Phoronis, besides other points, was issued by Caldwell (2). So far as I am aware, nothing further has been contributed by this worker except a short paper (3) on the early stages of the embryos, although no doubt the expectation of further contributions has caused the investigation of Actinotrocha and its earlier stages to be neglected for the last dozen years or so. In comparing my results with those of Caldwell there has been an important difficulty to contend with. In these days of rush for priority it is questionable how far a worker can be expected to stand by every assertion made in a "preliminary" note, and it is also difficult in a paper of this kind to discern how much of the statements therein contained are claimed as original. If Caldwell's paper does not, therefore, receive as much recognition below as it would seem to be entitled to, its preliminary and therefore tentative nature must be given as the reason and excuse.

To these we must add that Cobbold (4) describes, in 1857, the occurrence of Actinotrocha in the Frith of Forth. His paper is principally notable for the statement that the larvæ fixed themselves by their anal extremity.

Two years after (1859), Dyster (8) noted the eggs and early stages of Phoronis. We may add that in 1888 McIntosh (15) gave, in connection with his anatomical account of Phoronis buskii, two figures and a short description of some early stages of Actinotrocha from the tentacles of the adult.

We may only add to this short account the fact that Actinotrocha, after it was once proved to be the larval form of Phoronis, has in various text-books and general zoological articles been usually interpreted as a much "modified trochosphere" larva, whilst comparisons have been drawn between its structure and that of the Echinoderm larvæ. Although several authors, to be mentioned later, have drawn a comparison between the adult Phoronis and the Hemichordata, Harmer (10) is the only author, as far as I have been able to ascertain, who has attempted a tentative comparison between the organs of Actinotrocha and those of Balanoglossus. His deductions were limited in extent, owing to the supposed absence of the notochord, gill-slits, and mesenteries separating the cavity of the pre-oral lobe from those of the lophophoral region.

External Form.

The species of Actinotrocha referred to in this paper does not appear to differ in any essential respect from that originally described by J. Müller near Heligoland, and named by him Actinotrocha branchiata. The specimens here figured were caught by the bottom tow-net in St. Andrews Bay. In the absence of knowledge with respect to the adult in both cases the minor difference need not concern us here. Although the general appearance of Actinotrocha is familiar to every zoologist from the figures of Müller (16), Wagener (20), Metschnikoff (14), and many others, copied more or less accurately into nearly every illustrated text-book, yet I have

drawn the general appearance of the larva intact, and in several positions mainly for the demonstration of several newly described organs.

Pl. 18, fig. 2, gives the appearance of a young Actinotrocha with twenty arms, as seen in side view; the hood is in the position which perhaps may be described as normal. The front parts of a later stage are indicated, as seen from the dorsal surface, in Pl. 18, fig. 3; and a side view of the same parts at a yet more advanced stage is seen in Pl. 21, fig. 38.

Lastly, the general shape of the body is shown in Pl. 18, fig. 2. This is the side view of a larva with eighteen tentacles, introduced mainly to give an indication of the distribution of the principal nervous tracts. The posture of the pre-oral hood is very characteristic; indeed, most Actinotrochæ die in this position, or else with the hood turned still further back. The larva when alive is very active, and on provocation is capable of extraordinary contortions. Figs. 4 and 5 are two outline sketches which indicate two extreme positions assumed by one lively little fellow who lived for some hours in a small glass vessel, the only living specimen I have yet observed. The mobility of the pre-oral hood and its great prominence in the anatomy of the animal are remarkable features of its general anatomy.

It is not proposed here to follow out the development to any great extent, but there are indications that there is a great increase of size in the later stages, confined mostly to the trunk portion of the body. It is difficult to speak with certainty, but it appears likely that this increase is further followed by a contraction in size, a phenomenon of the same nature as that described for Tornaria by Morgan (17); at least, certain of the larvæ are smaller than usual, and in section all their epiblastic cells are closely aggregated, and form a thick epithelial wall, in marked contrast to the greatly attenuated cells which form the outer covering of the trunk in the larger larvæ. In these smaller forms the organs are closely packed together, and the cœlomic cavities are very reduced. The natural supposition would be that they are the

earlier stages of the larger larvæ; but this cannot be the case, for the structure of the notochord, of the nervous and circulatory systems, besides the number of tentacles, show clearly that they are at a more advanced stage. In the absence of direct observation of transition stages, there are only two alternative conclusions. These smaller larvæ are either later stages, reduced in size as above suggested, or they belong to a different species. Wilson (**22**) observed two distinct species of Actinotrocha, which he called A and B, differing mainly in the fact that the form A had a shorter intestine and stouter body.

The whole body of Actinotrocha has three natural divisions:

1. The pre-oral hood (syn. pre-oral lobe, cephalic lobe, protomere), whose position is essentially pre-oral, and from Metschnikoff's figures (**14**) of early stages it is primarily so. This hood is homologous in every particular with the "proboscis" of Balanoglossus, the "buccal disc" (buccal shield) of Cephalodiscus, and the "epistome" of Rhabdopleura.

This pre-oral lobe, whose shape and relationships are shown in the figures already referred to, is produced backwards into two lateral horns (cf. Balanoglossus), which fuse on to the body-wall, laterally to the mouth-opening.

2. The lophophoral area, or the part bearing the arms. It commences at the hind end of the pre-oral hood, the line of junction being marked by a radial nerve (see Nervous System), and extends backwards a very short way in the dorsal region, but a greater distance in the ventral. It is limited posteriorly by the line of tentacles, or rather by the nerve-ring lying posteriorly to them; and as this passes diagonally from the front dorsal region (nerve-ganglion) to the ventral side (see Pl. 18, fig. 2) it is easy to see that this lophophoral segment consists of a cylindrical area surrounding the part immediately behind the mouth, the ventral length of the cylinder being many times longer than the dorsal. This segment or area is, I hope to show, homologous with the "collar" of Balano-

glossus, and the lophophoral segment (already sometimes termed the "collar") of Cephalodiscus and Rhabdopleura. The whole segment may well be termed in all these animals the "mesomere" (see below).

3. The trunk, extending as an elongated cylindrical portion backwards from the mesomere, and bearing terminally the perianal band and the anus. It will probably be a matter of no great difficulty to show that this "trunk" segment ("metamere") is homologous with the trunk of Balanoglossus, Cephalodiscus, and Rhabdopleura.

The three segments are sufficiently well defined externally, but the arrangement of the internal organs, such as cœlomic cavities, is even more marked, and corresponds exactly with the divisions here indicated.

There are three prominent ciliated bands, the pre-oral (or prototroch), the collar-band following the line of the tentacles (mesotroch), and the trunk band (perianal or metatroch) surrounding the anus. Each is defined not only by the presence of very long cilia, but of elongated and densely aggregated epithelial ectodermal cells bearing them, and well supplied with nerve-tracts.

Of the three the perianal band is the most prominent, and functions as the locomotor organ of the larva.

The other general relations of the three divisions are diagrammatically shown in Pl. 22, figs. 47 and 50.

The Organs of the Epiblast.

The dorsal and ventral surfaces of the pre-oral hood are sharply separated by the pre-oral band of cilia and its thickened cells covering a nerve-ring. The whole dorsal surface is covered with thick glandular epiblast cells, which bear minute cilia (Pl. 19, fig. 6, and Pl. 20, figs. 18, 19, 20, 28, and 29). Each cell is elongated, with a nucleus near its middle. In some parts the longest cells are found in the mid-dorsal part, but in others the part next to the ciliated band is thickened.

In the small specimens referred to above, this very thick glandular epiblast is more conspicuous than in the larger

forms, and in preserved specimens is greatly folded on itself. On either side of the middle line, near the nerve-ganglion, a pore leads by a short canal into the cœlomic cavity of the pre-oral lobe. These two pores and canals are described in further detail below, and seem to be comparable with the "proboscis-pores" of Cephalodiscus.

The ventral surface of the pre-oral lobe has an epithelium of a very different character from that already described for the dorsal. The body-wall is extremely thin, and consists of attenuated cells (Pl. 19, fig. 6, and Pl. 20, figs. 18, 19, 20, 28, and 29), except in the region immediately anterior to the mouth. Here they are rather more cubical and bear cilia (fig. 6, *v. c. b.*). I have not been enabled to detect any cilia upon the rest of the ventral surface of the pre-oral lobe.

Almost the whole surface of the collar segment is covered with cilia, and the cells are fairly thick and cubical. Running down the mid-ventral line from the mouth region is found a ridge of cells with strong cilia, and on either side of this ridge, for some way backwards, the cells of the ventral surface of the collar are not ciliated, and are somewhat attenuated. Dorsally the thick epithelium of the pre-oral lobe is continued on to that of the collar on either side of the nerve-ganglion, and (Pl. 19, fig. 7) the dorsal surface of the tentacles is also covered with thick epithelial cells bearing long cilia.

In the larger larvæ the epiblast of the trunk becomes so attenuated as to defy complete analysis with the highest objective at my disposal (Pl. 21, fig. 39). The only exception to this is the epiblast of the perianal band, which is formed by the longest cells of the whole body, aggregated in enormous numbers, and bearing long flagelliform cilia. Seen in cross-section the ciliated ring or band appears lenticular, and just anteriorly to it the cells are slightly thickened, and form a nervous ring (Pl. 20, figs. 22 and 23).

This attenuation of the epiblastic cells of the trunk segment seems to be attended by and connected with an elongation of this region. Gegenbaur (9) has noticed that in the earlier stages (·35 mm. long) the trunk region was short, and that by

the time a length of ·5 mm. was reached it had greatly elongated, and formed a long cylindrical portion behind the tentacles. This was confirmed by Metschnikoff (14) and by others. A similar phenomenon is found in Tornaria, according to Morgan (17), the area between the post-oral and the perianal bands being very small in his younger stages, but elongating greatly afterwards.

It is a very necessary process in Actinotrocha in order that the requisite space for the accommodation of the ventral diverticulum may be forthcoming.

An inspection of the figs. 18—27 inclusive (Pl. 20) will sufficiently indicate the further relationships of the areas in which the epiblast is thickened to those in which it is attenuated. In fig. 28 is seen the marked contrast in this character of the cells on the dorsal and the ventral surfaces of the pre-oral lobe. In fig. 29 are to be noticed the two oral grooves (*o. g.*) passing out from the mouth. They are longitudinal depressions in the ventral collar area, and are also found in Cephalodiscus. In fig. 26 the right-hand groove is seen entering the side of the mouth. They can be traced on to the general surface of the collar. On the ventro-lateral side of the pre-oral lobe are also two grooves (fig. 24, *a. g.*), which pass from the antero-lateral corners of the mouth round the edge of the pre-oral hood. Their bounding cells, like the rest of the ventral surface of the hood, are without cilia and much attenuated. They may be termed the atrial grooves, and no doubt serve for the removal of the water which is brought into the mouth by the action of the ciliated oral grooves. In fact, they are the true analogues of the gill-slits of the Chordata, though perhaps not homologous with these.

The histology of the several areas is sufficiently indicated in a series of transverse sections (Pl. 19, figs. 6—10, 16, 17, and Pl. 21, fig. 39).

Nervous System.

The nervous system of Actinotrocha has been little studied, and some of the observations made with respect to it are without doubt erroneous. Wagener (20) describes in some

detail the appearances which he considered to be connected with the nervous system. He noticed, on the dorsal surface of the pre-oral hood, a "horn-shaped protuberance," and, posterior to this, a wart-like thickening. This latter is the true nerve-ganglion, and the former is the apical sense-organ, but they were not recognised by him as such. On the contrary, he describes a swelling (Wulst) on the ventral surface of the pre-oral lobe, which he conjectures to be the ganglion. Radiating from this, he says, are found a number of lines, and he indicates the course of these lines, and even figures the nervous system, as it appears when isolated (fig. 6). His "Wulst" is the aperture of the subneural gland, to be described later, and the radiating lines and commissures are, at any rate in large part, the mesenteries separating the cavities of the pre-oral lobe and the collar, together with the mesentery bounding the vascular space below the true nerve-ganglion. A comparison of Pl. 18, fig. 3, with Wagener's figure will make this clear.

One can readily imagine how an incautious inspection of the larva intact might cause this mistake, but how the observer referred to managed to "isolate" such a heterogeneous and unconnected collection of structures is not easy to understand.

Caldwell (2) speaks of the ectoderm becoming thickened in two regions:

1. In the pre-oral lobe.
2. In the form of a post-oral ring round the mouth.

The former, he states, becomes the future ganglion, and the latter the circum-œsophageal nerve-ring. Again, in the fully developed larva he remarks that "the nervous elements of the ectoderm of the pre-oral lobe in all species are concentrated into a ganglion. In some species a large number of nerve-fibres pass forwards from it to a sense-organ."

The central nerve-ganglion (figs. 1—3, 13, 14, 15, 20, 23, 28, *n.g.*) is a lenticular mass lying on the mid-dorsal line at the base of the pre-oral lobe. In fig. 1 it is seen to be at the front end of the body, but when the hood is directed forwards its true situation at the base of the pre-oral hood is evident

(Pl. 18, fig. 2, and Pl. 20, fig. 20). It consists of a mass of ganglion-cells and fibres in which the inner ends of the overlying epiblast take part.

The mesoblastic posterior wall of the pre-oral lobe can be seen passing downwards from its front border (figs. 1, 20, 23, &c., *mes.*), and the anterior mesoblastic wall of the collar cavities is in like manner to be observed passing from its posterior border inwards to the œsophagus (Pl. 18, fig. 2). The space lying immediately below the ganglion is therefore a large hæmocœlic cavity (fig. 2, *s.n.s.*). The position of this ganglion is exactly similar to that of the central nerve-ganglion of Cephalodiscus, as described by McIntosh, and the homology of the two cannot be doubted.

In early stages this ganglion lies just under the epiblast, which is continued forwards over the hood (Pl. 18, fig. 1); but as development proceeds a depression of the epiblast immediately in front of the ganglion takes place. The ganglion having a convex anterior border, the opening of this epiblastic pit is at first crescentic in shape, but afterwards it becomes only elongated from side to side. The depression becomes deeper, and eventually forms a long sac-like diverticulum lying under the nerve-ganglion (Pl. 18, fig. 2, Pl. 20, fig. 20, &c., *n. p.*).

The ganglion is, as already stated, a proliferation of the inner cells of the epiblast, and in almost every direction there radiate nerve-tracts formed by the inner ends of the epiblastic cells. This is especially the case in the anterior direction over the pre-oral hood. Here there are three main nerves, which run parallel to each other, one median and the other two on either side of it (Pl. 18, fig. 3, *a. n.*); and on either side of these again are a great number of smaller trunks, the course of which is indicated in fig. 2. They are seen to run forwards and outwards, and then to bend backwards and take a course to the posterior corners of the hood. All these are involved in the invaginated pit here referred to, and they appear in surface view to radiate from its front border. They are continued backwards, however, along the lower wall of the pit, and then round its base and up to the ganglion. The pit is

therefore a depression of nervous epiblastic tissue immediately in front of the main nerve-ganglion. It appears to be homologous with the "neuropore" of the Chordata in its inceptive stage. The same process of depression involving the nerve-ganglion, and carried further backward, would result in a tubular dorsal nervous system of the same type as that in Balanoglossus and the higher Chordata.

The three dorsal anterior nerves run forward, and the median one (Pl. 19, fig. 6, *a. m. n.*) continues into a large median nerve-swelling lying immediately under the sense-papilla. Beyond this, however, it can be traced together with the two dorso-lateral nerves (fig. 6, &c., *a. l. n.*) till they are lost in the pre-oral nerve-ring. This is a thick ring of nervous tissue, found immediately under the pre-oral ciliated band throughout its length. At the posterior corners of the pre-oral hood this ring terminates as such, and appears to branch out in several directions. One branch passes up again to the nerve-ganglion (Pl. 18, fig. 2), and numerous fibres also appear to pass on to the ventral surface of the collar region (Pl. 19, fig. 10, *p. o. n.*). The mass of nerve-tracts already referred to as leading out from the "neuropore," and bending back to this spot, appear also to pass on to the ventral surface of the collar, forming a pair of nervous areas on either side of the mouth. These spread out over the whole ventral surface of the collar as a series of branching fibres.

Posteriorly the nerve-ganglion passes gradually into a pair of thick nerve-tracts, which run along the dorsal collar region and then diverge. The main mass of fibres passes diagonally downwards immediately behind the tentacles, and, as is seen in sections, exactly at the posterior border of the collar region (Pl. 20, fig. 19, *c. n. s.*). The rest of the fibres pass mid-dorsally as a pair of tracts, giving off branches to the body-wall, and terminating in a nervous ring just anterior to the perianal band.

The collar-ring behind the tentacles is continued in the mid-ventral line to the same nerve-ring in front of the perianal band, and supplying the ciliated cells of this area with nerves.

In addition to these main nervous tracts the inner ends of the whole epiblast form a fine fibrillar nervous network in every direction. In the thin epiblast of the trunk I have not been able to make these out, but in surface view this area shows, as already described, fine nerves branching out in all directions from the ventral and dorsal trunks.

Pl. 18, fig. 2, is a surface view from the side of the whole larva, with the chief nerves indicated in black, whilst in fig. 1 are indicated the chief nerves of the hood in dorsal view at a slightly earlier stage, and figured as they actually appear, i. e. as fine unstained tracts.

The appearance of the nerve-tracts, as seen in section, is shown in Pl. 19, figs. 6 to 17.

In fig. 6 are to be noticed the three main dorsal nerves of the hood (*a. l. n.* and *a. m. n.*), which at the level of fig. 11 commence to pass down the ventral floor of the neural tube, and in fig. 13 the median nerve is seen to pass into the ganglion.

In fig. 15 the posterior end of the ganglion is reached, and it is seen to diverge into two main dorsal trunks situated (fig. 7) upon the dorsal surface of the collar area. These may be traced through fig. 8 at the level of the mouth, and fig. 9, which is almost at the posterior dorsal termination of the collar area. Here they diverge, and are found at the base of the tentacles, and they are seen to give off branches to the tentacles.

In fig. 9, immediately below the mouth, may be seen the ventral nervous area formed by the partial fusion of two tracts situated lateral to the mouth in fig. 8.

Fig. 10 shows the collar-ring (*c. n. r.*) still more diverging into two lateral branches, and the mid-dorsal region shows indications of the fibres running down to the perianal band, though the attenuation of the cells makes them difficult to follow. On the right of this figure is seen (*p. o. n.*) the termination of the pre-oral nerve-ring, and branches from it may be noticed passing dorsally and ventrally.

In fig. 16 the foregoing features of the collar-ring, &c.,

are seen, and in addition the ventral nerve-tract is seen to be now clearly separated into two ventro-lateral areas (*v.c.n.*) with columnar ciliated cells, though the nerve-fibres may be also traced somewhat prominently in the mid-ventral line. In Pl. 21, fig. 39, the trunk-area alone is seen in section, and ventral nervous areas can be just discerned, though their demonstration is easier in surface views. Thus the nervous system of Actinotrocha consists of—

1. A central ganglion lying in the front collar region and between this and the pre-oral lobe, with the epiblast immediately in front, depressed to form a neuropore.

2. A ring round the posterior part of the collar, continued from the ganglion dorsally and ventrally, giving off fine double groups of nerve-tracts to the anal end of the body.

3. Groups of fine nerve-tracts continued dorsally along the trunk from the hind end of the collar to the anal end of the body.

4. A ring around the anal end of the trunk, into which the dorsal and ventral tracts lead.

5. A ring round the edge of the pre-oral lobe, joined at each side to the ganglion, and in the median front region by three main tracts running in the mid-dorsal line forwards from the ganglion.

6. A diffuse plexus of fibres at the base of nearly all the epiblastic layer, conspicuous amongst which are the fibres of the ventral collar area, which pass forwards and dorsally to meet the ganglion.

A comparison of this nervous system with that of Balanoglossus and Cephalodiscus will be instituted later.

Sense-organs.—At a stage later than fig. 1 (vide fig. 2) the mid-dorsal surface of the pre-oral lobe is elevated into a cone-shaped process, which has slightly columnar cells, immediately under which is a swelling of nervous fibres protruding into the cavity of the pre-oral lobe (fig. 20, *s.p.*). The examination of fresh specimens is required for determination of the further structure of this organ.

Pre-oral Ciliated Ring.—Round the edge of the pre-

oral hood is an area of epiblastic cells which bear long and prominent cilia. The cells themselves are rather longer than broad (Pl. 21, fig. 44), and fine fibrils are readily distinguished passing into the mass of fibres which forms a nerve-ring immediately under the epiblast. These cells come to a sudden termination at the posterior angles of the pre-oral lobe, where they are continued on to the ordinary cells of the body.

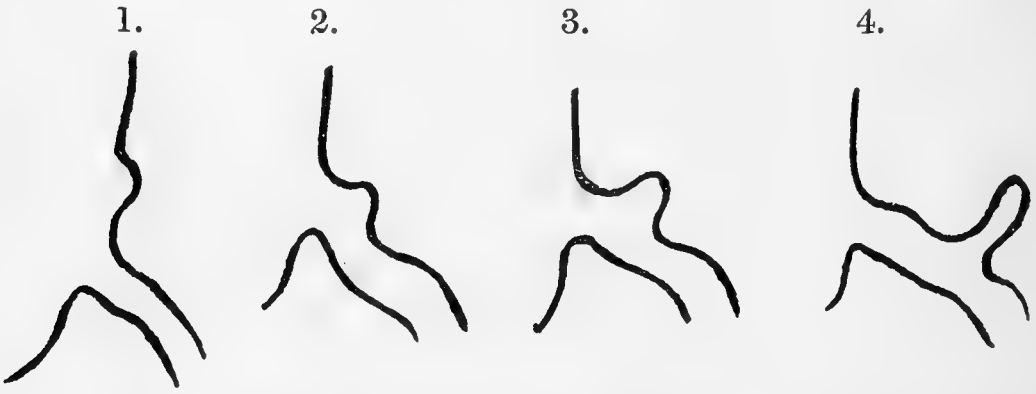
Tentacles.—The epithelium of the tentacles is of two types. Pl. 19, fig. 17, shows a succession of tentacles cut in transverse section, so that various stages are indicated. Starting from the right-hand side the tentacles are seen to arise as protuberances of the thickened ciliated cells of the collar area, with a diverticulum of the collar cœlom running down the centre. The nervous area at the base of the cells is prominent, especially at the outer angle of the thickened cells. As the tentacle becomes completely separated from the surface of the collar its inner wall is completed by a very thin non-ciliated area of cells. The transverse section of a tentacle (Pl. 21, fig. 43) is therefore somewhat ovoid, two-thirds of its epithelium having thickened ciliated cells, and the posterior one-third consisting of a thin cellular lamella. At the outer angle is a nervous area, which is probably a tentacular nerve seen in cross-section; and immediately internal to this is a hæmocœle space or vascular trunk, which, however, is only present at the base of the tentacle (cf. fig. 43). The rest of the cavity is cœlomic, and is lined by a mesoblastic wall, between which and the epiblast is a mesoblastic skeletal lamella, referred to later.

The Perianal Band.—The perianal band is composed of a dense aggregation of very long columnar cells (Pl. 18, figs. 1 and 2, *p. a.*) in direct continuity with the epiblastic cells of the trunk. In cross-section the band appears lenticular in outline (Pl. 20, figs. 18 to 23, and Pl. 21, fig. 45), and the cilia appear to be grouped more or less into masses. They are many times longer than any other cilia on the body, and are slightly curved towards the posterior end. Immediately in front of the band is a nerve-ring in a shallow depression, and fibres from this can be seen to enter the bases of the ciliated cells.

Proctodæum, Stomodæum, and Subneural Gland.

From a want of definite knowledge upon the point one cannot speak of a proctodæum with any degree of certainty, but Caldwell refers to a "slight invagination of ectoderm" in the formation of the anus. From a figure of an early stage, given by Metschnikoff (**14**), there can be little doubt that the organ usually referred to as the œsophagus is epiblastic in its origin. In Metschnikoff's figure (**14**, Taf. xix, 1) the connection between the œsophagus and stomach is apparently not yet established. In view, however, of no certain statement, the œsophagus will be described later.

Subneural Gland.—There remains an important organ about the origin of which there can be no doubt. During



1. Early stage of subneural gland in longitudinal section. 2. Later ditto. 3. Later than 2. 4. Latest stage ditto.

development the epiblast becomes tucked in at the mouth, and in the mid-ventral line of the hood, anterior to the mouth, there is formed a small depression (woodcut 1). As the depression deepens and increases in size it is carried further inside the buccal cavity (see woodcuts 2, 3, and 4), until the latest condition I have observed, seen in Pl. 21, fig. 38, *s. n.*, is arrived at. In median sagittal section the same organ is shown in Pl. 20, fig. 20 (*s. n.*). Its walls at this stage still exhibit a structure closely similar to that of the œsophagus. As referred to below, this may be termed the subneural gland, and may be compared to the organ of the Tunicata bearing the same name, and

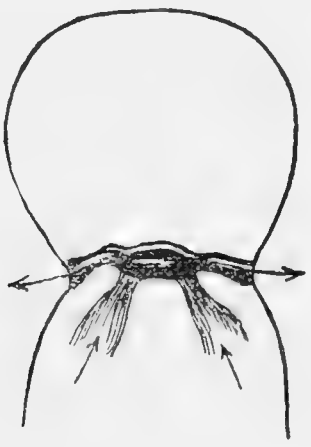
possibly to the hypophysis of the Vertebrata. Its relation to the blood-sytem, &c., will be described under that head.

Organs of the Hypoblast.

The alimentary canal is ciliated throughout, consisting of a tube of varying character, from the ventral mouth to the posterior anus. The mouth opens immediately under the pre-oral lobe, between it and the collar region. A mid-ventral ciliated area leads into it from the pre-oral lobe in front, and a broad ciliated area, depressed into two oral grooves (Pl. 20, fig. 29, *o. g.*), approaches it from the ventral surface of the collar area.

From the dorso-lateral corners of the mouth lead outwards the atrial grooves. The general arrangement, as seen in ventral view, is indicated in woodcut 5. At the edge of the mouth

5.



5. View of mouth-parts of Actinotrocha, from ventral surface. In the oral grooves the arrows point towards the mouth, in the atrial grooves they point away, and indicate the course of currents.

the cells on all sides pass by gradation to the long columnar ciliated cells of the œsophagus. The nuclei of these are evenly arranged at their centre (Pl. 19, fig. 8), and the inner half of each cell is clearer, and not so readily stained as the outer.

In fig. 7 may be noticed the base of the subneural gland, leading off from the "œsophagus;" and in figs. 9 and 10 there is shown the typical cross-section of the œsophagus below the mouth. On their outer face the cells are covered by a thin

layer of the mesoblastic lining of the collar cavities. The course of the œsophagus is first upwards towards the dorsal surface, and then curved backwards (Pl. 20, fig. 20; Pl. 21, fig. 38), and has a straight direction till it opens into the dorsal anterior end of the "stomach," which is partially differentiated into a pharynx.

The part of the "stomach" lying in the collar region is separated by a constriction from the part behind, and in fact in some cases the constriction forms a short tube running from the anterior part or pharynx to the posterior part or true stomach.

The pharynx has small cubical cells with regularly disposed nuclei; the protoplasm stains homogeneously. They have small cilia, but the contrast of these cells with the "œsophageal" cells is marked and the transition abrupt (cf. Pl. 19, figs. 9 and 10).

The cells situated in the mid-dorsal region of the pharynx are, however, for some way columnar and strongly ciliated (fig. 16), and altogether resemble more the "œsophageal" type. In the antero-lateral region the pharyngeal wall is produced into two remarkable diverticula, which in the fully developed larva lie, as a pair of elongated organs (notochords), laterally to the œsophagus (figs. 9, 22, *nch.*).

The cells are columnar, with homogeneous protoplasm, but in the course of development they undergo a remarkable transformation. The earliest stage yet found by myself (Pl. 21, fig. 30) presents the diverticulum as a hemispherical protuberance. Sections at the level of *a*, *b*, and *c* exhibit the characters shown in figs. 30*a*, 30*b*, and 30*c*.

In fig. 30*a* is seen a section through spherical vacuoles with a few nuclei squeezed in between them. The vacuoles are filled with a clear, homogeneous, non-staining substance. (I have failed to stain them in the slightest degree with hæmatoxylin, carmine, picric acid, iodine, and numerous aniline stains, such as eosin, methyl green, safranin, &c.)

In fig. 30*c* the central cavity is reached, and the cells are seen in longitudinal section. This reveals that the vacuoles are regularly disposed at the distal extremity of each cell, one

to each, and that the nucleus lies exactly internal to the vacuole in each case. The contrast between the even ring of light vacuoles and the inner ring of dark nuclei makes a beautiful and symmetrical section, which can be only poorly indicated in the figure. We cannot doubt that the presence of the vacuoles is due to the secretion of some fluid product at the distal extremity of the cell, which is further enclosed in a cell membrane. The close contact of the nucleus with the vacuole is suggestive in this connection.

In a later stage (figs. 31 and 32) there has arisen another vacuole in each cell, internal to the former. The whole organ is also more elongated. In fig. 31 the vacuoles are mostly alternate to each other, but in certain stages there can be discerned, especially at the area later developed (below *v.* in fig. 31), that the inner vacuole is first formed exactly internal to the first, and that later on a shifting takes place till the alternate arrangement is arrived at. The latter is evidently the more stable condition, and the assumption of it by these vacuoles tends to indicate that the vacuoles are turgid. In this stage the nuclei are still regularly disposed, the majority situated in an even row internal to the vacuoles, but a few are caught between the latter and are seen squeezed in the chinks here and there.

These changes may be indicated semi-diagrammatically by figs. 33, 34, and 35. The stage with three rows of vacuoles is not apparently to be obtained, because the vacuoles are later disposed rather more irregularly, and the next stage of importance is seen in fig. 36. Here the vacuoles have become squeezed together, like fish-eggs allowed to settle slowly in spirit, and some are no longer spherical. The majority of the nuclei are still confined to a small area of protoplasm at the inner end of each cell; they are not very regularly arranged. Fig. 37 is added to show the condition of this organ as seen in oblique section, within the young Phoronis, soon after the metamorphosis. Its subsequent fate is unknown, but it is not present in the adult. We may note in this final stage that the vacuoles are still further distorted by mutual pressure,

and altered in outline. I have found no indication that the fusion of the vacuoles with each other takes place. The position of the organs in situ is seen in Pl. 18, fig. 1, and Pl. 21, fig. 38 (*nch.*), in lateral view, whilst fig. 3 gives the dorsal view of the two notochords (*nch.*). In Pl. 20, fig. 22, their appearance in coronal section can be followed, whilst fig. 19 shows a sagittal section of one of them.

We may emphasise the fact that development of the notochords proceeds in two ways :—1. The organs themselves arise from a pair of evaginations of the antero-lateral walls of the pharynx, which gradually become longer and deeper till they extend forward in close contiguity with the mesentery between the collar and the pre-oral lobe. They have no connection whatever with the epiblast. 2. At the same time as this growth in length the cells undergo a remarkable metamorphosis into vacuolated tissue.

These organs will be compared to the notochord of the higher Chordata. As regards previous observations upon them, we may say at once that the majority of the numerous workers on Actinotrocha were quite content to describe them as "liver diverticula."

Thus Wagener (**20**) refers to the "liver-blind-därme," and Gegenbaur (**9**) as heaps of cells (liver-cells?) : Caldwell (**2**) remarks, "The stomach at its anterior end is produced into one or two ventral processes. In the vacuolated walls of these structures brown concretions are present" (p. 378). Claparède (**5**) described it as a dark mass with light balls embedded in it, no doubt in allusion to the appearance in the live animal, in which the dark areas are formed in the same way as the black rim of an air-bubble under the microscope. Schneider (**18**) accepts Claparède's interpretation, and gives up his former view that they were fat-globules, but does not clear up the point any further; he remarks that the "balls" are surrounded in Actinotrocha with black pigment, in allusion no doubt to the refractive effects above mentioned.

Metschnikoff (**14**) gives a figure (fig. 6) of a larva with four pairs of arms, in which the vacuolisation of the antero-lateral

region of the "stomach" has commenced, but the wall has not yet begun to protrude. He refers to the area as the "brown specks."

Lastly, Wilson (22) figures these organs in several positions, and refers to them as "glandular lobes of the stomach."

It will thus be readily seen that the organs have been repeatedly noticed by others, and a few sections could not have failed to reveal their true structure.

At its posterior end the pharynx becomes constricted, and then leads into the true stomach. The walls of this part consist, for the most part, of a single layer of hypoblast; the cells are cubical, with small cilia, and differ from the cells of the pharynx in that the nuclei are not so regularly arranged (Pl. 19, fig. 17). In the ventro-lateral region on each side they lose their cell walls, and are heaped up into two ridges or mounds extending the whole length of the stomach (Pl. 19, fig. 17, *d. a.*). Each of these ridges consists of an amœboid mass of nucleated cells without cell walls. They project some distance into the lumen of the gut, and there are always embedded in them the residue of various food-particles, usually surrounded by a clear vacuole. These ridges are their special organs for intracellular digestion, and may be termed the digestive areas. The mid-ventral wall of the gut between them differs slightly from that of the rest of the stomach, the cells being rather more columnar and numerous, and the nuclei more regularly arranged. In addition to this a certain portion of them, exactly opposite the "diverticulum" (Pl. 20, fig. 20, *v. ch.*), undergo a modification into vacuolated tissue in all essential respects like that of the notochords; but it does not apparently extend beyond the two-vacuole-deep stage. This area of chordoid cells is evidently developed as a supporting tissue to the parts in this region.

The stomach contracts to a small opening at its hind end which leads into the intestine, a fairly long bent tube which passes to the anus situated at the centre of the area surrounded by the perianal band. The cells of the intestine are single-layered, columnar, and ciliated, and call for no special

mention. Just before the anus is reached the lumen of the gut widens out into a larger vestibule, which may be termed the rectum.

The alimentary canal thus consists of a wide mouth leading into an "œsophagus," which is most likely epiblastic, and should be more correctly termed the stomodæum; the pharynx, with its notochords, leading by a short œsophagus to the stomach, with its two "digestive areas;" and the intestine, with the distal end widened into a rectum and terminating in a posterior anus.

Organs of the Mesoblast.

The mesoblastic structures of Actinotrocha are highly developed, and indicate that this larva must be regarded in its highest development as at a high morphological grade. Thus there is little indication of "mesenchyme," and the cœlom is present with splanchnic and somatic layers. In addition to this the vascular and muscular systems are well differentiated.

Cœlom.

The mesoblast is in the condition of a thin layer of protoplasm (Pl. 19, figs. 6—10, and 15, &c.), with a few nuclei dotted here and there, but at certain places this layer is thickened by a massing together of mesoblast cells which form simple contractile muscle-cells, and at others again are found protoplasmic processes of the same nature, extending across the body cavity. This thin layer of mesoblast is closely applied to the hypoblast along the gut, except at certain places where the blood lacunæ are formed by the spaces left between the two layers. On the outer wall the somatic mesoblast is also in close contact with the epiblast, though between them is a thin layer of mesoblastic chondroid tissue.

The cœlom is segmented into three parts,—the cœlomic cavity of the pre-oral lobe, that of the collar, and that of the trunk.

The cavity of the pre-oral lobe (Pl. 19, fig. 6) is of the same size and shape as the hood itself. Posteriorly it is produced

into two horns running back laterally (Pl. 20, fig. 24) to meet the collar cœlom. Here the mesoblastic walls of each cavity meet each other and form a well-marked mesentery, which is of considerable firmness, and probably has in its centre a thin lamina of chondroid tissue.

In the mid-dorsal line the posterior wall of the pre-oral cœlom borders the subneural sinus (Pl. 20, fig. 20, *mes.*) ; and just where the pre-oral mesoblastic wall slopes away on either side of the sinus (Pl. 21, fig. 42) there are a pair of thickenings, *p. p.*, which, traced forwards, show themselves to be the commencement of a pair of internal openings. Further forward they become cellular tubes (Pl. 20, fig. 28, *p. p.*), and after a short course parallel to the body-wall they open into grooves on either side of the nerve-ganglion. A cross-section of the tube shows a single layer of columnar ciliated cells, closely similar to the cross-section of the collar nephridium (Pl. 21, fig. 41). The pre-oral cœlom, therefore, opens to the exterior by a pair of ciliated pores, identical in position and structure with the proboscis pores of Cephalodiscus (see below).

The pre-oral cœlom is traversed in every direction by fine protoplasmic filaments, with here and there, especially at the unction of the threads, a nucleus (Pl. 20, fig. 28, *b. c.*, 1).

The collar cœlom is a spacious cavity separated in front by the mesentery (*mes.* in the figures) from the pre-oral cœlom, and behind by the mesentery (*mes'.* in figs. 22 and 23) from the trunk cavities. It is produced into each tentacle, and is everywhere bounded by typical cœlomic epithelium. A dorsal mesentery is present throughout their length, in connection with which is a dorsal vessel. We may specially notice that as the notochords grow forward they push the splanchnic cœlomic epithelium before them, and they are thus, like the rest of the hypoblast, surrounded by a layer of mesoblast, in this case forming a mesoblastic sheath to the notochordal diverticula (Pl. 21, figs. 30, 31, *sh.*). The mesentery (*mes'.*) separating the collar cœlom from that of the trunk starts just posterior to the tentacles and collar ring, and runs forwards and inwards to meet the gut. Its course is shown in trans-

verse sections in Pl. 19, figs. 16 and 17, and in longitudinal sections in Pl. 20, figs. 19—23 and 27, *mes'*.

At the ventral posterior part of this collar area are seen in the view of the whole larva (fig. 17, *c. n.*, in section) a pair of large bodies, apparently aggregations of amœboid cells with large nuclei. Their presence has been observed, figured, and remarked upon by almost all the workers upon this larva, and various interpretations have been put upon them. It has been noticed that the masses break up at the metamorphosis, and the constituent cells have in different cases been seen to float freely in the cavity, pass into the tentacles, or into the vascular trunks.

In fig. 17 there is seen nothing but a mass of protoplasm and large nuclei, but in sections further back we notice that the cells are grouped round a few intercellular canals which open into the collar cavity by broad funnels, join each other, and pass outwards and downwards between the two layers of the collar-trunk mesentery (*mes'.*) to open to the exterior near the mid-ventral line immediately on either side of the "diverticulum."

This is the organ referred to as the "nephridium" by Caldwell (2), as follows :—"The pair of nephridia lie on either side of the body, their numerous excretory cells floating freely in the body-cavity in front of the septum. The external openings are placed one on either side of the opening of the foot." He further describes in some detail the excretory cells, and remarks, "At no time during the free-swimming life of larva does the excretory canal system open into the body cavity." This statement does not apply to the species I have investigated, and it is improbable that it does so to any other Actinotrochæ. The internal funnels, without doubt, open into the collar cavities, and not into the trunk cœlom, as do the adult nephridia. Pl. 21, fig. 40, shows a longitudinal section of one of these collar nephridia as constructed from a series of sections. Each is suspended and held in position by a long cord of mesoblastic cells running from the front wall of the collar. A transverse section of the duct is seen (fig. 41) to

consist of a single layer of columnar ciliated cells enclosing a fine lumen.

Wagener (**20**) figures one of these organs, and describes its appearance in the live larva. He says that "from its free broad sides there project many small stumpy pointed knobs on long stalks, which spread about in all directions like a bouquet of flowers" (translation). This description plainly indicates that the mass of cells seen in prepared sections around the tubes is formed by a number of cells attached by long processes to the mouth of the funnels. In fact, a few preserved specimens give indications of this arrangement. We thus arrive at a pair of collar nephridia resembling almost identically the structure of the nephridia as described by Boveri (**1a**) in Amphioxus. A point which cannot be fully entered into here is the fate of these collar nephridia, so evidently homologous with the collar pores of Cephalodiscus and Balanoglossus. If they become the nephridia of the adult the openings into the collar region must be lost, and fresh openings must be acquired into the trunk cœlom. Caldwell (**2**) says, "The large canals remain as the paired nephridia of the adult." We may, however, recollect that the adult nephridia open on the dorsal side and the collar nephridia on the ventral side. Further than this, there are described below other organs, which may possibly be the rudiments of the true nephridia.

Trunk Cœlom.—The cœlom of the trunk has the same characters as that of the collar. It is continued dorsally forwards for some way almost to the level of the notochords. There is apparently no dorsal mesentery, but a ventral mesentery is continued throughout the length of the trunk, and suspends the intestine. At the perianal area are a pair of organs which I have not fully made out, but they may be the rudiments of the trunk nephridia. They lie in the hæmocœle space immediately below the ciliated band, and are thin-walled. They have an internal opening into the trunk cœlom, and apparently open to the exterior on either side of the anus. Their walls are of the same nature as those of the cœlom,

and appear to be portions of the cœlom, with the trunk nephridial tubes cut off from the rest of the trunk cœlom.

These two organs are seen in a longitudinal section in Pl. 21, fig. 45. A study of other stages can alone show their true significance.

Skeletal System.—The skeletal elements are difficult of demonstration, but a very thin layer of mesoblastic chondroid tissue, homologous with that of the adult, appears to be present immediately under the epiblast. In carefully macerated specimens the epiblast of the tentacles can be removed, and the skeletal system is then evident as a thin hyaline layer. In the trunk region, again, it is difficult to account for the rigidity of the body-wall, considering its extremely attenuated epiblastic and thin mesoblastic layers, without the assumption of the skeletal supporting layer.

Muscular System.—The muscles are all in the condition of simple mesoblastic cells with long contractile processes. They are most prominent in the interior of the hood, where they form a fine meshwork. The thickest and most regularly arranged are inserted in the mesentery, between the pre-oral- and collar-cavities, and radiate outwards on either side to the edge of the hood (figs. 23 and 50). A few strands also connect the further side of this mesentery with the sheath of the notochord. In the collar there are two muscular bands, already referred to, attaching the collar nephridia to the collar wall, and there are a few muscle-cells forming a sheath around the ventral blood-vessel (Pl. 19, fig. 17). In the trunk region a few fine circular muscle filaments can be discerned in surface view, and a dorsal longitudinal trunk (fig. 16). The trunk mesoblast, forming a sheath round the "diverticulum" ("foot," Caldwell), is very thick, and mesoblast cells are becoming differentiated into true muscular elements (Pl. 20, fig. 19, *div.*).

The splanchnic mesoblast over the dorsal blood-vessel is thickened by an accumulation of simple contractile cells (figs. 16, 17, and 20, *d. l. v.*). The contractile nature of the dorsal blood-vessel has been noticed by many workers. Thus Schneider found it to be rhythmically contractile, and remarked

upon the presence of transverse lines due to the contraction. He compared the structure of the muscle-cells to that of those lying over the "diverticulum."

Blood System.—The vascular system has received a great deal of attention by former observers. Wagener's first observations were corrected in some particulars by Schneider. The latter found a dorsal vessel on the stomach with indications of its double nature. He also described a number of blind sacs which, as finger-like processes, project into the cœlom at the junction of stomach and intestine. Their presence had been noticed prior to this by Leuckart and Pagenstecher.

Schneider, along with some others, states that the blood-corpuscles arise from the masses of cells referred to above in connection with the collar nephridia.

Metschnikoff (**14**) states the vascular system to be in direct communication with the body cavity, apparently as an inference from the fact that these masses of cells, at first in the body cavity, are found later in the blood-sinuses. Krohn, again, gives a description of the dorsal and ventral trunks and the cæcal vessels, he observed the contraction and expansion of the cæcal vessels; and Wilson (**22**) figures the main features of the vascular system as determined by his predecessors.

Caldwell (**2**) confirmed the results of his own work and that of previous workers as follows:—"1. Blood-corpuscles aggregated in two or more masses, lying free in the body cavity of the pre-oral lobe, i. e. in front of the septum. 2. A blood-vessel formed on the dorsal wall of the stomach—a marked structure in the larva. 3. The splanchnopleure sac, which in the region of the stomach forms a loose sac surrounding the gut. 4. Cæcal prolongations of this sac. 5. Cæcal prolongations into the rudiments of the adult tentacles."

I have been enabled not only to confirm some of the above observations, but to complete the account of the blood system. It consists essentially of a system of sinuses and fissures lying outside the mesoblast and between it and the epiblast or hypoblast. These may be regarded as vestiges of the segmentation

cavity. In every case in which a true vessel is present it is formed by the thickened mesoblastic walls surrounding the vessels.

The vascular system is, therefore, morphologically in a very primitive condition.

There is just under the nerve-ganglion a large sinus caused by a want of contiguity between the mesoblastic walls of the pre-oral cavity in front and the collar cavities behind. This sinus, which may be termed the subneural sinus (*s. n. s.*), is seen in figs. 1, 3, 20, 23, and 38, in which its relationships can be readily made out. Into its cavity projects upwards from below the inner end of the subneural gland, so that the lining cells of this organ are in direct contact with the hæmal fluid. At the posterior extremity of the sinus it leads by a chink or fissure between the gut wall and that of the two collar cavities, along the dorsal wall of the œsophagus (figs. 7, 8, *d. b. v.*) to the hind extremity of the collar region. Here it falls into the large dorsal vessel with contractile walls. This vessel is continued along the dorsal wall of the pharynx and stomach (Pl. 19, figs. 16 and 17, *d. b. v.*) till it meets the intestine. Here a small ring-sinus is formed connecting it with the ventral vessel with cæcal prolongations into the body cavity. At the anterior extremity of the dorsal vessel, at the front end of the pharynx, two lateral trunks are given off, which, passing downwards round the base of the œsophagus and between this and the notochords, meet again in the mid-ventral line, forming a post-oral ring sinus (Pl. 20, fig. 21, *r. s.*). From the point of junction (fig. 20, *v. b. v.*) there originates a mid-ventral vessel which may be traced throughout the length of the gut (figs. 16 and 17, *v. b. v.*) into a large hæmal ring immediately internal to the perianal ciliated band. Lastly, there are some not very distinct indications of hæmal sinuses passing down the tentacles; but these are not very decided.

This blood system is closely comparable to that of Balanoglossus and of Cephalodiscus, as I hope to show later.

Caldwell makes the statement, "Free communication exists between the body cavity in front of the septum and the split

in the splanchnopleure, which will form the blood-sinus and vessels of the adult." I can find no confirmation of this statement; on the contrary, I find the vascular system a completely closed system of sinuses, bounded in various parts by epiblast, mesoblast, or hypoblast, but in no way connected with the cœlomic spaces.

General Considerations.

In the foregoing pages has been given a brief account of the structure of the larval Phoronis, the investigation of which was undertaken mainly to assist in deciding the true systematic position of this group. As before stated (**24, 25, 26**), the facts clearly point to a close genetic relationship with Balanoglossus and Cephalodiscus, especially the latter.

We therefore have, firstly, to institute a comparison of Actinotrocha and Phoronis with these; and secondly, to see whether, this comparison being held valid, the anatomical facts will throw any further light upon the relationship of these groups collectively to others above and below them in the morphological scale.

Comparison of Phoronis and Balanoglossus.

Under this head we may compare (1) Actinotrocha with Balanoglossus, (2) Actinotrocha with Tornaria, and (3) Phoronis with Balanoglossus.

(1) Actinotrocha and Balanoglossus.

General Shape of Body.—In each case we have an organism of an elongated shape, and showing clearly external indications of a division into three segments, following one another from before backwards. One of these, the pre-oral lobe, is situated in front of the mouth, and forms a prominent organ overhanging the oral aperture. It is narrowed at the base, presenting a stalk in Balanoglossus, and a contracted portion bounded by the atrial grooves in Actinotrocha (cf.

Pl. 22, figs. 50 and 51). This is followed by a post-oral segment, which in Actinotrocha is produced into tentacles, and in Balanoglossus is not. This at first sight is a marked difference, but the fact that the collar region in Cephalodiscus is produced into tentacles, and also that in some Tornariæ tentacular processes are found post-orally as well as pre-orally, must be taken into consideration, and the secondarily acquired adult habitat of Balanoglossus renders an atrophy of tentacular processes at least a plausible hypothesis. Behind this is the third portion of the body or "trunk," an elongated cylindrical segment with a terminal (or subterminal) anus.

These segments are, in each type, covered with a unicellular layer of epiblast or ectoderm, which is for the most part ciliated and glandular.

Nervous System.—The nervous system is in both cases of a primitive type. The inner ends of the outer layer cells form a fine diffused network of fibres underlying the whole surface, but special concentrations can be distinguished in various parts.

In both Actinotrocha and Balanoglossus (Pl. 22, figs. 46, 47) the chief nervous concentrations are found in the dorsal region of the collar, and form a thick nervous mass extending throughout this region. The differences are of degree only, for in Actinotrocha the dorsal collar region is narrowed from before backwards, and with it the nervous area, and the more or less hollow tubular condition in Balanoglossus is seen in a more nascent condition in the neuropore of Actinotrocha (cf. figs. 48, 49). In each there extend forwards from the main ganglion, on to the pre-oral lobe, three main nerve-tracts, one median dorsal (in the condition of three secondary groups in Actinotrocha) and two lateral bands, skirting the lateral edges of the pre-oral segment and meeting in front.

Posteriorly the collar nervous area gives off a pair of lateral branches which encircle the extreme hind edge of the collar, with a nervous ring, and from the mid-ventral point of this

ring are given off fibres forming a ventral nervous tract in the trunk. In addition there is a mid-dorsal nervous tract along the trunk, concentrated into a cord in Balanoglossus, in a more diffused condition in Actinotrocha. Lastly, there is the perianal nervous ring in the latter.

It is difficult to follow this comparison with the semi-diagrammatic figures (figs. 46, 47), and to account for the resemblances as accidental or homoplastic.

Alimentary System.—We have seen that there is some reason for suspecting the presence of a stomodæum in Actinotrocha, whereas, so far as evidence goes, such is scarcely present in Balanoglossus. Again, the hypoblastic system is complicated in the latter, by a forward movement of certain of the organs, into the proboscis, giving them a secondary pre-oral position. Thus it cannot be denied that the notochord is secondarily displaced into the proboscis, probably in connection with a burrowing habit. The proboscis is primarily pre-oral, and therefore de facto has no hypoblast in its cavity; any hypoblastic organ must, therefore, invade it secondarily. Thus Bateson states that the notochord arises in the collar region and moves forward later in development.

This moving forward phyletically of the notochord must necessarily involve the displacement of other structures, and together with the absence of a stomodæum must be allowed for in our comparison.

At the front end of the pharynx in Actinotrocha are a pair of organs which have been described and referred to above as "notochords."

The presence of a "notochord" has always been so insisted upon as an essential character of the Chordata that at first sight one might be led to suppose that the proving of the identity of these organs of Actinotrocha with the notochord of Balanoglossus is a sine quâ non for the assumption of their close genetic connection. This does not, however, appear to be the case, for the anatomical likeness is so marked, quite apart from the "notochord" question, that it merely serves to form an additional reason for regarding these latter as

of notochordal value. However, the question of the actual homology of these organs has been carefully investigated, and the balance of evidence seems distinctly in favour of regarding them as the primitive paired representatives of the notochord of Balanoglossus.

There can be no question of their analogy to notochords. Their histological structure and their relationship to the pre-oral mesentery indicate a function of support, as in the case of the true notochord. They are undoubtedly formed from hypoblastic tissue, and arise normally as diverticula of the primitive gut wall. They also arise in the front end of the collar region, a position identical with that of Balanoglossus. The point of difference, therefore, resolves itself into this,—that there are in Actinotrocha two lateral organs, whereas in all the other Chordata, as far as is known, there is but one median dorsal notochord.[1]

If we can assume a fusion of two lateral organs in the median line as having taken place, the change from lateral to dorsal would follow as a matter of course, so that the difference resolves itself into the difference between "paired" and "median unpaired."

To any one who can appreciate the principles upon which a bilaterally symmetrical animal is constructed this difference will not bear any importance. Indeed, it is more difficult to conceive in such an animal of the presence of an organ which arises primarily in a strictly unpaired condition; and it is at least a defensible hypothesis that in the higher Vertebrata every asymmetrical unpaired organ arose by atrophy of its fellow, and every symmetrical one either by survival from a radially symmetrical condition or by fusion, in the middle line, of paired rudiments.

The atrium in Tunicata arises by paired lateral rudiments, in Amphioxus by a median ventral invagination, in spite of which difference we do not find the homology of the two called in question, and one might multiply instances from the study

[1] The two lateral notochords of Cephalodiscus were found subsequently to the writing of this statement.

of the arteries, veins, central nervous axis, skull, swimming bladder, genitalia, &c.

Scarcely any process in the phylogeny of the higher animals is more clearly indicated than this, unless it be the ease with which primitively unpaired organs may become impressed with bilateral symmetry.

In the case of the notochord itself we may remark that in Balanoglossus it tails off in the hind region (collar) into paired rudiments, which are more marked in the young stages. The figures of Morgan (17) in this connection are very striking, and we cannot explain the fact that the part of the notochord which is least displaced from its primitive position is found in the paired condition, otherwise than as indicating a paired phyletic origin.

It may be urged that if the notochord of the Chordata arose as a paired organ there should be indications of this in the ontogeny of the higher types.

I think there is one reason why the vertebrate notochord does not as a rule show traces of the paired condition, and this is that in the higher Chordata the development of the notochord is greatly hastened. Thus in Amphioxus and in several higher types the rudiment of the notochord is pinched off from the gut at quite as early a stage as are the mesoblastic pouches. A transverse section of a larval Amphioxus at a suitable stage shows the pair of dorso-lateral mesoblastic pouches still in continuity with the gut wall, and in the median line the notochordal rudiment is in the same condition.

So much is this the case in some Vertebrata, e.g. the chick, that certain observers have claimed that the notochord arises primitively as a median rod of mesoblast.

A careful study of the more primitive forms, however, clearly shows that the notochord naturally arises from the hypoblast which remains after the mesoblast has been developed from it (secondary endoderm). Without offering any explanation of this precocious development of the notochord, it is plain that, with such ontogenetic conditions, the retention of a phyletic process of development from paired rudiments is impossible. The mesoblastic pouches force the notochordal

rudiments into the median line from their very inception, though even in Amphioxus the organ is formed by the "dovetailing" together of two lateral rows of cells.

I am indebted to Dr. Gadow for calling my attention to the work of Mitsukuri (15a) on the ontogeny of Emys, in which he has described paired rudiments of the notochord, which he styles "hemichords," and which fuse together in the median dorsal line to form the notochord. The observation is significant in the light of this discussion.[1]

There is one other possible interpretation of these "notochords" of Actinotrocha, namely, that of a pair of abortive gill-slits. In the light of the work on Cephalodiscus which follows, it will be seen that this is perhaps a tenable hypothesis, but it will be discussed under that head.

The significance of the ventral chordoid tissue will be referred to later. Assuming, then, that these organs may be regarded as primitive paired rudiments of the Chordate notochord, I have depicted for the further comparison of the two types one of these notochords in fig. 49, as bent into the mid-dorsal line, in order to have it included in a median sagittal section.

In fig. 48 is seen a similar section of Balanoglossus, compiled, in a semi-diagrammatic way, from the works of Spengel (19a), Köhler (12a), and Morgan (17). A comparison of the two will show the way in which it is here suggested that the notochord of Balanoglossus has moved forwards. In doing this it has come into peculiar relation with the pre-oral cœlom, which will be referred to again.

We have in Balanoglossus the "proboscis vesicle" of Morgan (17) ("sac of proboscis gland" of Bateson [1] and Köhler [12a], Herz-blase" of Spengel [19a]), with its relationship to the front end of the dorsal blood-sinus and to the pre-oral cœlom, and in Actinotrocha the subneural gland with precisely similar relationships to these parts. This is even more clearly

[1] Davidoff (7a) has shown that in certain Tunicata the notochord and nervous system arise from paired rudiments, and Brooks (1b) finds a similar origin for the eleoblast of Salpa. These facts may have more phyletic significance than Brooks is inclined to allow.

shown in coronal sections of the two types (figs. 50 and 51). In each case the organ has precisely similar relationships to the pre-oral cœlom, collar cœlom, and subneural sinus (or heart), though there is a striking difference in the fact that the subneural gland has a duct to the exterior, whilst the proboscis vesicle has none in the adult, though it arises in close contact with the epiblast, even if not actually from this layer. We have assumed a forward movement of the notochord in Balanoglossus, and this movement must have considerably altered the environment of the proboscis vesicle. It is not inconceivable that this organ might be carried forwards and lose its connection with the exterior, as is shown in fig. 48.

The subneural gland is undoubtedly epiblastic in origin, and arises in the mid-ventral line, whereas there is considerable difference of opinion regarding the origin of the proboscis vesicle. Spengel (19a) maintains that it is epiblastic in origin, and Morgan (17) is inclined to believe that it is mesoblastic. Morgan's figures show the early rudiment of the vesicle in close contact with the epiblast; and I think that although Morgan (17) interprets the organ as formed from mesenchyma, there is as much testimony in his figures to its epiblastic origin as is found in the case of the segmental duct of Vertebrata and the nerve-cord of Teleostei. Although the organ in Actinotrocha arises ventrally, it comes into connection with the dorsal sinus and other parts in such a way that it would not be surprising to find the mid-ventral invagination method in this type altered to the modified process of development in Balanoglossus. With the forward movement of the notochord a mid-ventral development of the proboscis vesicle would be an ontogenetic process of great difficulty. (We might here compare Willey's [23] suggestion of the alteration in position of the mouth of Amphioxus, correlated with a forward growth of the notochord.) The structure of Cephalodiscus is especially instructive with regard to the subneural gland.

Gill-slits.—I have not as yet been able to find any trace of gill-slits or their homologues in Phoronis or Actinotrocha, though their analogues are present in each case,

represented in Actinotrocha by the atrial grooves already described.

Stomach.—After the pharynx is found the digestive portion of the stomach; and though the intracellular digestive areas may be probably regarded as the first rudiments of the hepatic diverticula of Balanoglossus, this suggestion cannot be enlarged upon here.

Intestine and Anus.—An intestine suspended by a ventral mesentery terminates in each case in a posterior anus.

Mesodermic Organs.

Vascular System.—As in the case of the nervous system, the vascular tissues in both Balanoglossus and Actinotrocha are in what is undoubtedly a primitive condition. The veins and arteries, in fact, are merely sinuses and chinks between the mesoderm on the one hand and the ectoderm or endoderm on the other.

In each case (figs. 50, 51) there is a large sinus (the subneural sinus [*s. n. s.*] of Actinotrocha, the heart [*ht.*] of Balanoglossus), which lies just below the junction of pre-oral lobe and collar, a position with a special significance. This is continued along the dorsal endodermic wall between the collar walls as a small fissure into a larger sinus, with more or less muscular walls, differentiated from the trunk cœlomic walls, which are produced forward (perihæmal, Spengel). In each case there is a ring sinus in the collar-region which is continuous with a ventral median sinus below the gut. Such resemblances, in so plastic and adaptable a system as the vascular, speak for themselves (cf. figs. 48 and 49).

Cœlom.—The condition of the cœlom in Actinotrocha bears so remarkable a resemblance to that of Balanoglossus and Cephalodiscus that a genetic relationship might be claimed upon this count alone.

The pre-oral lobe is in each case lined internally by the mesodermic wall of the pre-oral cœlom, the cavity of which is itself partially filled by muscular tissue, passing in various directions, though in each case the main strands can be

traced from the lateral walls in a converging manner to the posterior angles, where in Actinotrocha they are inserted in the "pre-oral collar" mesentery, and in Balanoglossus in the sub-notochordal skeletal rod. The arrangement of these muscles will be shown to be in Cephalodiscus identical with that of Actinotrocha. In Actinotrocha there are two proboscis pores opening on either side of the sub-neural sinus (fig. 51) in identically the same manner as in Cephalodiscus. In Balanoglossus the pores are somewhat further away from the middle line, but in an homologous position. The collar-cavities are closely similar, with a tendency in each case to a production of the dorsal part forwards into the neck. The collar pores have already been compared to the collar nephridia of Actinotrocha. The relationships of the collar cœlomic walls to the vascular system and the gut are practically identical.

The trunk cavities are continuous dorsally, and ventrally they form a mesentery. From their walls are derived in each case the gonads (Phoronis). The front dorsal part of the trunk cœlom is produced into a pair of perihæmal spaces, embracing the dorsal blood-vessel.

We thus see that not only is there in both types a segmentation of the cœlom into five pouches, one pre-oral and unpaired (leaving out of consideration for the present the possible cœlomic value of the proboscis vesicle), and two pairs of post-oral pouches, but these pouches are similar, to a large extent, in their anatomical relationships, though perhaps the presence of tentacles in Actinotrocha tends to make the resemblance to Cephalodiscus even closer.

Thus a very general and brief comparison of the nervous, alimentary, vascular, skeletal, and cœlomic systems brings out a close agreement, the genetic origin and value of which can hardly be denied.

(2) Actinotrocha and Tornaria.

The comparison of these two larvæ is interesting in that it leads one to the conclusion that the fully developed Actino-

trocha is more nearly allied to Balanoglossus than to its larval form, Tornaria. Thus a closer agreement would probably be found between the latter and an earlier stage of Actinotrocha than is here described. There are, however, certain general points of similarity which bear out the conclusions already arrived at in the former comparison of Balanoglossus and Actinotrocha.

Each has a mouth overhung by a large pre-oral hood, at the posterior mid-dorsal border of which is a thickened nervous area. From this runs a pre-oral ciliated band round the edge of the hood, and a post-oral band behind the mouth. In Tornaria the bands are more convoluted, but the arrangement is well seen in Stage IV, figured by Morgan (**17**). The terminal anus is surrounded by a powerful perianal band, which has long lash-like cilia, and forms the main locomotive organ of the body (cf. **26**).

The condition of the cœlom is similar, though Tornaria is less developed in this respect. It, however, shows an unpaired pre-oral cœlom with a dorsal pore, a pair of collar cœloms, and a pair of trunk cavities. In the course of development the trunk region is in each case greatly elongated.

(3) (The Comparison of Phoronis with Balanoglossus must be left to another Paper.[1])

We may now inquire if the acknowledgment of Phoronis as closely connected with the chordate stock sheds any further light upon the origin of the Chordata, or of organs peculiar to this group.

As regards the notochord a very primitive condition is met with. In Actinotrocha the notochords are little more than vacuolated areas of the gut wall. Their lumen is in continuity with that of the gut, and the process of vacuolisation is very simple and instructive. Each cell can be observed to be gradually filled with turgid vacuoles, and these are not

[1] We may state that the differences in habitat and their resulting modifications obscure most resemblances in protomere and mesomere, but that the arrangement of the parts in the metamere or trunk is closely similar. (See **24**.)

further complicated by fusions. Just as in tracing down, e. g., the digestive organs into lower types, we find that stages are reached in which the function is more diffuse, and is confined only by the extent of the hypoblast layer (indeed, not even by this in Cœlenterata), so in tracing down the hypoblastic skeletal function we find it in the Diplochorda distributed over certain areas in which it is no longer found in higher types. Thus in Actinotrocha it is found in the mid-ventral wall (in the form of chordoid tissue) exactly at the spot where most support is required, in addition to the normal area of chordoid tissue in the collar region.

Again, in Cephalodiscus, chordoid supporting tissue is developed round the pharyngeal clefts, where, again, it is obvious that support is specially essential (see next paper). It seems that in the Diplochorda the supporting function of the hypoblast has been active, and chordoid tissue developed in whatever position was requisite for the needs of the organism.

The development of turgid vacuoles for support seems to be the special character of the hypoblast (or endoderm), and is also found in the Cœlenterata, e. g. in the tentacles of certain Hydroids and in some larvæ, such as that of Lucernaria (R. S. Bergh). We may suppose that a feature of the chordate stock is the retention of this ability to form hypoblastic skeletal tissue by vacuolisation.

The subneural gland is apparently to be compared with that of Tunicata bearing the same name; and if we assume that the line from subneural gland to nerve-ganglion marks the posterior boundary of the pre-oral lobe, we may be helped in the comparison of these Urochorda with other Chordata. (I see no sound reason for holding that the large suboral process with papillæ for attachment, found in the larvæ of sedentary Ascidians, is the pre-oral lobe, but would rather regard it as an hypertrophied ventral sucker belonging to the post-oral region.)

Lastly, this organ may be compared with the hypophysis of Vertebrata, and the relationship of the latter to the front end of the notochord and to the subneural mesodermic tissue would thereby receive a ready explanation.

The notochord must be regarded as arising in the collar-region, and the prolongation forward of this organ in Amphioxus is usually acknowledged to be a secondary adaptation, as is also the pre-oral position of Balanoglossus.

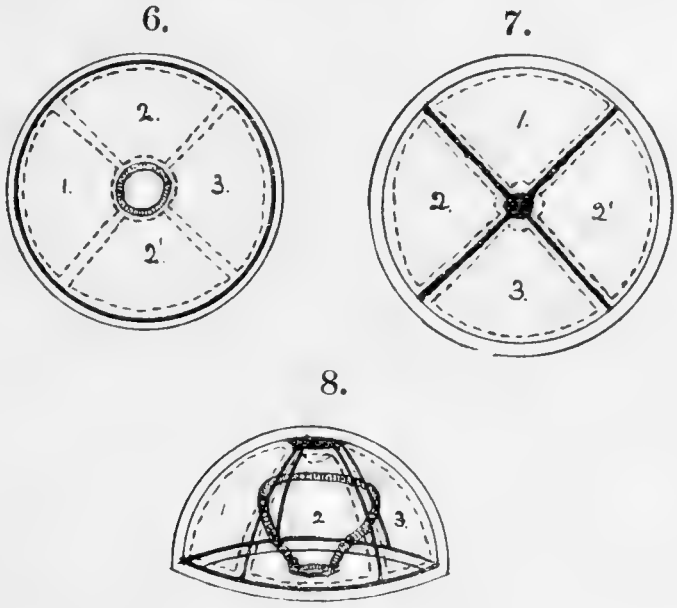
The true Vertebrata, primarily free from a burrowing habit, have probably retained at least the front end of the notochord in its primitive position. The front end of the notochord, therefore, indicating the anterior collar-region, the hypophysis is in the same relation to it as the subneural gland in Actinotrocha. In the former case its inner end comes into close relation with mesodermic vascular tissue lying close under the brain, and in the latter case the subneural gland has similar relations with the subneural sinus under the main ganglion. It is even possible that the base of the "neuropore" might be compared with the infundibulum. As in the case of the Tunicata, it is probable that the line from brain (infundibulum) to hypophysis marks the posterior boundary of the pre-oral lobe.

It is not so safe to attempt to draw the posterior boundary of the collar-region. In both the Cephalochorda and Vertebrata there can be no doubt that the trunk region is hypertrophied, and also that the position of the anus has changed, perhaps more than once. At the same time, in these forms and in the Urochorda there are always traces of hypoblast other than the notochord (post-anal gut, &c.), which indicate that the prolongation of the notochord in a posterior direction was part of the process of a movement of the whole gut towards the posterior end of the body. There is no difficulty in reconciling the relations of the notochord to gut and blastopore in the embryos of Vertebrata, and the idea of a primitive condition of collar notochord. In fact, the elongation of the notochord posteriorly by a proliferation of cells at the anterior end of the blastopore is readily interpreted by this assumption.

Again, a careful consideration of the structure of Actinotrocha may make it possible to trace the chordate stock practically to the Cœlenterate stage, or at least to a rudimentary triploblastic type.

Rather than work back to simpler organisms, I would attempt to follow the indicated course of evolution from simple to complex.

In woodcuts 6, 7, and 8 I have depicted the leading characters of Stage 1, a pelagic, triploblastic, radially symmetrical ancestor



6. Ventral view of Stage I. 7. Ventral view of ditto. 8. Lateral view of ditto. N.B.—In this and succeeding woodcuts the general ectoderm is unshaded, the endoderm shaded, nervous areas black, and mesoderm dotted. The cœlomic pouches are numbered.

of the Cœlomata, or at least of those Cœlomata to which I wish to refer. With four cœlomic pouches it is quite easy to imagine this organism as derived from a pelagic Cœlenterate with four gastric pouches. The origin of the radial symmetry must be assigned to the conditions of free pelagic (floating) existence, and the presence of the apical nervous system precludes a sedentary origin. The environment, in a horizontal plane, of a free pelagic organism with only perpendicular automatic locomotion should be as capable of impressing a radial symmetry upon an organism as that of a sedentary habit.

We may note that there is an apical ganglion with four radial nerves, meeting a nerve-ring round the mouth.

Below the nerve-ganglion is a subneural sinus which com-

municates with a system of intercœlomic sinuses, the primitive vascular system.

The four cœlomic pouches all retain the muscular, reproductive, and nutritive functions appertaining to the primitive cœlom.

The axis of symmetry is perpendicular, and the central nerve-ganglion is situated at the termination of this axis in the direction of motion.

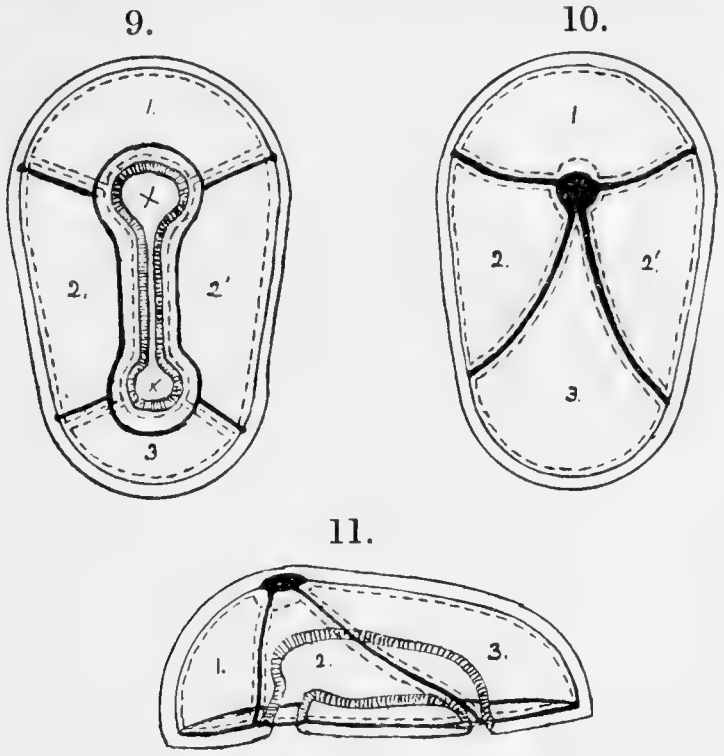
I would specially emphasise the fact that the mesoblastic pouches are evolved in this stage (Protaxonia, Hatschek), so that their segmentation into four is due to radial determining factors, and it follows from this that they cannot strictly be regarded as "paired." This segmentation of the mesoderm, for reasons given below, we may term the "archimeric segmentation."

With horizontal motion forwards the axis of symmetry is replaced by a perpendicular plane of symmetry in the direction of motion. The Heteraxonia (Hatschek) stage is now commenced, and, as has been shown by Sedgwick, the blastopore elongates and forms mouth and anus.

Under these conditions the mesoblastic "archimeres" are placed under fresh symmetry-determining factors, and they are now no longer under the same environmental conditions. In woodcuts 9, 10, and 11 are seen three views of this stage (Stage 11). The new mouth and anus are almost completed, and it is now seen that one of the archimeres (1) is pre-oral in position, two are lateral (2, 2′), and the last (3) is now post-anal.

The first of these (the protomere) is now subjected to the environment incidental to the front end of the body and to the pre-oral position. No longer in a suitable position with respect to the digestive part of the gut, it will lose its nutritive and digestive function, which implies also a loss of that of reproduction. In other words, its vegetative functions will be lost whilst its animal functions will be markedly developed. This protomere is evidently homologous with the pre-oral lobe, hood, proboscis, &c., of so many of the Cœlomata. It

appears to be destined to play a very secondary part in the higher Cœlomata in any case. In animals which take up a sedentary existence it is no longer required to any great extent,



9. Ventral view of Stage II. 10. Dorsal view of ditto. 11. Lateral view of ditto.

for the "animal" functions are at a low ebb. Again, in those which do not thus retire from the arena of pelagic or littoral life it is replaced by the shifting forwards of the "metameric" segments from behind.

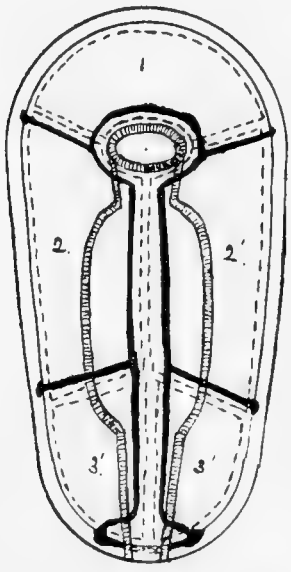
The cavities (2, 2′) of the two lateral segments are the most suited, by their position, for performing the functions of digestion and excretion; and at the same time their position immediately behind the mouth causes these segments to be adapted (by tentacles, &c.) for ingestive functions. They may be termed the "mesomeres," and are recognised in Hemichorda as the collar-segment. The last (3) or post-anal is only found in this position in rare cases, for the next stage is soon reached.

The nerve-ganglion is seen to be making its way forwards

to the new animal apex, and with it the radial nerve-tracts are altered somewhat in position, as also are the cœlomic pouches, in correlation to the forward motion of the sub-neural sinus.

In the next stage, Stage III (woodcuts 12, 13, and 14), further changes are indicated. The nervous system is somewhat

12.



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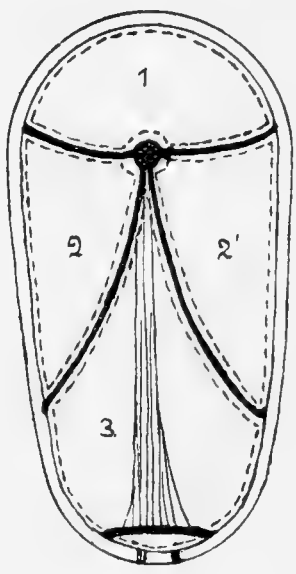

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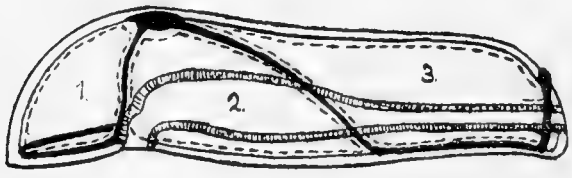


12. Ventral view of Stage III. 13. Dorsal view of ditto. 14. Lateral view of ditto.

altered. The nerve-ganglion has still progressed forwards, and it is seen that the longitudinal nerve-ring and radial nerves are now tending to form a secondary system of rings in planes at right angles to the axis of motion. Thus two radial nerves and part of the primitive ring form the "pre-oral ring," two radial nerves form the "post-oral ring," and part of the primitive ring forms the "perianal ring." In other words, these rings are formed to correspond to the three archimeric segments—protomeric, mesomeric, and metameric.

We note that the anus has travelled to the posterior end, in obedience to general laws of symmetry.

This change from radial to bilateral symmetry, though hypothetical, is paralleled in a remarkable manner in the Echinoids, and the above woodcuts will forcibly recall the transitional symmetry of such forms as Spatangus.

The change in position of the anus is important, for it places the metameres (3, 3′), now separated by the alimentary canal into two, in an advantageous position in regard to the processes of nutrition, and hence we find that not only do these metameric cœlomic cavities early monopolise the reproductive function to the exclusion of the protomere and the mesomeres, but they elongate enormously; and in them is instituted in higher forms a secondary bilateral segmentation, which is already termed "metameric." The study of the segmented forms leads one to believe that the metameric segments grow forward, at least the cœlomic elements in them, and to a large extent replace the archimeric protomere, and mesomeres.

The stage we have now reached is practically identical with that exhibited by Actinotrocha (without taking into consideration the notochordal rudiments). I need not go into a detailed comparison of the two types,—Actinotrocha is so closely represented by this stage that its study suggested the hypothesis here laid down. Woodcuts 12, 13, and 14 would almost pass for diagrammatic representations of Actinotrocha. These are the bare outlines of a suggested ancestry of the Chordata, tracing the lines of evolution nearly to the Cœlenterata. We must assume that the archimeric segmentation is predominant till above the Hemichorda, and that in Amphioxus the metameric segmentation comes into existence, and the protomere and mesomeres become of secondary importance or disappear altogether. I find, in looking at the literature of Balanoglossus, that Morgan has suggested that the condition of the mesoderm in Amphioxus is arrived at through secondary segmentation of the trunk of Balanoglossus, so that I must adopt

his hypothesis, and put no claim to originality in this particular point.

If the metameric or secondary segmentation took place between the stages of Balanoglossus and Amphioxus it is evident that, as Bateson has emphasised, the "segmented" Invertebrata and the segmented Vertebrata must be genetically distinct, in spite of the most elaborate anatomical resemblances. The recognition of this archimeric segmentation and its relationships to the secondary or "metameric" segmentation will, I venture to think, not only clear up many difficulties in tracing the origin of Vertebrata, but will furnish a sound basis for the phyletic classification of the Cœlomata.

Although the archimeric segmentation would form the basis of the group Archicœlomata, here suggested, a primitive condition of the other systems is characteristic. Thus we might state the following characters of this group:

1. The body is divided into three archimeric segments, the protomere, the paired mesomeres, and the metamere, either throughout life or in early stages,[1] when the relative prominence of one or more of these may be altered.

2. The metamere is never definitely divided up by a secondary bilateral segmentation, usually known as "metameric" segmentation.

3. The nervous system is usually in a primitive condition, and is still in continuity with the ectoderm throughout life.

[1] The coronal section of Bateson's early larva of Balanoglossus clearly shows indications of primitive equivalent radial value of the four pouches though the mesomeric elongate very early. In ontogeny one need not expect the four pouches to, in every type, be separately invaginated from the gut. A difference in the relative time of development of different parts would readily account for the variations in this respect seen in the different groups of Echinodermata and the Chætognatha, though the separate invagination of each from the hypoblast must be regarded as the more primitive condition, just as the elongation of blastopore to join both mouth and anus is regarded by many as the more primitive condition, and the survival of this aperture as the anus or mouth only is conceded to be an ontogenetic adaptation.

4. The vascular system is primitive, consisting, when present at all, of sinuses and splits between the cœlomic walls.

5. The protomere (pre-oral lobe) is usually present throughout life, or is distinctly in evidence at one time in the ontogeny of the individual.

6. The cœlom is segmented into a protocœle in the pre-oral lobe, a pair of mesocœles, and a metacœle which tends to be secondarily more or less divided up into two. Each cœlomic cavity has primitively a pore leading to the exterior, which primarily acts as a gonaduct and nephridiopore. The protocœlic pore is divided usually into two proboscis pores, the mesocœlic pores are usually termed collar pores; all these early lose their gonaducal function. The metacœlic pores usually become specialised for gonaducts.

These will indicate a few characters of the Archicœlomata, and it will be seen that the Archichorda (Cephalodiscus, Phoronis, and Balanoglossus), Echinodermata, Chætognatha, and Brachiopoda, all readily group themselves under this head, and there can be little doubt that the Sipunculoidea and Polyzoa will be found to conform to the same character.

As might be expected of animals preserving a primitive type of structure, nearly the whole of these groups are essentially of sedentary or burrowing habits, though the Chætognatha are a remarkable exception. The position of the anus and the retention of the reproductive function in the mesocœles in this group indicate an early assumption of the group-characters, and the very existence of this group shows that, under certain conditions,[1] the archicœlomate type has been able to maintain its existence in competition with metamerically segmented forms. Amongst these Archicœlomata we may notice one group, the Archichorda, which are nearest the direct line of the true metamerically segmented Chordata; and others again, such as the Sipunculoidea and Brachiopoda, which show, in their ontogeny, stages in the evolution

[1] The Chætognatha are probably a primitively pelagic group.

of the metamerically segmented Invertebrata, the Annelids and Arthropoda.

One cannot here enter further into these ideas, but they may be appropriately expressed by the accompanying tree.

Though it would be difficult to imagine a more rudimentary condition of the notochord than that of Actinotrocha, the ontogeny of the Echinodermata and Brachiopoda, or even the Sipunculoidea and Polyzoa, may yet furnish interesting rudiments of chordate organs.

Another suggestive line of thought is the gradual abolition of the archimeric segmentation and elaboration of the metameric in the Annelida, Arthropoda, and Eu-chorda. The nerve-ganglion of Actinotrocha is probably homologous with the trochophoral apical ganglion, and hence with the supra-œsophageal ganglion of the Annelida.

The protomere is most likely represented by the prostomium, the mesomeres by the peristomium, and the post-oral or mesomeric nerve-ring by the circum-œsophageal ring. The mesomeric cœlomic cavities may possibly break down, leaving the trocophoral head-kidneys as mesomeric nephridia, and there are indications that all the cœlomic cavities (and appendages in Arthropoda) are post-oral, probably metameric.[1]

It is not difficult to understand why the metamerically segmented animals have replaced, in nature's highways, the Archicœlomata, retaining only the archimeric segmentation into three parts.

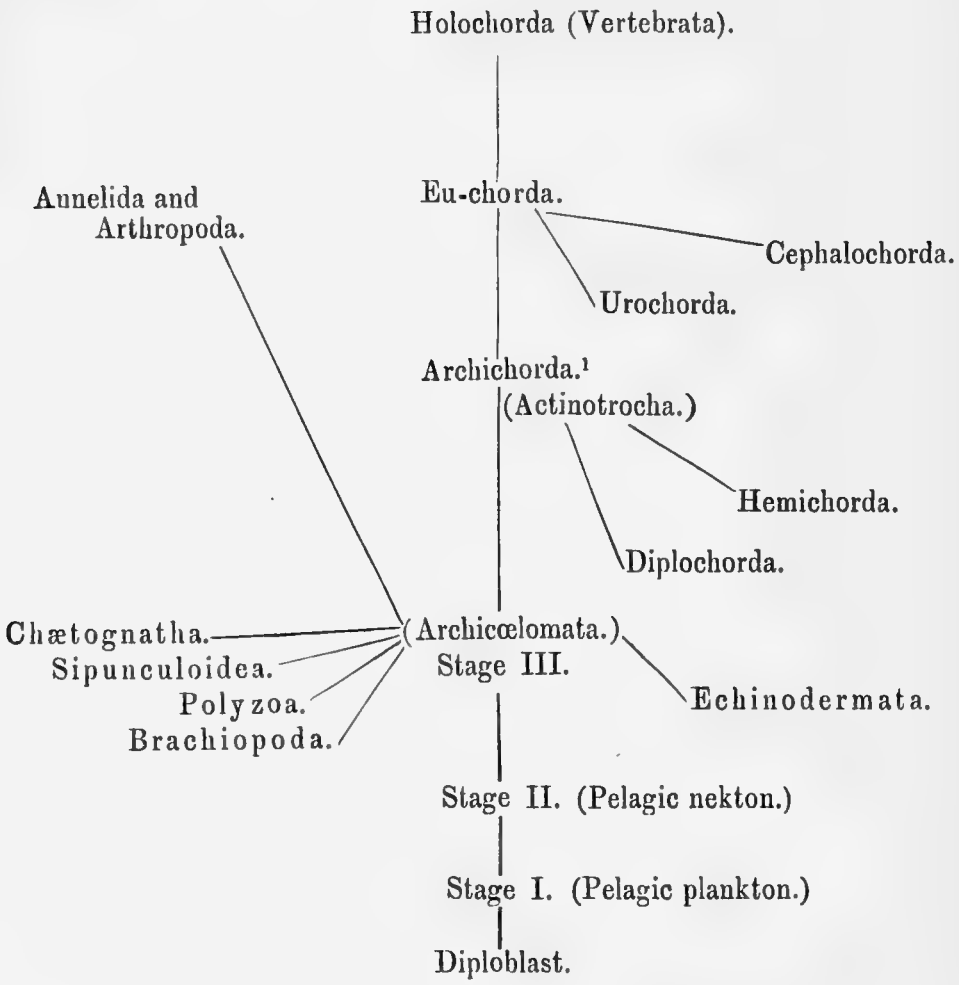
Just as a limit is placed to the organic capabilities of single-celled individuals, which is easily surpassed by the Metazoa, so the animals starting with a greater number of segments,

[1] "It appears that the antennæ of Peripatus and the antennæ of Myriapods and Hexapods, as well as the two pairs present in Crustacea, are all of them post-oral parapodia shifted in relation to the mouth. . . . . The conclusion to which embryological study (as to segments of body-cavity, so-called cerebral ganglion, and position of early rudiments of antennary appendages of Arthropods) tends is that in the various branches of Arthropod descent . . . . there have been forward movements or shiftings of post-oral segments and their parapodia."—E. Ray Lankester, 'Natural Science,' April, 1897, p. 265.

and other suitable conditions, are capable of more elaborate differentiation to suit a more complex environment than those which are limited in the number of their morphological units, i. e. segments in this case.

The same environmental factors acting on the metamerically and the archimerically segmented animals produce a close homoplastic resemblance in the two groups of organisms. Thus the metamerically segmented Arthropoda, and even Vertebrata, show a grouping of segments into three regions, the head, thorax, and abdomen, which in the general plan of their several form and functions have a close resemblance to the protomere, mesomere, and metamere of the Archi-cœlomata.

On the other hand, in the parallel evolution of metameric segmentation in the Eu-chorda, the tracing of archimeric segments in them is fraught with interest.



In the above tree the idea of the ancestry of the Chordata is a long line of pelagic organisms up to the stage reached in Actinotrocha, at which stage two groups took to degraded habits, the Hemichorda and the Diplochorda, and thereby saved themselves from extinction. Balanoglossus, with its burrowing habitat, shows a few remarkable modifications and further differentiation in organs, and even a commencing metameric segmentation, and is less degenerate than the Diplochorda. In this group Cephalodiscus has sought refuge in a deep-sea habitat and a protecting house; whilst Phoronis also resorts to a sedentary and, in most cases, tubicolous habit. Hence the former retains a more

[1] Differing in the main from Stage III by the evolution of paired chordoid rudiments, rudimentary pharyngeal clefts, and a subneural gland.

primitive character, whilst the latter exhibits extreme degeneration in the adult.

The main chordate stem then underwent metameric segmentation, and gave rise to the Eu-chorda. Of these, those still retaining an active pelagic or littoral life gave rise to the Vertebrata or Holochorda (Gadow); whilst others again, falling out of the ways of progress, suffered the same fate as the Hemichorda and Diplochorda. The Cephalochorda, taking to a burrowing habitat, suffered like modifications (such as pre-oral extension of the notochord) to the Hemichorda; whilst the Urochorda adopting the sedentary habits of the Diplochorda, and especially those of Phoronis, like this group suffered extreme degeneration. The metameric segmentation of the Urochorda is not beyond dispute, but the consensus of workers on the group appears in favour of regarding them as metamerically segmented, at least primarily.[1] The views here propounded may be expressed in this classification.

Chordata.

I. Archichorda.

Archimeric segmentation into protomere, paired mesomeres, and metamere; little or no metameric segmentation. Notochord in primitive continuity with the walls of the gut throughout life. More or less connected with the protomere, the main animal organ of the body. Nerve-ganglion between protomere and mesomere or dorsal to mesomere. Main nerves are protomeric ring, mesomeric ring, and dorsal and ventral trunks. A mesoblastic chondroid skeleton and an ectodermal chitinoid tube or skeleton.

1. Hemichorda.—Notochord fused in middle line and protruding far into the protomere. Commencing metameric segmentation in gill-slits and gonads.

2. Diplochorda.—Notochord in primitive paired condition. In close connection with the two posterior protomeric

[1] The position of Urochorda is undoubtedly nearer the Eu-chorda than the Archichorda.

mesenteries. Mesomeres produced into numerous tentacles. Metamere with dorsal flexure.

(1) Phoronidea.—Loss of pre-oral lobe and notochord in adult. No gill-slits.

(2) Cephalodiscida.—One pair of gill-slits with chordoid walls; persistent notochords.

3. Rhabdopleurida.[1]

II. Eu-chorda.

Archimeric segmentation replaced by metameric. Single dorsal notochord loses connection with the gut-wall, and dorsal nervous system with the ectoderm. Protomere and mesomeres reduced. Notochord extends into metamere (Urochorda, Holochorda), and also into protomere (Cephalochorda).

1. Urochorda (?).
2. Cephalochorda.
3. Holochorda.

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EXPLANATION OF PLATES 18—22,

Illustrating Mr. A. T. Masterman's paper "On the Diplochorda: Part I. The Structure of Actinotrocha."

List of Abbreviations.

a. g. Atrial grooves. *a. n.* Anterior nerve. *a. m. n.* Anterior median nerve. *a. l. n.* Anterior lateral nerve. *B. c.*[1] Pre-oral body-cavity. *B. c.*[2] Collar body-cavity. *B. c.*[3] Trunk body-cavity. *c. p.* Collar-pore. *c. n.* Collar nephridium. *c. n. r.* Collar nerve-ring. *d. b. v.* Dorsal blood-vessel. *v. b. v.* Ventral blood-vessel. *d. n.* Dorsal nerve. *d. a.* Digestive area. *ht.* Heart. *mes.* Mesentery between pre-oral and collar cavities. *mes'.* Mesentery between collar and trunk cavities. *m.* Mouth. *nch.* Notochord. *n. g.* Nerve ganglion. *np.* Neuropore. *œs.* Œsophagus. *o. g.* Oral grooves.

pb. Proboscis vesicle. *p. a.* Perianal band. *p. a. n.* Pre-oral nerve-ring. *p. s.* Perianal sinus. *p. p.* Proboscis pore. *r. s.* Ring sinus. *sh.* Sheath of notochord. *s. n.* Subneural gland. *s. n. s.* Subneural blood-sinus. *s. p.* Sense papilla. *st.* Stomach. *t.* Tentacle. *v. c.* Ventral collar area. *v. c. r.* Ventral ciliated ridge. *v. ch.* Ventral chordoid area. *v.* Vacuoles. *v. c. n.* Ventral collar nerves. *v. m.* Ventral mesentery.

PLATE 18.

FIG. 1.—Lateral view of entire larva with ten pairs of arms.

FIG. 2.—Lateral view of later larva, showing the distribution of the main nervous tracts.

FIG. 3.—Dorsal view of larva a little later than Fig. 1.

FIGS. 4 and 5.—Two positions commonly assumed by living larva.

PLATE 19.

FIG. 6.—Transverse section of fully developed larva in region of pre-oral hood.

FIG. 7.—Transverse section of same larva behind the nerve ganglion.

FIG. 8.—Ditto, through mouth.

FIG. 9.—Ditto, through notochords and œsophagus.

FIG. 10.—Ditto, through collar region.

FIGS. 11—15.—Transverse sections of dorsal wall of same larva, between Fig. 6 and Fig. 7, to show nerve ganglion.

FIG. 16.—Transverse section of same larva through collar and trunk.

FIG. 17.—Ditto ditto, further back.

PLATE 20.

FIGS. 18—20.—Sagittal sections of fully developed larva. Fig. 20 is nearly median.

FIGS. 21—27.—Coronal sections of ditto, with hood turned back.

FIG. 28.—Transverse section of hood.

FIG. 29.—Obliquely transverse section to show oral grooves.

PLATE 21.

FIG. 30.—Surface of notochord in early larva.

FIGS. 30A, 30B, 30C.—Transverse sections of notochord in Fig. 30 at A, B, and C respectively.

FIG. 31.—Transverse section of later stage of notochord.

FIG. 32.—Longitudinal section of later stage of notochord.

FIGS. 33—36.—Series of semi-diagrammatic figures illustrating the process of vacuolisation.

FIG. 37.—Oblique section of notochord in young Phoronis.

FIG. 38.—Side view of front parts of fully developed Actinotrocha.

FIG. 39.—Transverse section of same larva as Figs. 6—17, in trunk region.

FIG. 40.—Longitudinal section of collar nephridium, as reconstructed from serial sections.

FIG. 41.—Transverse section of duct of collar nephridium.

FIG. 42.—Transverse section of nerve ganglion and subneural sinus.

FIG. 43.—Ditto of tentacle (larval).

FIG. 44.—Ditto of pre-oral nerve-ring.

FIG. 45.—Longitudinal section of hind end of larva to show the doubtful organs.

PLATE 22.

FIG. 46.—Semi-diagrammatic dorsal view of nervous system of Balanoglossus.

FIG. 47.—Ditto of Actinotrocha.

FIG. 48.—Semi-diagrammatic median sagittal section of front end of Balanoglossus.

FIG. 49.—Ditto of Actinotrocha.

FIG. 50.—Semi-diagrammatic median coronal section of Balanoglossus.

FIG. 51.—Ditto of Actinotrocha.

List of Abbreviations.

b. c.[1] Pre-oral body-cavity. *b. c.*[3] Trunk body-cavity. *b. s.* Blood-sinus. *c. p.* Collar-pore. *d. mes.* Dorsal mesentery. *d. b. v.* Dorsal blood-vessel. *ep.* Epistome. *g. s.* Gill-slit. *int.* Intestine. *l. b. v.* Lateral blood-vessel. *l. c. c.* Left collar cavity. *m.* Mouth. *n. g.* Nerve ganglion. *n.* Nuclei. *nch.* Notochord. *ov.* Ovum. *p. o. l.* Post-oral lamella. *ph.* Pharynx. *p. p.* Proboscis-pore. *r. c. c.* Right collar cavity. *st.* Stomach. *sh.* Sheath. *s. n. s.* Subneural sinus. *s. n. g.* Subneural gland. *t.* Tentacle. *v. b. v.* Ventral blood-vessel. *v. mes.* Ventral mesentery.

PART II.

On the Structure of Cephalodiscus dodecalophus, McIntosh.

As already indicated in the previous work upon Actinotrocha, the comparison of some of its organs with those of Balanoglossus indicated that a renewed study of Cephalodiscus and its "notochord" was desirable. The work here referred to was done upon material from the "Challenger" collection.

The former work upon Cephalodiscus is confined to a description of the chief anatomical features by Professor McIntosh (2) in the "Challenger" series, and an appendix to it by Mr. S. F. Harmer (1), the principal feature of which is a detailed comparison of this species with Balanoglossus, as a result of which the group Hemichorda has by many been made to include these two species in addition to Rhabdopleura.

As already mentioned in the preceding paper on Actinotrocha, work on this animal and considerations leading therefrom caused me to suspect the presence in Cephalodiscus of a notochord different in form and position from that already described as such by Harmer. I must here express a deep sense of gratitude to Professor McIntosh, LL.D., F.R.S., for the gift of specimens of this unique animal, taken from the few now remaining in his hands. I hope to be able to publish later the results of labour upon the buds and young forms, but this note must be confined to the description of some organs not hitherto noticed, and to the inferences to be drawn therefrom. It need only be mentioned that my pre-conceived view that Harmer's "notochord" is the homologue of the subneural gland, and that Cephalodiscus has a paired notochord, has been confirmed to a remarkable degree.

Of the two preceding workers, the keynote of McIntosh's (2) views was a close resemblance to Phoronis and Rhabdopleura; of Harmer's (1), an alliance with Balanoglossus. Whilst accepting the views of the latter, I think the work below will justify the placing of Phoronis and Cephalodiscus together under the title Diplochorda.

We may add that Professor A. Lang (4), in an article upon the organisation of Cephalodiscus, accepts its affinities with Balanoglossus. Further than this, he considers that the organs of the former show an earlier stage of evolution than those of the latter. On the other hand, he comes to the conclusion that the simplicity in structure of Cephalodiscus is not primitive, but has been secondarily acquired by a degeneration due to its habit. I fail to see any indications that this is the case. It seems to be the more natural conclusion, and that most in accordance with the facts—more especially since I hope to show (below) that a distinct vascular system and also paired notochords are present—to regard this animal as retaining a primitive Archichordate organisation, always excepting the dorsal flexure of the gut, and the hypertrophy and forward direction of the tentacles, which cannot be regarded as characters of a free-swimming chordate ancestor. This will be further referred to below.

Lang considers the resemblance of Cephalodiscus to Phoronis and the Bryozoa to be convergent. Whilst leaving out of the question at present the latter, I hope to show sufficient anatomical resemblance between Cephalodiscus and the free-swimming Actinotrocha to outweigh such suggestions.

The absence of pharyngeal clefts apparently extends to Rhabdopleura as well as Phoronis, but the value of this feature cannot be gauged till we know more concerning the origin of these organs.

Nervous System.—McIntosh (2) described the main nervous ganglion on the dorsal side of the collar region, and remarked, "It extends for a considerable distance laterally on each side along the basal region whence the plumes

spring, and for some distance on the dorsal surface of the buccal disc." He also noticed that the general arrangement was similar to that of Phoronis. Harmer (1) showed further that nervous branches passed up the dorsal sides of the arms. As will be seen by inspection of the accompanying figures, in which the nervous system is coloured yellow, my sections confirm the previous results, and make further statements possible. The main nerve-ganglion (Pl. 23, fig. 3, *n. g.*) lies over the subneural blood-sinus (*s. n. s.*), which is a hæmal space immediately between the mesodermal wall of the pre-oral body-cavity (epistomial) and those of the collar cavities. In other words, it is in identically the same position as the nerve-ganglion of Actinotrocha. On the walls of the sub-neural sinus arise the inner ends of the "proboscis pores" (or canals) (*p. p.*), which then lead forwards and upwards on either side of the ganglion to the exterior (see Pl. 23, figs. 2 and 3, *p. p.*). This arrangement of proboscis canals, subneural sinus, and ganglion is precisely similar to that of Actino-trocha.

Traced backwards (fig. 4), the main ganglion gives off the thick lateral branches to the arms, whilst further back it continues its course, overlying the thin dorsal blood-sinus between the two collar cavities (figs. 5—8). By the time fig. 9 is reached (cf. Pl. 24, fig. 13) the pharynx, or more possibly the basal part of the subneural gland, comes to lie close under the nervous mass, which then divides into two lateral nerves (fig. 12, *l. n.*). These give off a post-oral ring at the posterior edge of the collar region (Pl. 25, fig. 15), which becomes lost ventrally in a mass of nerve-fibres, scattered over the inner surface of the ectodermal layer of the post-oral lamella (fig. 13). The lateral nerves are then continued as a pair of rather broad nerve-tracts, lying laterally in a position similar to those of Phoronis.

Anteriorly, the ganglion is continued as a mass of fibres along the upper surface of the epistome, in the mid-dorsal line and somewhat to each side of it (figs. 1, 13, 14), round the apex, and then unites with a very broad flat ring which lies at

the base of the long glandular cells, covering the "front" of the epistome.

Laterally and anteriorly, the ganglion gives off a pair of large nerves (Pl. 24, fig. 13, *p. o. n.*) which pass downwards and outwards along the "inner" surface of the epistome, where they unite (fig. 12), and passing round the base, they meet the broad ring.

This arrangement, allowing for the peculiar modification of the epistome, is closely similar to that of Phoronis (Actinotrocha) and Balanoglossus.

Lastly, along the mid-ventral surface of the trunk there are several nerve-fibres running longitudinally, and some of these pass into the "pedicle" in a peculiar manner, where they appear as fine nervous cylinders. The detailed description of these and of the pedicle will be given in future work.

The mid-dorsal and two lateral epistomial branches have their homologues in Balanoglossus and Actinotrocha, whilst the pre-oral nerve-ring of the latter can be compared to the "front" ring of Cephalodiscus, especially when the relationship of both to the cœlomic muscle-strands is considered. One can assume that the condition of the pre-oral nerve-ring of Cephalodiscus has been arrived at by a process of centralisation by which the ring, and with it the origin of the muscles, has been moved into the centre of the disc, for the formation of a sucker.

I do not feel justified in giving a complete diagram of the nervous system of Cephalodiscus until I have confirmed certain points not dealt with here. It has been shown that the lateral trunks of Phoronis may be regarded as the two halves of the dorsal nerve of Balanoglossus, in the trunk region, and that their peculiar position can be accounted for by displacement due to reduplication of the trunk. The same reasoning would apply to the case in hand, the lateral nerve trunks of Cephalodiscus occupying the same position as in Phoronis.

Sense-organs.—The consideration of the curious masses of pigment surrounding the oviducts, which were at first

described as eyes, together with that of the reproductive organs, must be left to a later paper (in which I hope to give an account of the buds and young forms), but a careful examination has led me to believe that Cephalodiscus is provided with no less than a dozen large eyes of a very primitive compound type.

Each plume terminates in a globular enlargement, the appearance of which has been described and figured by McIntosh (3). Many of the cells of this enlargement appear to contain a large clear globule of an ovoid shape. McIntosh (3) remarks, "The rugose appearance, however, is due to large gland-cells containing granules and globules, which are arranged in a somewhat regular manner round a central cavity, and which present a deep yellowish tint in the preparations" (p. 11). If the parts be subjected to partial maceration the clear globules can be obtained free (Pl. 26, figs. 28 and 29), and they remind one irresistibly of a crystalline refractive lens. Two different common shapes are here indicated. In longitudinal section of the bulb (fig. 30) it is seen that the epithelium is single-layered, and consists of elongated tapering cells, with their nuclei mostly situated at the base. All have fine pigment granules scattered throughout their interior, and a great number of them contain the crystalline lenses referred to. With Meyer's carmine and with hæmatoxylin these latter stain readily, one or more areas in the centre staining more deeply than the rest. An axial part of the protoplasm immediately internal to these bodies, and containing the nucleus, usually appears darker than the rest of the protoplasm. The lens may often be observed protruding through the cuticle to the exterior, evidently an abnormal condition.

Fig. 31 gives a view of one of the cells in longitudinal section (unstained). It is bounded externally by a definite but fine cuticle, and its inner end tapers to a fibre-like thread, which I believe to have in some cases traced into the main nerve of the plume.

The whole structure here described seems to indicate that these organs are rudimentary monostichous compound eyes,

which bear a remarkable resemblance, both in appearance and structure, to the "branchial organs" found in the sedentary Annelids, such as Potamilla and Sabella. The figures and description of these organs as given by Andrews (3) (and a comparison of them with these organs of Cephalodiscus) can leave little room for doubt that the functions in each case are similar. It seems most reasonable to regard them tentatively as primitive eyes, though the presence of compound eyes in the Chordata is rather remarkable.

Andrews conducted experiments with Potamilla to determine if the "branchial organs" were possibly phosphorescent organs, but apparently with a "negative result." The presence of hairs (sensory?) in the branchial eyes of some of the Annelida, such as Myxicola and Filigrana, led him to suspect a possible sensory function other than that of vision, but there is no indication of hairs or cilia in the branchial eyes of Cephalodiscus.

Branchial Plumes (Lophophore).—The collar region, as is well known, is produced on each side into six arms or plumes, and each of these has a double row of smaller pinnæ, borne laterally.

The structure of a plume is very similar to that of the rest of the collar region. A layer of epithelial cells rests upon the mesoblastic chondroid tissue (Pl. 26, fig. 26), which encloses a well-defined cavity. Across this are stretched many fine strands of protoplasm, with nuclei dotted here and there in their course. The transverse section of a plume is crescentic in outline, and the dorsal side is convex. The epithelium on this side is thinner than that on the concave ventral surface, and consists of closely set cubical cells; it is continuous with the epithelium of the dorsal collar region. In the mid-dorsal line a flattened nerve runs throughout the length of the plume, terminating distally in the "branchial organ," and joining proximally with the other five to enter the nerve-ganglion. Immediately internal to this nerve (in the plume) runs a main blood-vessel, usually somewhat triangular in transverse section (fig. 26), and continuous with the main dorsal blood-vessel in the collar region.

On the ventral side the ectoderm consists of long glandular cells closely crowded together, and sometimes thrown into folds. They are covered with minute cilia, and are continuous ventrally with the epithelium lining the mouth-cavity.

Ventro-laterally are given off, from each plume, the rows of pinnæ on each side. A cross-section of a pinna is shown in fig. 27. The dorsal and ventral surfaces of the pinna are covered by similar cells respectively to the same two surfaces of the plume, and under the dorsal cells is a crescentic (in transverse section) blood-vessel whose lumen is continuous with that of the plume. The rest of the internal cavity is cœlomic, and is also continuous with that of the plume. A few intersecting strands of mesoderm are to be seen. There does not appear to be any definite nerve in the pinna.

The way in which the six plumes are arranged is peculiar, and although one or two may be, and usually, in sections, are slightly displaced, it is easy to demonstrate the principle upon which they are disposed. Pl. 26, figs. 32—36, are meant to illustrate this, and they are selected from a series of sections cut perpendicular to the long axis of the buccal shield.

The areas occupied by glandular ciliated epithelium, and belonging morphologically to the ventral surface, are indicated by the thicker lines, whilst the thinner lines indicate the morphological dorsal surface with its characteristic non-ciliated epithelium. In fig. 32 the right side shows the base of the united plumes and the post-oral lamella behind them, whilst the left side shows indications of a separation of the two most posterior plumes (5, 6). In fig. 33 these two are seen to be free from the main axis, and very little further up (fig. 34) the next (4) branches off. Higher up still these three arrange themselves in linear series, so that their morphological dorsal surface is opposite to that of the main axis, which has not yet divided into three. Fig. 35 shows the separation of (3), and further up again the last two diverge. Fig. 36 indicates the arrangement some way further towards the tips, and the six plumes are here seen to be arranged in more or less of a circle,

though they still show indications of two rows, an anterior and a posterior.

These sections are all transverse to the epistome, and are not quite parallel to the dorsal surface of the collar. It is probable that in a section in the latter direction the six plumes would diverge from the main branch at about the same level, a conjecture further borne out by the examination of uncut specimens.

Practically the plumes and their pinnæ form a large funnel-shaped framework, with the interior lined by the thinner or atrial epithelium, and the exterior with its twelve grooves running down into the mouth, covered by the glandular and ciliated branchial epithelium.

In Phoronis a precisely analogous arrangement holds, but in this case the tentacles surround the mouth in the mid-ventral line, and are then produced laterally to form a double row, which, bending backwards, forms an imperfect atrium, in which is situated the anus. In this case there is a similar disposition of branchial and atrial epithelium, but, as the tentacles here surround the mouth, the former lines the branchial cavity, with the mouth at its base, whereas the latter lines the less complete atrium. It is evident that if the outer row of tentacles, meeting mid-ventrally in Phoronis, were removed, and the epistome restored to something like its Actinotrochan proportions, the similarity would be striking.

One cannot doubt that in Cephalodiscus the cilia of the ventral surface cause currents down the ventral grooves of the plumes, and thence into the mouth, as has already been suggested, carrying food particles, entangled in slime, into the alimentary canal, a mode of alimentation analogous to that of Phoronis, and indeed of the Urochorda.

Organs of the Mesoderm.

Muscular System.—The muscles are little differentiated, and often consist histologically of simple protoplasmic strands with nuclei. In the pre-oral lobe, or protomere, a great number of these strands cross the cœlomic cavity in a definite radiat-

ing direction (figs. 1, 9, 14),—cf. McIntosh (3). They originate from the thickened chondroid skeleton lying between the posterior cœlomic wall of the epistome and the anterior wall of the collar, and run forwards and outwards to be inserted into the thin chondroid tissue immediately underlying the anterior nerve-ring of the epistome (Pl. 25, fig. 15). Their contraction would result in the drawing inwards of the central part of the epistomial ventral surface; and hence, if this organ were applied to a flat surface, they would convert it into an efficient sucker.

The cœlomic cavity of the collar and plumes is also traversed by a number of protoplasmic filaments which are much finer than those of the epistome; they may or may not be contractile (figs. 15, 16). In all cases they run from one part of the chondroid tissue lining the cœlom to another, and appear indefinite in arrangement. The largest and best defined run from the lateral skeletal bodies to the head of the notochords (fig. 15).

In the trunk there is (contrary to Phoronis and Balanoglossus) a marked absence of muscular tissue, with the exception of the mid-ventral region, in which there are definite muscular bands running from behind the mouth to be inserted into the "ventral sucker" (Pl. 25, fig. 17, *v.m.*), as described by McIntosh. In the ventral sucker itself these muscles show a fibrous structure.

Skeletal System.—Closely underlying the ectodermal cells is found the so-called "basement tissue," which varies greatly in thickness in various parts. A thin layer of this tissue lines the whole cavity of the epistome, and where its mesodermic wall is in contiguity with that of the collar cœlom there is formed on either side (fig. 15) a thick mass of the same tissue, from which long muscular fibres run forwards through the epistomial cœlom and backwards to the front end of the notochords. They have been referred to as the lateral skeletal masses.

The chondroid tissue is also easy to trace in the collar region, —in the post-oral lamella, the plumes and pinnæ, and the

proximal area of the collar. In the trunk it is thinner, though slightly thickened in the ventral sucker. It is only present in connection with the "somatic" part of the mesoderm in contiguity with the ectoderm, except in the case of the gonads and the lateral masses.

At the inner posterior end of the gonads there is a thick wall of this tissue surrounding their tapering extremities. The structure of this skeletal tissue is the same throughout, a hyaline supporting but elastic mass, staining easily. There can be little doubt that it is mesoblastic in origin, considering that parts of it, such as around the gonads and in the lateral skeletal bodies, are far removed from the ectoderm or endoderm, and that the tissue in question is in such intimate connection with the muscular system. In this case it could be homologised with the chondroid tissue of Phoronis, and even with the mesoblastic cartilaginous skeleton of the Vertebrata.

In the regions in which there is no chondroid tissue, such as the trunk mesenteries and mesodermic (splanchnic) layer over the endoderm, the mesoderm appears in the form of a fine protoplasmic layer (Pl. 25, figs. 18, 19, *sh.*), with nuclei here and there. In the parts directly enclosing blood-sinuses there is often a thickening of this layer, due to an accumulation of branched cells, which are probably contractile (see Vascular system). It is possible that a thin layer of mesoderm may line the cœlomic cavities internally to the chondroid tissue, but the protoplasmic filaments have the appearance of actually arising from the substance of the chondroid tissue.

With the exception of the muscle-fibres of the ventral sucker, therefore, the skeleto-muscular system of Cephalodiscus appears to remain in the undifferentiated condition of protoplasmic strands or branched cells and a hyaline skeletal matrix, as in the early stages of other forms, such as Phoronis.

McIntosh (**3**) has suggested that the elasticity of the chondroid tissue ("basement membrane") may compensate for the absence of circular muscles in the ventral sucker and other parts, and, in addition, it evidently imparts a rigidity to the

plumes—and, in the case of the two lateral skeletal masses, forms a fulcrum for the pre-oral muscles. These lateral masses, by their structure, origin, and function, can be homologised with the chondroid tissue of Balanoglossus (Marion).

Vascular System.—There is no prior reference to a vascular system. A system of sinuses, bearing a close similarity to that of Actinotrocha, is capable of easy demonstration. The walls of the sinuses are in all cases formed by the mesoderm of the cœlomic cavities, and in some cases either the ectodermal or endodermal wall. In certain parts, especially in the dorsal vessel, there are thickenings of the walls caused by nucleated mesodermic cells which bear a close likeness to those described in Actinotrocha, and it can hardly be doubted that these are contractile as in the latter. Immediately under the nerve-ganglion lies a large sinus, which, as in Actinotrocha, we may term the subneural sinus. It is very constant in outline (figs. 2 and 3), and has, in transverse section of the epistome, a quadrilateral shape. Its dorsal wall is formed by the nerve-ganglion, and the other three sides of the rectangle (i. e. laterally and ventrally) are formed by the cœlomic wall of the epistomial cavity. Anteriorly it is blind, and posteriorly it tapers off into the mid-dorsal blood-vessel, the greater part of which is filled up by the long cæcal subneural gland (*s. n. g.*) (see below). Its course can be followed in the series of sections from fig. 1 to fig. 9, in which it is tinted red. The dorsal blood-vessel in this region (*d. b. v.*) is bounded laterally by the collar walls, and these are thickened and, as already mentioned, probably contractile.

At the level of fig. 10 this vessel separates into two, which encircle a part of the gut to be referred to later, and again join up beyond this to form a single median vessel (fig. 15, *d. b. v.*). Dorsally to the pharynx this vessel can be traced (fig. 16) till it reaches the stomach, round which it breaks up into a system of sinuses, which, more or less disconnected in the preserved specimens, is probably one large blood-sinus in the living animal. From this sinus branches, with indications

of contractile walls, are given off to the gonads, and there are traces of a ventral vessel which also passes out into the pedicle (fig. 17, *v. b. v.*). There is a hæmal vessel in each pinna of the tentacles, but I have not traced these further (see "Branchial plumes").

Many of these vessels have undoubtedly shrunk in size, and in some cases lost all lumen; so that in the living animal, especially if the blood be tinted, they probably form a very prominent system. The same condition is met with in preserved Actinotrocha, in which the vessels are difficult to trace, though followed with ease in the living larva.

The vascular system here described corresponds, even to minute detail, with that of Actinotrocha and Phoronis.

Subneural Gland.[1]—In his comparison of this species with Balanoglossus, Harmer (1) drew up a list of features in which it closely resembled the latter. Amongst these he compared an anterior diverticulum of the gut to the notochord of Balanoglossus. Whilst confirming the other features he described, and further adding thereto the very similar vascular system, I think that the homology of this organ to the enteropneustan notochord (or to that of any other animal) cannot be maintained. Its origin (from epiblast or hypoblast) cannot be determined till the sexual development is followed, for I have every reason to believe that the entire gut in the young buds is formed from the ectoderm of the parent. It presents no histological features resembling those of every other notochord yet described, and it is difficult to conceive of its performing any supporting function. As already suggested, I propose to homologise it with the subneural gland of Actinotrocha (and in part to the proboscis vesicle of Balanoglossus.[2]

In view, however, of its "notochord" claims, I have worked

[1] Described here to follow 'vascular system,' though of course not mesodermal.

[2] In section the subneural gland consists of a single layer of elongated ciliated cells surrounding a small lumen, and in many cases there may be seen, in the centre of the lumen, a rod of hardened darkly staining mucus.

out its relationships in the adult with some detail. Figs. 1 to 12 are drawings, by camera, of sections selected from a consecutive series, cut nearly transversely to the long axis of the epistome. In the sections in front of fig. 1 the whole interior of the epistome is lined by the cœlomic epithelium of the pre-oral body-cavity (*b. c.* 1). At fig. 1 are cut the tips of the two collar cavities, and in the centre the front end of the subneural sinus. In fig. 2 there is further seen a space in the centre of the subneural sinus, which is continuous with the pre-oral body-cavity (see Pl. 24, fig. 14), and the walls of which are thickened and contractile. In life this space, in all likelihood, only exists when its walls are in a state of contraction. In fig. 3 the tip of the subneural gland is seen, and in fig. 5 this organ is to be noticed embedded in the sinus. The condition seen in figs. 6, 7, and 8 may be found in a number of sections, and probably represents the state of affairs when the walls are collapsed. They all indicate the dorsal blood-vessel, with the subneural gland lying in its lumen, though mostly resting against the ventral cœlomic wall. In fig. 9 the tip of the gut-wall is recognisable, and in Pl. 24, fig. 10, the subneural gland is observed to pass into it. Figs. 11 and 12 illustrate further stages, which are readily understood with the help of fig. 13. I would regard this dorsal diverticulum of the gut which underlies the collar nerve-mass, and into which the subneural gland is continued, as the enlarged base of the gland, and it is at least possible that the stomodæum extends as far as the dotted line in fig. 13.

The relationships of this subneural gland and its structure are so closely identical with those of the similarly named organ in Actinotrocha that a want of definite proof of an epiblastic origin can hardly militate against the homology of the two organs. If further evidence were required, the discovery of other organs which have good claims to be regarded as of notochordal value can be added.[1]

What little light is thrown upon the question by study of

[1] The above description has been checked by sagittal and coronal sections, which, however, it would be superfluous to reproduce.

the buds tends to show that the subneural gland is, from the first, pre-oral in position, and does not move forward from the collar region, like the notochord of Balanoglossus.

Organs of the Endoderm.—There are a conspicuous pair of grooves converging to the mouth (Pl. 24, fig. 12, *o.g.*), which correspond with the oral grooves of Actinotrocha.

The mouth (*m.*) leads into an area which I would consider a stomodæum, giving off its subneural gland, and thence into a pharynx (*ph.*). This is a spacious chamber which extends some way down the body and finally opens through a short œsophagus into the large stomach (*st.*), which, in its turn, passes to the exterior by a broad intestine (*int.*). These parts have all been described in more or less detail elsewhere, and the pharynx alone need here detain us.

Its walls are folded transversely into many sac-like outgrowths which present a somewhat regular arrangement, though they may be largely due to shrinkage (fig. 13), but throughout its whole length extends a pair of lateral vacuolated areas which I would compare to the notochords[1] of Actinotrocha. Though almost lateral in the extreme front end (Pl. 25, fig. 15, *nch.*) they come to occupy a dorso-lateral position at the posterior end (fig. 17, *nch.*), the whole pharynx being in this region, partially divided into ventral and dorsal halves by lateral ridges.

In tracing out the course of one of these notochords, we first observe it in transverse section at the extreme front end of the pharynx, covered by a mesodermic sheath formed mainly by the collar cœlom, and in this sheath originate strong muscular bands which extend forward to be inserted in a thickened mass of chondroid tissue lying between the epistomial and collar cœlomic walls. In the opposite face of this chondroid skeleton are inserted the muscles which are con-

[1] In this case, and in Actinotrocha, the term notochord is, strictly speaking, a misnomer; but, considering Bateson's name for Enteropneusta, we can scarcely adopt the term "hemichord" (Mitsukuri). With those who object to the term notochord, applied to a lateral organ, the name "pleurochord" might find favour.

nected with the central disc of the epistome, and which no doubt subserve the function of attachment by suction. Thus, in an indirect way, the front end of the notochord acts as a fulcrum for the epistomial muscles, and before the evolution of the chondroid tissue it would do so directly.

Lower down (fig. 16, left side of), at the level of the mouth, the notochord is seen as a diverticulum abutting on the nerve-tract and the collar-pore (*c.p.*); whilst a little below this (fig. 16, right side of) the pharyngeal cleft is seen to lead forwards and downwards in front of the lower end of the collar-pore (*c.p.*). This will be referred to later.

With very little change in character the notochord is continued throughout the length of the pharynx, as a vacuolated longitudinal groove, with a wide lumen in continuity with the pharyngeal cavity. Here and there it is folded or thickened, but this is probably an artefact. Fig. 17 shows very nearly the hind end of the pharynx with the notochords. The mid-dorsal pharyngeal wall is thickened, arched into a ridge, and strongly ciliated, whilst in the mid-ventral line a part of the pharynx tends to become constricted off as a spacious ventral groove.

Behind this the notochords gradually lose their vacuolated cells, giving place by degrees to the ordinary cells of the pharynx, and a very few sections after this the pharynx passes into the narrow œsophagus.

In fig. 19 is seen the appearance of the notochord in transverse section. The vacuolisation extends throughout, and the nuclei are seen dotted about, especially at the junctions of the vacuoles.

Amongst those who accept the genetic relationship of Balanoglossus and Cephalodiscus, I do not think there will be any hesitation in regarding these as a pair of notochords (pleurochords), together homologous with the single notochord of the other Chordata. The remarks made concerning the notochords of Actinotrocha apply equally well to these organs, and the homology to each other cannot be doubted, especially as the small differences, such as the presence of a

thin unmetamorphosed layer of protoplasm in the notochord of Actinotrocha, can be traced to the fact that only undeveloped stages are found. The notochord of the young Phoronis most nearly approaches that of Cephalodiscus in structure.

The same supposition as was put forward in the case of Actinotrocha will hold for the comparison of Cephalodiscus and Balanoglossus. It seems likely that in Balanoglossus the two notochords have protruded still further forward into the proboscis and have fused in the middle line, and that the two thickened masses of mesoblastic skeletal tissue (chondroid) in Cephalodiscus have met in the mid-ventral line in Balanoglossus to form the skeletal rod.

Pl. 26, fig. 20, gives a diagrammatic sagittal section of the front end of Cephalodiscus, comparable to those of Balanoglossus and Actinotrocha, previously given. A comparison of the three figures will show the close similarity between the three types.

The only previous observation, so far as I am aware, of the chordoid tissue of Cephalodiscus is in the work of McIntosh (2), in which, referring to the walls of the pharyngeal clefts, which are also of chordoid character, he speaks of "the translucent wall of the slits which seems to be a modified continuation of the pharyngeal mucous membrane. The granules are finer, and the whole tissue is more translucent" (p. 16).

As a matter of fact, the walls of the pharyngeal cleft present identically the same histological structure as those of the notochords, except that the vacuoles are smaller. The chordoid tissue of the notochord is continued directly into that of the pharyngeal cleft (fig. 16), and meets the ectoderm at the distal end of the cleft. In fig. 19 the left-hand end of the notochord is continued into the commencing pharyngeal cleft.

Fig. 18 is a transverse section of the cleft, drawn from a section of the same series as, and a little further out than, fig. 14. Just as the notochords in the pharynx subserve a supporting function, so here the chordoid tissue of the gill-slits evidently has also a similar function analogous to that of the

tracheal thickenings of Insecta and Vertebrata. The maintenance of an open pharyngeal cleft must be an important factor in the well-being of this little animal, or the water currents would be diverted into the stomach.

The existence of this chordoid tissue surrounding the pharyngeal cleft is, I think, very instructive in its bearing upon the evolution of the chordate organism.

Firstly, in regard to the later history of gill-slits in the rest of the Chordata, we note that in every type of vertebrate the gill-slits are supported by branchial bars developed from mesoblast. The mesoblastic cartilaginous skeleton consists of a central axis of cranium and vertebral column, and of an appendicular skeleton of visceral bars. The former are usually regarded as a secondary skeleton, replacing phyletically in great part the primary hypoblastic axis or notochord; but so far as my knowledge extends, there has not been described a primary hypoblastic chordoid basis preceding the branchial bars of the gill-slits. This may be partly accounted for by the earlier evolution of these bars in comparison with the vertebral column.

The chordoid skeleton of the pharyngeal clefts of Cephalodiscus can, however, be interpreted as the primary hypoblastic skeleton of this region, which remains the permanent supporting structure of the pharyngeal cleft in this type, just as the notochord remains as the permanent supporting axial structure of such forms as Amphioxus and the lowest Vertebrata.

In other words, Cephalodiscus has the chordoid forerunner not only of the vertebrate axial, but also of the appendicular skeleton.

In the absence of any ontogenetic evidence, this chordoid condition of the pharyngeal clefts continuous with the true notochordal tissue, renders possible two theories with regard to the evolution of gill-slits (primarily pharyngeal clefts).

In the first place we may assume that, in earlier forms, certain areas of the pharyngeal wall became chordoid for the purpose of support, and that these diverticula came in close contact with the ectoderm, as in the case of the base of the

subneural gland and the notochordal diverticula (fig. 16), and that a breaking-down of the terminal cells gave rise to the clefts. Vacuolisation is certainly a process of cell-degeneration, and disintegration of the vacuoles would be but a step further in the process. On this hypothesis the notochordal diverticula of Actinotrocha might possibly be interpreted as a pair of blind pharyngeal clefts in process of formation.

The objection to this theory is that it implies a discontinuity in function, the pharyngeal clefts being incapable of performing their true function until they were actually formed, an objection which applies equally heavily to the hypothetical formation of a new mouth and a new anus in the Vertebrata, and the annelid derivation of this group.

The other alternative is to suppose that the pharyngeal clefts were already present without chordoid support, and that the lining cells became vacuolated later.

It is in these lowest Chordata that one would expect the chordoid tissue to be diffuse and occurring in any part of the hypoblast where it is specially needed (cf. Actinotrocha). This explanation of the chordoid pharyngeal clefts appears to me to be safe, and to leave out of the question the primary origin of gill-slits.

It is remarkable that there are no gill-slits in Phoronis, though reasons have been given elsewhere for their absence in the adult (see below). In Actinotrocha there are a pair of atrial grooves (see Actinotrocha) which function for the removal of water brought along the oral grooves by ciliary action. These grooves, therefore, have the function which the pharyngeal clefts perform in Cephalodiscus, and it is conceivable that these grooves travelled back on either side in the course of phyletic history of the Chordata, performing their function the more efficiently as their point of exit from the pharynx became carried further backwards. The tissue behind their track would close up, and no trace of the migration of the grooves (now clefts) would remain. There seems to me to be no more difficulty in this process than in the migration of the origin of a blood-vessel from one point to another of the parent vessel.

In ontogeny one would hardly expect to find the exact process repeated, for other structures may have been evolved in the areas lying in the course of the migration, which appear early in the ontogeny, but there are certain facts in the development of gill-slits which may be explained in the light of this hypothesis. Thus the gill-pouches of Tornaria first appear as paired sac-like outgrowths of the œsophagus close to the mouth region, so much so that if they reached to the overlying epiblast immediately on their appearance and perforated it, the gill-slits would open through the lateral wall of the proboscis (cf. Morgan, 'Journal of Morphology,' January, 1894). Later on they shift along with the gut itself posteriorly, so that by the time they reach the epiblast they do so at a spot behind the collar-pores, and in the anterior part of the trunk region. This is the kind of modified repetition we should expect, in ontogeny, of a phyletic migration of apertures from one part to another (cf. formation of new mouth in Antedon and other types).

At any rate tentatively, I would regard the atrial grooves of Actinotrocha as the early rudiments of pharyngeal clefts as found in Cephalodiscus.

The structural condition of the endoderm in the pharyngeal region of Actinotrocha, Cephalodiscus, and Balanoglossus may be compared as follows:—In Pl. 26, fig. 21, is seen a diagrammatic transverse section of the pharynx of Actinotrocha. In this case differentiation of the endoderm has taken place. Two lateral areas are modified into chordoid tissue for supporting function, whilst the ventral ciliated area is more directly connected with alimentation. The two crosses indicate the position homologous to that in which pharyngeal clefts are situated in the other two types.

In Cephalodiscus (fig. 22) the same two lateral chordoid areas are seen, though more approximated in the mid-dorsal line. In addition, the two chordoid pharyngeal clefts open at their ventral border, and below these, in the mid-ventral line, is the special alimentary part or true gut.[1]

[1] The right-hand side is supposed to indicate the condition behind the gill-slits (cf. Actinotrocha, fig. 21).

In Balanoglossus there are very similar structures (fig. 23), but the chordoid structure is no longer formed in the two lateral diverticula of the respiratory portion, nor in the pharyngeal clefts, which have now acquired the function of gill-slits. In the former the chordoid tissue is only formed in the anteriorly situated coalesced part, and in the latter case the chordoid walls have been superseded or forestalled by the cuticular branchial skeleton.

Fig. 24 is a diagrammatic transverse section of the pharynx of a vertebrate, in which the development of the notochord is assumed to resume its primitive order of appearance after the mesoderm has been developed from the endoderm and has taken up its proper position. The two rudiments of the diplochordate condition have fused into one median dorsal notochord, still, however, in continuity with the gut. Below this on either side are the widely open gill-slits, and in the mid-ventral line the ciliated groove, destined to be later reduced into the thyroid gland (Gegenbaur).

In fig. 25 the adult condition of the notochord is reached, in which it has (Eu-chorda) separated from the gut, and becomes an organ entirely distinct therefrom in form and function. In doing so it pushes dorsalwards into the hæmocœle space, and the aorta is thus formed beneath it. (A portion of the pharynx [stomodæum?] of Cephalodiscus in similar manner protrudes dorsalwards, to come in contact with the ectoderm of the dorsal surface, and in doing so divides the dorsal blood-vessel into two lateral vessels [cf. Pl. 24, fig. 12]. An extension of this process would lead them to fuse in the mid-ventral line. See Vascular system.) A further stage could be instanced from the higher Vertebrata in which the thyroid also, under the changed conditions of alimentation in the Gnathostomata, loses its connection with the gut-wall.

We may thus trace the stages from the archichordate pharynx with its walls supported by chordoid tissue, with pharyngeal clefts for escape of the water-current, and with a ventral alimentary portion for the conduction of food particles to the highest Eu-chordate pharynx with the pharyngeal clefts formed

into true gill-slits (branchial) with mesoblastic skeletal bars, the chordoid tissue evolved into a supporting organ for body-muscles, and eventually itself replaced by chondroid vertebral tissue, and the ventral groove, the true alimentary part (gut) in the Archichorda, losing its connection with the gut, and becoming more or less vestigial as the thyroid gland. We can clearly see some of the lines in relation to function along which these changes have proceeded. In the Archichorda the method of food ingestion is, typically, by ciliary action, causing a current of water and minute food particles. The former has to be removed by pharyngeal clefts (Harmer, Brooks), or by atrial grooves, in Actinotrocha, whilst the latter is entangled in currents of slime, and thus retained and passed down the gut for digestion. In Phoronis the same method of ingestion is effected, but the water-current is got rid of by a special adaptation of the epistome, to be described later, before the mixed currents reach the true mouth, so that true pharyngeal clefts should be superfluous ; and not only so, but as the pharynx is an organ especially evolved for the effectual separation of these two currents, it has entirely disappeared in Phoronis, carrying with it its chordoid walls.

The same method of ingestion is pursued in the Urochorda and even the Cephalochorda, though in each of these, as in the Hemichorda, the simple pharyngeal clefts are elaborated into a complicated system of branchial slits.

In the Holochorda the mesoblastic gill-bars are impressed into the service of food ingestion, and the Gnathostomata are evolved. The true jaws and elaborated locomotory system enable more bulky prey to be secured, and the primitive function of pharyngeal clefts, that of removal of the water-current, is no longer existent, so that the more recently acquired branchial function alone warrants their maintenance. A few exceptions are noteworthy. In the herrings and their allies, the diet of Copepoda has given rise to a return of this function, and the gill-slits and gill-rakers are again requisitioned for removal of water-current. Again, in the Cetacea, a return to the pelagic diet involves the elaboration of a system

of whalebone bars, which, in an animal whose ancestors long since lost their pharyngeal clefts, perform the primitive function of these organs.

To this change of diet (made possible by the mesoblastic branchial bars [1]) we may trace the change of function of the pharyngeal clefts, and upon a loss of this later function (branchial) their extinction. On the other hand, the notochord may be traced from chordoid tissue supporting the ingestive pharynx to a fused chordoid rod forming the main skeletal axis until it also is replaced by a mesoblastic skeletal organ.

The mid-ventral alimentary area of the Archichordate pharynx, adapted for the secretion of mucus and the passage of it, together with food-particles, eventually down the gut (cf. endostyles, hypopharyngeal groove), loses its function in the Gnathostomata, and retaining some other function of which little is known, and which is not connected directly with that of alimentation, is no longer in continuity with the gut.

As already mentioned, I propose to include Balanoglossus, Cephalodiscus, and Phoronis together in one group, the Archichorda, dividing this secondarily into two sub-groups:

1. Diplochorda,—Cephalodiscus, and Phoronis.
2. Hemichorda,—Balanoglossus.

Such differences as may hold between these three types can be traced to their differing environment. Their common meeting-ground is in a pelagic ancestor, very nearly represented by Actinotrocha. We may suppose that this ancestor on taking to life on the bottom developed a sucker on the ventral surface, by which it was capable of fixing itself, more or less permanently, to foreign objects. In the case of Phoronis, the sedentary habit became complete, with a consequent loss of pre-oral lobe, notochord, and sense-organs, and an approximation of mouth and anus at the end furthest away from the foreign object. In this particular case, the fixing organ being ventral, the approximation is dorsal. In the case

[1] Other factors in the evolution of the Gnathostomata are the increase in size, made possible by greater correlation of parts and elaboration of the muscular system.

of Cephalodiscus the ventral sucker was also adapted for a fixing organ, but in addition the pre-oral lobe became modified for a like function, and the animal, protecting itself in a spacious house or cœnœcium, is most probably enabled to travel about, somewhat after the manner of a leech, although the budding function of the ventral sucker (or pedicle) points to the fact that it is largely sedentary and fixed by this organ. It is possibly the very intermediate character of this animal's habits, partly sedentary, partly locomotory, that has given it its peculiar structure. In the reduplication of the gut and trunk and the pre-oral position of the branching tentacles are to be seen very marked sedentary characters, whilst the persistence of the epistome and the notochords are to be accounted for by a still functional locomotory capacity.

In the case of Balanoglossus, although the presence of the ventral sucker in the young individual, and of a like organ of attachment on the proboscis (Bateson), indicates a transitory adoption of the distomial mode of progression, a burrowing habit and locomotion in a longitudinal direction seems to have been early adopted, with a loss of the ventral sucker and of tentacles. At the same time, the burrowing habit is correlated with the migration forwards of the notochord, with its connected structures; whilst the elongation of the trunk-region and commencing metamerism are at least made possible by a locomotory habit.

It is possible that the Echinodermata may be descended from a form in many respects not unlike Actinotrocha, in which fixation took place, not by the ventral sucker, but by the pre-oral hood.

Cephalodiscus, isolated in its deep-sea cœnœcium, has therefore been removed from the active arena of life, where fresh organs are evolved and primitive organs are modified, whilst its own peculiar method of progression has saved it from the fate following upon a completely sedentary habit, a fate involving the loss of many important organs, as is shown by the anatomy of its sedentary ally, Phoronis.

The resemblances of Cephalodiscus to Phoronis and to

Actinotrocha will have been noticed throughout this paper, and that upon Actinotrocha. A detailed comparison of the two species could be given, but the same purpose will be served more succinctly by the subjoined list of characters of the Archichorda and its two sub-groups.

Archichorda.

Body composed of three archimeric segments, protomere, mesomeres, and metamere. Ectoderm simple, in great part ciliated and glandular, secretes mucoid exoskeleton (tube, cœnœcium). Nervous system still in connection with the ectoderm, consisting of central dorsal ganglion, pre-oral ring, post-oral ring, dorsal and ventral cords, and, in addition, a more or less diffuse nervous plexus. Mesoderm in four cœlomic pouches, the protocœle and metacœle showing secondary indications of a paired condition. Protocœle opens to exterior, usually by two proboscis-pores; the mesocœles and collar-pores and the metacœles have either paired nephridia, functioning as genital ducts, or closed genital ducts. Muscular system prominent in protocœle (the "animal" organ), and in some a circular and longitudinal layer in the metacœles. A mesodermic skeleton of chondroid tissue—a vascular system of hæmocœle spaces, consisting mainly of subneural sinus (heart) [1] near the dorsal ganglion, dorsal and ventral vessels, and a sinus round the gut. A simple digestive tube, with paired lateral (or early fused into one) notochords, never free from the gut, and one or more pairs of pharyngeal clefts. A subneural gland, opening primarily into stomodæum, gonads confined to metacœles. Metamere bears a ventral organ of attachment, ventral sucker. Habitat burrowing or sedentary.

1. Hemichorda.—Body, especially the metameres, elongated, and the latter showing in gonads and gill-slits traces of true metameric segmentation. Well-developed muscular system in metameres. Ventral sucker present only in young. Notochords fixed and protruding forwards into protomere.

[1] Only in the Eu-chorda, when the branchial gill-slits appear, is the typical ventral heart of the Vertebrata found.

Distal portion of subneural gland detached to form "proboscis-vesicle." Burrowing habitat.

2. Diplochorda.—Mesomeres produced laterally into a number of ciliated branchial tentacles, which in the adult point upwards in front of the mouth, are supported by a chondroid skeleton, and subserve ingestion of food. Metameres reduplicated by a dorsal flexure. Stomodæum with subneural gland still opening to exterior, and extending into the subneural sinus. Paired proboscis-pores near median dorsal line, arising internally along the wall of the subneural sinus. Paired notochords in pharynx, not displaced forwards. A short œsophagus, stomach, and intestine. One pair of pharyngeal clefts may (Cephalodiscus) or may not (Rhabdopleura, Phoronis) be present, with chordoid walls. Ventral sucker forming the organ of attachment throughout life.

(1) Cephalodiscida.—Protomere persistent throughout life as adhesive organ. Twelve pinnate plumes with eyes. Notochords and chordoid gill-slits persistent. Ventral sucker forms budding organ. Habitat, creeping, sedentary, and cœnœcial.

(2) Phoronida.—Loss of protomere, atrial grooves, subneural gland, and notochords in adult. Great development of lophophoral tentacles (unbranched) and of chondroid tissue. Paired nephridial apertures in metameres. Metamere elongated, with circular and longitudinal muscles (as in Balanoglossus). Permanent fixation by ventral sucker. Habitat sedentary and tubicolous. Phoronis.

(3) Rhabdopleurida.—Protomere persistent. No notochord (?)[1] nor pharyngeal clefts (?) in adult. Two pinnate plumes. Attached by hypertrophied ventral sucker. Habitat creeping, tubicolous.

[1] In the light of this interpretation of the organs of Cephalodiscus the "notochord" of Rhabdopleura requires renewed investigation, as it evidently corresponds to the subneural gland of the former. Rhabdopleura should, at one time in its life, have paired pleurochords, as in Cephalodiscus.

List of References.

1. S. F. Harmer.—'"Challenger" Reports,' vol. xx, Appendix to (2).
2. W. C. McIntosh.—'"Challenger" Reports,' vol. xx, on Cephalodiscus dodecalophus.
3. E. A. Andrews.—'Journ. Morph.,' vol. v, 1891.
4. A. Lang.—'Jenaische Zeitschrift,' xxv (1890), pp. 1—12.

KEY TO PLATES 23—26,

Illustrating Mr. A. T. Masterman's paper "On the Diplochorda: Part II. The Structure of Cephalodiscus."

PLATE 23.

Fig. 1.—Transverse section of the epistome of Cephalodiscus dodecalophus, showing only the central area.

Fig. 2.—Ditto, from same series, next section.

Fig. 3.—Ditto, ditto, third section.

Fig. 4.—Ditto, ditto, fourth section.

Fig. 5.—Ditto, ditto, sixth section.

Fig. 6.—Ditto, ditto, ninth section.

Fig. 7.—Ditto, ditto, twelfth section.

Fig. 8.—Ditto, ditto, seventeenth section.

Fig. 9.—Ditto, ditto, eighteenth section.

PLATE 24.

Fig. 10.—Ditto, ditto, nineteenth section.

Fig. 11.—Ditto, ditto, twentieth section.

Fig. 12.—Ditto, ditto, twenty-third section.

Fig. 13.—Sagittal longitudinal section (nearly) to one side of median line to cut notochord, &c.

Fig. 14.—Ditto, of same series as Fig. 13, left side nearly median.

PLATE 25.

Figs. 15, 16, 17.—Nearly transverse sections, in order from before backwards.

Fig. 18.—Transverse section of pharyngeal cleft, from a sagittal section of same series as Figs. 13 and 14.

FIG. 19.—Transverse section of lateral notochord, passing to pharyngeal cleft on the left and pharyngeal wall on the right.

PLATE 26.

FIG. 20.—Diagrammatic median sagittal section of front part of Cephalodiscus.

FIG. 21.—Diagrammatic transverse section of pharynx of Actinotrocha.

FIG. 22.—Ditto of Cephalodiscus.

FIG. 23.—Ditto of Balanoglossus.

FIG. 24.—Diagrammatic transverse section of stage in evolution of Vertebrate (Eu-chordate) pharynx.

FIG. 25.—Ditto of pharynx of Eu-chordata.

FIG. 26.—Transverse section of a plume of Cephalodiscus.

FIG. 27.—Ditto of a pinna of Cephalodiscus.

FIGS. 28, 29.—Refractive globules isolated from the "eyes."

FIG. 30.—Longitudinal section of branchial eye of Cephalodiscus.

FIG. 31.—Ditto of single cell from branchial eye of Cephalodiscus.

FIGS. 32—36.—Serial transverse sections of plumes and collar region.

ABBREVIATIONS.

*b.c.*1. Cœlomic cavity of epistome. *b.c.*3. Trunk cœlomic cavity. *b.s.* Blood-sinus. *c.p.* Collar-pore. *d.b.v.* Dorsal blood-vessel. *d.mes.* Dorsal mesentery. *ep.* Epistome. *gd.* Gonaduct. *g.s.* Gill-slit (pharyngeal cleft). *int.* Intestine. *l.b.v.* Lateral blood-vessel. *l.c.c.* Left collar-cavity (cœlomic). *l.n.* Lateral nerve. *m.* Mouth. *nch.* Notochord. *n.g.* Nerve ganglion. *n.* Nucleus. *o.g.* Oral grooves. *ov.* Ovary. *ph.* Pharynx. *p.o.l.* Post-oral lamella. *p.o.n.* Pre-oral nerve. *r.c.c.* Right collar cavity (cœlomic). *sh.* Sheath of notochord. *st.* Stomach. *s.n.g.* Subneural gland. *s.n.s.* Subneural sinus. *t.* Plume.

Note on a New British Echiuroid Gephyrean, with Remarks on the Genera Thalassema and Hamingia.

By

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With Plates 27 and 28.

On the dredging expeditions of the Liverpool Marine Biology Committee we endeavour as much as possible to try new methods of collecting, or to modify old methods, with the object of securing animals whose special habitat renders them difficult of capture. With this end in view, when dredging last Easter (April, 1896) in the deep depression that runs down between the Isle of Man and Ireland, it occurred to me to try a haul of the large fish-trawl (50-foot beam) which we had on board, but to steam very much more slowly than is customary when trawling, so as to allow the foot-rope to dig deeply into the stiff blue mud which I knew we were on. We went dead slow with plenty of the heavy steel warp out, the result being—as the event proved—that a good deal of the warp lay on the bottom, and so made the pull on the trawl practically a horizontal one. As a consequence of this we must have sliced off the top six inches to a foot, possibly, of the bottom deposit as we went along. When the trawl came up it was half full of the blue mud, and we found it impossible to get the net on board. After steaming round for a little a good deal of the mud was washed out through the meshes,

and the cod-end was then hoisted on board by means of the tackle, and ripped up so as to precipitate upon the deck a seething mass of mud, spawning fish,[1] and Invertebrates, which spread over a considerable area to a depth of about a foot.

While the cod-end was still hanging from the tackle, dripping mud and fish-spawn, I noticed and managed to secure a soft, green-coloured, worm-like object which was protruding from one of the meshes. A careful search through the mud on deck afterwards gave me two other similar green objects—evidently all of them pieces of a large Gephyrean worm. They were still alive when we landed that evening at Port Erin, and were at once transferred to a vessel of fresh sea water in the Biological Station. It was too late to see anything more of them that night, and the next morning they were so feeble that after watching their sluggish movements for a time and making a few sketches, I considered it best to kill them. I put two of the pieces in a 5 per cent. solution of formol with the view of preserving the beautiful green colour, while the third was put in strong spirit for anatomical purposes.

The particulars of the locality where the specimens were obtained are as follows:

Twelve miles south-west of the Chicken Rock, Isle of Man, bottom "reamy" mud, depth 40 to 50 fathoms, beam-trawl, on L.M.B.C. expedition of April 5th, 1896.

Identification.

On the first glance I supposed that the specimens were a species of Thalassema, and I spoke of them as such to Mr. Gamble and the other naturalists who were with me. But the following day, being struck by the difference in appearance from the common British Thalassema Neptuni, and influenced by the striking green colour, and by the observation that the proboscis was not perfect, I thought that they more probably belonged to a Bonellia. So in writing shortly

[1] This locality, on the mud, about March and April, is a very important spawning ground for food fishes. On this occasion we obtained ripe haddock, ling, hake, plaice, and witches, with the eggs running out freely.

afterwards to Canon Norman I told him of the specimens, and stated that I thought they were probably pieces of two or three large Bonellia viridis. On looking at the specimens more carefully, however, and on talking the matter over with Professor Lankester, I came to the conclusion that, from the proportions of the body and proboscis (even supposing the latter to be imperfect at the extremity), it was impossible for the species to be a Bonellia, and that it was much more likely to belong to Thalassema, as I had at first supposed. The detailed anatomical examination which I have since made proves this latter view to be correct. The specimens belong either to a new species of Thalassema, which comes near T. gigas, M. Müller, from the Adriatic, or represent a new genus close to Thalassema in the direction of Hamingia. I incline to the former view (see below, p. 381), and shall describe the species as a Thalassema.

Anatomy.

The three specimens in my possession are all imperfect. They are certainly pieces of two, and possibly of three distinct worms. I believe they make up between them a complete body, or very nearly so; there may be a very short piece in the middle of the body missing, but it cannot be much, or of any anatomical importance. Consequently the restoration shown in fig. 4 must be fairly correct. The dimensions and positions in the body of the fragments are as follows:

A. Anterior half (?) with proboscis (probably incomplete at tip); body about 3 cm., proboscis about 9 cm. in length; preserved in formol (see Pl. 27, fig. 1).

B. Posterior two thirds (?) of the body, measuring about 8 cm. in length; preserved in formol (Pl. 27, fig. 2).

C. Anterior half or more (?) with proboscis (probably complete); body about 8·5 cm., proboscis about 8 cm. in length; preserved in spirit (Pl. 27, fig. 3).

Possibly A and B may be the two halves of the same specimen, and even if the body be longer than I take it to be, B and C together probably represent the whole of it, even if

they do not belong to the same specimen. If the proboscis of A, which seems to have a natural appearance and shape (formol specimen), such as it had when alive, is incomplete at the tip, that of C, which is somewhat distorted (spirit specimen) about the middle, has every appearance of being perfect at its extremity. Consequently I believe that the three fragments represent all parts, and show us the full characters of the species so far as is shown by the adult female (see fig. 4). The description is as follows:

Shape.—The body is elongated and irregularly cylindrical, rather wider anteriorly than posteriorly, with somewhat undulating outline along the sides, and with occasional irregular swellings (see fig. 2). At the anterior end it is rounded, and narrows to join the constricted base of the proboscis (fig. 3). The posterior end is evenly rounded. The proboscis springs from the anterior end of the body by a narrow neck, which rapidly widens, while a slit appears in the median ventral line, which widens to form a shallow groove, and then opens out to become the flattened ventral surface of the greater part of the proboscis (fig. 5). Along its whole length the edges of the proboscis are slightly incurved, so that the ventral surface is somewhat concave and the dorsal somewhat convex. The distal portion may be rolled up (see fig. 1), and when unrolled the tip is excavated in the middle, so as to leave two projecting horns at the sides (fig. 3). In transverse section the proboscis is at its base cylindrical, then becomes a deep groove nearly closed in, then an open groove, and finally a nearly flat plate with incurved edges (figs. 1 and 5).

Surface.—That of the body is tuberculated all over; that of the proboscis is smooth, and when alive of glutinous and slimy appearance.

Colour.—The body is of a beautiful and nearly uniform apple-green,[1] which has the appearance of being quite on the surface of the integument. The proboscis is not so deeply

[1] Described by different experienced observers as "chrome" green and "apple" green. Professor Lankester tells me it is exactly the colour of Hamingia.

coloured, and the colouring is not so uniform, but is rather streaky. It is greener along the edges and paler (pale greyish green, becoming in places almost a dirty white) along the centre.

Size.—The length of the body when complete was probably about 12 cm.; the thickness varies from 5 to 15 mm. The length of the proboscis is fully 9 cm., and its breadth in the flattened part varies from 15 to fully 20 mm. The narrow basal part is about 5 mm. in diameter.

Apertures.—The mouth is placed at the anterior end of the body, in the median ventral line, beneath the base of the proboscis (which is a pre-oral lobe). The cloacal opening is median and posterior (Pl. 27, fig. 6).

The two genital apertures are placed ventrally, one at each side of the middle line, and a little behind the mouth. Each genital aperture has, projecting from the integument beside it, a large curved seta, or genital bristle, of a golden colour (figs. 5 and 13).

Integument and Muscles.—The body-wall is thick, especially at the posterior end. It varies from 1 mm. on the thinnest part of the sides to as much as 4 mm. near the cloacal aperture; and has the usual layers—cuticle, epidermis, connective tissue, three muscle layers, and the lining of the cœlomic cavity. Under the epidermis there is a thick gelatinous dermal layer (Pl. 27, fig. 14, *d.*, in which the green colour is found), which may be up to 3 mm. in thickness. It is this layer which gives rise to the variations in thickness of the body-wall. The pigment is present in the form of accumulations of small rounded masses (fig. 14, *p.*). These may extend to the bases of the epidermal cells, or even up between them, but always belong to the dermis. Inside the gelatinous dermal layer come the muscular layers of the body-wall, two circular layers separated by a longitudinal, which are not broken up into definite regularly arranged bundles as in the case of some Gephyrea, but form a practically continuous layer of muscle-fibres over the whole inner surface, becoming rather thicker at the posterior end around the cloaca. Special muscle bundles run in radial fashion from the wall of the cloaca to the

neighbouring body-wall. Special bundles are also attached to the inner ends of the genital setæ, which project freely for the greater portion of their length into the cœlomic cavity.

Mesenteries containing delicate muscle-fibres project inwards from various points on the body-wall, and sling the loops of the alimentary canal in position (fig. 15). The mesenteries are delicate and silky in appearance, and are much folded and crumpled so as to be wisp-like. This appearance is partly caused by the thickening of the cœlomic epithelium in irregular ridges and masses on the mesenteries (fig. 12). The end of the mesentery which arises from the body-wall is clear, transparent, and membranous, while the end which is attached to the wall of the gut is grey, opaque, and like a wisp of spun silk (figs. 10—12).

Alimentary Canal.—The gut is long and much convoluted (figs. 7—9, 15). It can scarcely be divided into regions. The anteriorly placed mouth leads into a pharynx which cannot be called dilated—one of the points in which this form differs from Hamingia arctica, D. and K. The gut performs several close convolutions in the neighbourhood of the genital setæ, and then stretches backward to the level of the posterior ends of the anterior nephridia (uteri), where it again coils upon itself (fig. 9). It then extends backward once more and enters the posterior much convoluted part of its course, in which it runs almost to the posterior end of the cœlom, and then forms first one and then another loop directed anteriorly before becoming the rectum, which extends down to the cloacal aperture (figs. 15 and 16). The intestine as a whole is not so wide relatively to its length as in the case of Hamingia arctica.

Posterior Nephridia.—These dense tufts of white twigs are placed one at each side of the rectum, and open into the cloaca. Each organ consists of a single, central, thick-walled, opaque brown tube, about 12 mm. in length, which gives off an immense number of delicate, opaque, white-coloured twigs, which interlace with one another so as to give rise to the bush-like appearance (figs. 15—18). These white twigs, however,

do not, so far as I can determine, ever branch again. They are very long, and are closely intertwined in their proximal parts (see figs. 19—21), so as to be difficult to follow; but all those I have teased out seem, like the one shown in fig. 22, to be free from the neighbouring twigs. The appearance of repeated branching seen in figs. 19 and 20 is due to frequent crossing of the twigs. This is a case, then, of what Rietsch[1] calls a simple ramification, in distinction from both the unbranched tube of some other species of Thalassema[2] (e.g. T. Neptuni) and the doubly ramified organ of Bonellia viridis. Rietsch figures and describes the posterior nephridium of Bonellia minor as being simply ramified, but the twigs in that case are not nearly so long nor so numerous as in the present species. In anatomical condition, then, so far as this organ is concerned, the present form lies between Bonellia viridis and B. minor, and is certainly in this one character more like that genus than like the typical Thalassema. The posterior nephridia of Hamingia arctica seem, from the figures and description of Danielssen and Koren,[3] to resemble very closely our form.

Each of the milk-white twigs bears a little bell-shaped funnel or nephrostome at its distal end (figs. 21 and 23). The margin of the funnel has a thickened ciliated rim (fig. 24). This arrangement is very like that of Bonellia minor as figured by Rietsch. The further details of appearance are sufficiently shown by my figures (Pl. 28, figs. 19—24). The nephrostomes, then, are very numerous, and open freely by wide ciliated mouths into the body-cavity.

Anterior Nephridia.—These also are a single pair. They lie one at each side of the alimentary canal ventrally, in the anterior one fourth or so of the body (figs. 7, 8, 25, *n*). They are large, and in both my specimens are distended with

[1] 'Recueil Zool. Suisse,' t. iii, 1886.

[2] T. gigas has nephridia which, from Müller's figure, seem to resemble those of our form.

[3] 'Gephyrea of Norwegian North Atlantic Expedition, 1876–8,' Christiania, 1881.

ripe ova. They have the usual structure of uterine nephridia in the Echiuroidea, and consist of (see fig. 26) (1) a simple external aperture (nephridiopore), (2) a much-coiled slit-like internal opening (nephrostome, *n. s.*), (3) a short wide tube leading to (4) a globular dilatation (*u.*), in which the eggs lie, followed by (5) a long, narrower cæcal tube hanging down posteriorly into the body-cavity. The eggs are chiefly in the globular uterine dilatation (figs. 25, 26, and 31), and are very distinctly seen through the transparent but rather tough walls. The nephrostome is a striking object. The narrow slit is drawn out to a great length to form two horns, which are then each coiled up spirally (as shown in figs. 26—28), to form a structure closely similar to the dorsal tubercle at the opening of the hypophysial gland in a Cynthiid Ascidian. As in the case of the Ascidian dorsal tubercle, however, there seems to be considerable individual variation. My second specimen is not so much drawn out laterally, and not so much coiled (see figs. 29 and 30). The external apertures of the anterior nephridia (genital) are placed close together near the ventral middle line of the body, and each is provided with a strong genital seta, embedded in the body-wall and provided with special muscle bundles. These genital setæ are of a burnished golden appearance where they project to the exterior. The shape is shown in fig. 13, and the entire length of the seta is 5·5 mm. The inner end, which projects into the body-cavity and has muscle bundles attached to it, is wider, softer, and of a white colour.

Gonad, &c.—Both my specimens were females. The ovary in each case was distinctly visible, running along the upper surface of the posterior part of the nerve-cord and ventral vessel (figs. 17 and 18, *ov.*). A few ova were found floating freely in the cœlomic cavity, and, as has been noted above, the anterior nephridia or uteri contained large numbers of ova. The globular dilatation was in one specimen packed full (fig. 31), and in that case about 120 ova were visible on the one side of the vesicle. The ovarian ovum not quite ripe has a central more granular and opaque part, in which the germinal

vesicle and spot are distinctly visible (fig. 33), and a peripheral clearer zone of considerable width. The ripe ova from the uterus (fig. 32) are more opaque, and are almost filled with yellow granules, the germinal vesicle being no longer visible, and the clear peripheral zone very narrow (fig. 34). I was able by slight pressure to squeeze a line of ova out from the uterus through the nephridiopore, as shown in fig. 26.

Nothing specially noteworthy was noticed in connection with the blood-vessels, the nerve system, and other organs.

No coloured corpuscles were found in the cœlomic fluid, and no rudimentary males were seen. These and other morphological points are more fully discussed below in determining the systematic position (see p. 378).

The Green Pigment.

One of the most remarkable and interesting points about this worm is certainly its beautiful green pigmentation. When alive the colour was a well-marked apple-green, and the specimens preserved in formol have retained a good deal of the colour, although the fluid has also become coloured green. The specimen put in spirit has lost its colour altogether, and the spirit has not acquired any green tint; while in the formol, on the other hand, the fluid has become coloured, and the specimens have retained their green appearance. I have used the green formol solution for an examination of the spectroscopic characters of the pigment, and my friends Professor Sherrington and Dr. Noël Paton have kindly investigated samples for me, and have given me their results. I am indebted to these physiologists and their assistants for the information that follows. Professor Sherrington states:

"The dilute formol in which the Thalassema has been lying was clear, but tinted a pale greenish blue,—in fact, a "sea" colour. Examined in a layer 20 centimetres deep before a Hilger single flint-prism spectroscope illuminated by a Welsbach incandescent gas lamp, the following localisation of absorption was obtained (see Pl. 28, fig. 35). From the violet end up to nearly as far as the solar line F, from the red end up to the solar *a*, between the solar lines C and D a single very definite broad band of shadow. By diluting the fluid or diminishing the thickness of the layer examined until the band between

C and D just disappeared, the absorption at the ends was not greatly diminished. On again increasing the thickness of the layer the point of first appearance of the single band was rather nearer to D than C, i. e. at λ 617.

"The measurements obtained were as follows:

Stronger solution, absorption from violet end to λ 468.
absorption from red end to λ 716.
absorption between λ 630 and λ 602.

Solution just too weak to give any absorption between C and D gave
absorption from violet end to λ 428.
absorption from red end to λ 725.

"The colour was not affected by weak reducing or weak oxidising agents, and no evidence has been obtained indicating that the pigment is of respiratory function. It is not easily bleached; in formol solution exposure at a south window for nine weeks has made no perceptible difference to its depth of tint, as compared with a similar tube-full preserved during that period in a dark cupboard. The localisation of the absorption points to the pigment being one not hitherto met with, at least not hitherto recorded."

Later Professor Sherrington added:

"Hæmoglobin in formol solution exhibits the spectrum of reduced hæmoglobin. There is no similarity between the spectrum of the pigment here examined and that of hæmoglobin. On the other hand, the position of the band recalls that of the strong band given by 'bonellein,' λ 643 to λ 617 (Sorby). But bonellein was not examined in formol solution. No other definite absorption band was given by the Thalassema pigment in formol. The substance is not a respiratory pigment. The spectral band-shadow suggests alliance with bonellein. . . . . Of course there is no question of the identity of this with bonellein; the only thing the spectral map does tell us is that this pigment cannot be the same as bonellein, unless—which is very unlikely—bonellein has a single band in formol solution, or this a multiple shadow in CS_2 or C_5H_6O."

Dr. Noël Paton writes to me:

"Dr. Milne Murray and I have examined the solution after evaporating to about one half and placing it in a 3-inch tube. We find a single band with ill-defined edges in the red, with its centre at λ 640. It was impossible to fix the exact position of the edges. There is very little absorption of the red end of the spectrum, which can be seen up to λ 790; but there is considerable absorption of the violet end—up to about λ 496. I shall send the fluid back to you, unless you would like me to ask Miss Newbigin, who is working at the pigments of Invertebrates, to see if it has any of the characters of a lipochrome."

I asked Dr. Noël Paton to hand the sample of fluid over to Miss Newbigin, who has since sent me the following report:

"Report on Green Pigment of Thalassema.

"The pigment was received in the form of a solution in formalin. The solution was a dull green colour, and did not display any fluorescence, as do solutions of bonellein or chlorophyll.

"Action of Heat.—The solution underwent no change on boiling. When evaporated to dryness in a water-bath a dull green pigment was left behind.

"Action of Ether and Alcohol.—When shaken in a separation funnel with ether, the ether did not remove any pigment from the solution. The addition of alcohol to the solution produced a green stringy precipitate, soluble in water, slightly soluble in excess of alcohol. The pigment obtained by the evaporation of the formalin extract is soluble in alcohol.

"Action of Acids.—Acetic, hydrochloric, and nitric acids produced no change. This is in marked contrast to the reactions given by bonellein to acids. According to Krukenberg ('Vergleich. Physiol. Studien,' II^{te} Reihe, II^{te} Abtheil., pp. 70—80), that pigment in alcoholic solution turns violet with strong acids, whether organic or inorganic; and on the further addition of acid turns blue, both solutions having definite spectroscopic characters. The green colour was restored on the addition of alkali.

"If the green Thalassema pigment be boiled with concentrated nitric acid, the solution turns first yellow, and then evolves nitrous fumes and becomes a clear green.

"Action of Alkalies.—Alkaline solutions, such as caustic soda and ammonia, in large part precipitated the green pigment as a green stringy mass. The precipitate was insoluble in excess of alkali, and only slightly soluble in water or methylated spirit.

"When this methylated spirit solution was evaporated to dryness it left a yellowish rather than a green residue. When this residue was treated with strong nitric acid the acid became yellow, and then nitrous fumes were evolved and the pigment became green. The residue obtained by evaporating the formalin solution to dryness did not give this reaction so readily.

"H_2S seemed to have no effect on the pigment.

"Conclusion.—If the absence of the red fluorescence, and of the reaction with acids described by Krukenberg, are to be relied upon, then this green pigment is not bonellein. Again, the absence of fluorescence, the absence of an associated lipochrome, and the colour are evidence against the supposition that it is chlorophyll. The only reaction given by the pigment which is at all distinctive is the vivid green coloration with concentrated nitric acid. This is a reaction given apparently by all of a little known series of pigments, forming the hepatochromes of Krukenberg and the enterochlorophylls of MacMunn, which occur in the livers and digestive glands of many Invertebrates, and are either of a green or a yellow colour. The green does not give the reaction with nitric acid so well as the yellow, but seems to be readily convertible into the yellow. According to Krukenberg, there is no evidence

that they are really of the nature of chlorophyll. They are apparently useless substances, eliminated either by the gut or by the skin, or sometimes by both.
"M. T. NEWBIGIN."

Professor Sherrington and Dr. Noël Paton, as is shown above, differ somewhat in their localisation of the absorption band, but that may possibly be due to a change caused by the evaporation, to about one half, of the formol solution.

The result appears to be that this substance, which may be appropriately named "Thalassemin," is a remarkable and apparently unknown pigment, which is not allied to hæmoglobin nor to chlorophyll. It is not a respiratory pigment, and is apparently nearer to "bonellein," described by Dr. Sorby in 1875,[1] from the Gephyrean Bonellia viridis, than to any other known pigment; but differs markedly from bonellein in several respects, besides having a single in place of a multiple band, and so cannot be identical with that substance. Possibly thalassemin is more closely related to the green pigment of Hamingia.

The chart showing the spectral characters drawn up by Professor Sherrington is given in Pl. 28, fig. 35. I may add that this pigment is so intense that even in the case of the specimen from which the formol solution referred to above had been extracted, the green colour is still distinctly visible in the pigment masses of the dermis in thin sections (about 8 μ) under Zeiss's $\frac{1}{12}$ oil immersion.

Systematic Position.

In 1883 Professor Lankester gave an account of some specimens of Hamingia arctica, Kor. and Dan., from the Hardanger Fjord, and discussed fully the relations of the genus Hamingia to Thalassema and Bonellia. He considered Horst's H. glacialis to be the same as Koren and Danielssen's H. arctica; and from the additional specimens he and Canon Norman dredged in the south of Norway he was able to add considerably to our knowledge of the structure of this form, and of the differences between it and the

[1] 'Quart. Journ. Micros. Sci.,' N. S., vol. xv, p. 166.

neighbouring genera Thalassema and Bonellia. He discovered and figured the minute worm-like male living as a parasite in the dilated pharynx of the female Hamingia. Lankester regards Hamingia as having a closer resemblance to Bonellia in internal organs, and to Thalassema in external characters; while it is quite peculiar in having the genital pores on prominent papillæ, and in the absence of genital setæ in the female. He gives a useful tabular statement of the characters of the three genera, which I shall make use of in discussing the position of our new form.

The specimens I am describing from the Irish Sea are intermediate in their characters between the genera Thalassema and Hamingia as defined by Lankester, but in my opinion they come nearer to Thalassema—so near, in fact, that I think they must constitute a species of that genus. I shall take up the characters seriatim as given by Lankester in his tabular statement.[1]

1. In shape of body the new form agrees equally well with Hamingia (since Lankester showed that that form possessed a large proboscis, as long as the body) and with various species of Thalassema. It perhaps most nearly resembles in size and shape the T. gigas of M. Müller, from the Adriatic.

2. The proboscis agrees equally with the characters of the two genera.

3. The female genital pores in Hamingia are one or two in number, and open on well-marked papillæ. In Thalassema (according to Lankester) the female genital pores are four to six, and are not placed on papillæ. In our new form there are no papillæ, but only two pores are present. In this character, then, our form is intermediate between the two genera. In Thalassema gigas and T. faex, however, there is said to be only one pair of "segmental organs" (see Greef, Selenka, and K. Lampert), and therefore, I take it, only one pair of female apertures. I should alter, then, this character[2]

[1] 'Ann. and Mag. N. H.,' vol. xi, 1883, p. 42.

[2] This extension of the diagnosis is recognised by Rietsch, Selenka, and others.

of the genus Thalassema, as given by Lankester, to read, "Uteri (enlarged nephridia) and female genital pores from one to six pairs, not opening on papillæ." That would admit our new species.

4. In Hamingia the male is a minute parasite on the female, as in Bonellia. In Thalassema (so far as is known) the males and females are alike in size and appearance. The three pieces of our new form all belong to mature females, and after a careful search in uteri, pharynx, and proboscis, I am unable to find any minute males. This character, then, does not help us. Minute males or full-sized males may yet be found.

5. A pair of strong genital setæ are present in our species, and in this respect it agrees with Thalassema, and differs from Hamingia.

6. The ova are not enclosed in follicle cells. This is in agreement with other species of Thalassema.

7. There are no distinct "zones" in the mature ovum, such as are present in Hamingia.

8. The uterine pouches when distended have hyaline, transparent walls, but still they are firm and resistent. In this respect our form seems to combine the characters of Hamingia and Thalassema, as given by Lankester.

9. The internal opening of the uterine pouch (nephridium) is drawn out into a spiral trough, as in Thalassema.

10. The anterior part of the pharynx is not dilated, thus agreeing with Thalassema.

11. The cloacal nephridia are densely covered with long intertwined branches, so as to look like little bushes, and the nephrostomes are on the ends of the twigs. In this respect our form comes nearer to Hamingia and Bonellia, but does not exactly agree with either genus as previously described.

12. No red-coloured corpuscles were found in the cœlomic fluid. The fluid was slightly milky in colour. In the absence of hæmoglobin our form differs from Hamingia, and agrees with at any rate some species of Thalassema.

To sum up: our new species agrees with Hamingia in

having branched cloacal nephridia; while in all other respects it either agrees with or comes nearer to Thalassema.

Professor Lankester, after seeing one of my specimens and hearing the details of structure as given above, has suggested that I should describe this form as a new genus intermediate between Hamingia and Thalassema, and forming a term in the series: Echiurus, Thalassema, the new form, Hamingia, Bonellia. Professor Lankester's opinion must always carry great weight, especially in regard to this group which he had already done so much to elucidate; but in the present matter I am inclined to think, from the consideration of the characters given above, that my new form is distinctly nearer to Thalassema than to Hamingia, and may without any violence be included in the former genus. Consequently I prefer to describe it as a new species of Thalassema, related to T. gigas, M. Müller, rather than to form an independent genus for its reception. I desire to associate Professor Lankester's name with this species. It seems appropriate, as he has written on the two genera to which this form is related. The specific diagnosis will run as follows:—

Thalassema Lankesteri, n. sp.:

Length about 20 cm. Proboscis nearly as long as trunk, and in most of its extent wider. Tip of proboscis truncated and slightly indented. Surface evenly tuberculated all over. Colour apple-green on the trunk; paler on proboscis. Longitudinal musculature not divided into bundles. A single pair of anterior nephridia; nephrostomes spirally twisted. Cloacal nephridia branched, with numerous ciliated funnels on the ends of the branches. Females alone known. Off Isle of Man, fifty fathoms.

As a species, T. Lankesteri is undoubtedly distinct from the previously described species of Thalassema. Apart from the anatomical peculiarities noted above, the external characters sufficiently distinguish the species. According to Lampert's and Rietsch's synoptic tables our form would be grouped, from the characters of the body musculature and the

nephridia, along with T. gigas,[1] M. Müll., taken off Trieste, from which it may be distinguished by the proportions and shape of the proboscis, which is much wider compared with the body than in T. gigas, and is not trilobed at the tip.

The other species which have been described as being more or less of a green colour are—

(1) T. Baronii, Greef, from the Canaries; but there the proboscis is relatively much smaller, and, moreover, there are important anatomical differences. T. Baronii has two pairs of anterior nephridia, and a different arrangement of muscles.

(2) T. Moebii, Greef, from Mauritius, with three pairs of anterior nephridia.

(3) T. viridis, Verrill, off north-east coast of America, with small (6 mm.) swollen body and long slender proboscis.

The remaining species of the genus which have been found in the North Atlantic or Mediterranean are T. Neptuni, Gaertner (British), T. Frohmanni, Diesing (Sicily), and T. faex, Selenka (between Scotland and the Faroe Islands). The first of these has two pairs of anterior nephridia; the last agrees with our species in having only a single pair, but differs totally in the structure of the cloacal nephridia, as well as in colour, shape, and appearance generally; while T. Frohmanni, from the Mediterranean, is insufficiently known, and may be the same as T. Neptuni. However that may be, T. Frohmanni cannot be confused with T. Lankesteri; the short description given by Diesing is sufficient to show that our form differs from his both in colour and shape. As a matter of fact, although several of the above-named species have been referred to as more or less green, none of them, judging from the coloured plates given by Greef and others, are so intensely and completely green as our specimens. T. Lankesteri in its coloration is probably more like Hamingia arctica than any other known form.

[1] 'Observationes anatomicæ de Vermibus quibusdam maritimis, Berlini.' T. gigas was originally found at Trieste, and is still taken there. I believe it has not been found elsewhere; and, as in the case of T. Lankesteri, only females are known.

EXPLANATION OF PLATES 27 & 28,

Illustrating Professor W. A. Herdman's paper on "A New British Echiuroid, Thalassema Lankesteri, n. sp. (Herdman)."

Reference Letters.

cl. Cloacal aperture. *c.* Cuticle. *c. n.* Cloacal nephridia. *ep.* Epidermis. *d.* Dermal layer of skin. *f.* Ciliated funnels of posterior nephridia. *int.* Intestine. *l. n.* Left anterior nephridium. *m.* Mouth. *mes.* Mesentery. *musc.* Muscular layer of body-wall. *n.* Anterior nephridium. *n. c.* Ventral nerve-cord. *n. s.* Nephrostome. *œs.* Œsophagus. *ov.* Ovary. *p.* Groups of rounded pigment masses of a green colour in the dermal layer of skin. *p. l.* Pre-oral lobe or "proboscis." *r. n.* Right anterior nephridium. *r.* Rectum. *s.* Genital seta. *u.* Uterine part of nephridium. *v. v.* Ventral blood-vessel. *w.* Body-wall.

PLATE 27.

Thalassema Lankesteri, n. sp. (Herdman).

Fig. 1.—Specimen A. Natural size, from left dorsal side.

Fig. 2.—Specimen B. Natural size, showing the green colour.

Fig. 3.—Specimen C. Natural size, from ventral side.

Fig. 4.—Restoration, to show probable appearance of the species.

Fig. 5.—Ventral view of anterior end of Specimen A, to show base of proboscis and genital setæ.

Fig. 6.—Posterior end and cloacal aperture, to show papillæ on surface. × 2.

Fig. 7.—Anterior end of specimen, dissected from right side, to show alimentary canal and other organs.

Fig. 8.—Anterior part of alimentary canal, with anterior nephridia, &c., isolated, from ventral surface.

Fig. 9.—Diagram of course of anterior part of alimentary canal.

Fig. 10.—A few convolutions of alimentary canal, to show the silky mesenteries.

Fig. 11.—Piece of a mesentery, enlarged.

Fig. 12.—Small part of same mesentery, magnified (× 50).

Fig. 13.—One of the genital setæ, enlarged.

Fig. 14.—Part of a section through the skin, to show the position of the pigment masses (*p.*) which give rise to the green colour (× 300).

PLATE 28.

FIG. 15.—Dissection to show posterior part of alimentary canal.

FIG. 16.—Dissection to show cloaca and posterior nephridia.

FIG. 17.—Rectum reflected to show posterior nephridia opening into cloaca.

FIG. 18.—Posterior nephridium and ovary.

FIG. 19.—Posterior nephridium isolated and enlarged.

FIG. 20.—Same cut across, enlarged.

FIG. 21.—Small portion of same teased and mounted (× 50).

FIG. 22.—Single branch bearing ciliated funnel, teased out and isolated (× 100).

FIG. 23.—Two of the ciliated funnels.

FIG. 24.—Single ciliated funnel, highly magnified (× 300).

FIG. 25.—Dissection from right dorsal side, to show pair of anterior nephridia in situ.

FIG. 26.—Single anterior nephridium, enlarged.

FIG. 27.—Vesicle and nephrostome from one end.

FIG. 28.—Same nephrostome from the side (× 50).

FIG. 29.—Vesicle and nephrostome of another specimen (× 50).

FIG. 30.—Same nephrostome from the other side.

FIG. 31.—Vesicle (uterus) of anterior nephridium, packed full of ripe ova.

FIG. 32.—Ova from uterus.

FIG. 33.—Immature ovum from ovary.

FIG. 34.—Ripe ovum.

FIG. 35.—Chart of the spectrum of the green pigment ("Thalassemin") of Thalassema Lankesteri (kindly drawn up for me by Professor Sherrington).

The Placentation of Perameles.

(Contributions to the Embryology of the Marsupialia—I.)

By

Jas. P. Hill,
Demonstrator of Biology in the University of Sydney, N.S.W.

With Plates 29—33.

Introduction.

In a preliminary note (1) communicated to the Linnean Society of New South Wales I recorded the occurrence of an allantoic placenta in Perameles obesula, and gave a short account of its structure based on an examination of a single stage, at that time the only one in my possession. Since then, the acquisition of certain important earlier and later stages has enabled me to study the placentation of Perameles in some detail, and the results of this investigation are presented in the following pages. I am now able not only to amplify the short statement of the preliminary note, but to give a fairly connected account of what I believe are the most important phenomena in the evolution of the Perameles placenta. I am also now able to state that an allantoic placenta in all respects similar to that of P. obesula occurs in the closely related P. nasuta. The stages described consecutively in the following pages have been derived from these two species indifferently, just as pregnant individuals chanced to come to hand; but so far as I have been able to make out, there are no recognisable differences in the details

of the development in the two forms. Even now, after nearly two years' collecting, the material at my disposal is by no means large. It comprises altogether six stages, of which two are post-partum. I take this opportunity of acknowledging my great indebtedness to the following gentlemen for most generous aid in the by no means easy task of securing the material on which this paper is based:—Messrs. J. B. Cooper, A. G. Hamilton, A. M. Lea, Thomas Steel, and Dr. A. E. Mills. To all these gentlemen I tender my sincere thanks.

To my friend Professor J. T. Wilson I am under a very deep debt of gratitude for invaluable help in the way of suggestion, criticism, and advice during the whole course of my work. I am also indebted to my friend Professor C. J. Martin for much kind advice.

I have further to thank Mr. R. Grant, of the Physiological Laboratory, for very great help in photography, and for assistance in other ways.

Lastly, I desire to thank my honoured chief, Professor W. A. Haswell, F.R.S., for his uniform kindness and consideration, and for much kindly interest in my work.

Methods.—As fixing fluids picro-sulphuric and picro-nitric acids were used. Most satisfactory staining results were got by staining sections fixed on the slide by Mann's albumen method, first with a weak watery solution of Renaut's hæmatoxylic glycerine for eighteen to twenty-four hours, followed by an alcoholic solution of eosin. Even better results were obtained by the substitution of hæmatëin for hæmatoxylin in Renaut's formula. By this method of double staining the fœtal and maternal vessels can be beautifully differentiated.

GENERAL SUMMARY OF RESULTS.

Before entering upon the detailed description of the various stages, it may conduce to clearness if a brief résumé of the main facts of placental development be here presented.

I. Changes in the Uterine Wall.

The mucosa undergoes marked hypertrophy over its whole extent; the uterine glands increase both in transverse diameter and in length; the interglandular connective tissue forms a loose open network of anastomosing cells, and becomes permeated by abundant lymph; the vessels of the mucosa increase greatly in size and in number. These changes in the corium are accompanied by the transformation of the whole of the uterine epithelium into a vascular syncytium. This is ushered in by the disappearance of the cell outlines between the cells and the active proliferation of the nuclei. The uniform nucleated syncytial layer thus produced increases in thickness by the growth of the protoplasm; the nuclei also increase in number, and eventually become, in greater part, grouped together in nests, situated in lobular projections of the deeper surface of the syncytium. At the same time maternal capillaries pass up between the syncytial lobules, penetrate the syncytial protoplasm, and form a network on and just beneath its surface.

The uterine wall is now prepared for the attachment of the embryo.

II. Embryonic Changes.

(a) Fixation of the Embryo.—(1) The embryo becomes attached to the maternal syncytium by means of the enlarged ectoderm cells over the discoidal area of true chorion with which the allantois fuses. The ectoderm, when the attachment is complete, consists of a single layer of greatly enlarged cells, roughly cubical or columnar in shape. Their irregular outer ends accurately fit into the irregularities of the surface

of the syncytium, and are firmly adherent thereto. In correlation with this close adherence of the chorionic ectoderm, this area of the uterine syncytium is markedly thicker than the remainder, and forms the allantoic placental area. In the allanto-chorionic mesenchyme, and in close relation to the inner surface of the chorionic ectoderm, run the allantoic capillaries. (2) Outside the discoidal allanto-chorionic area a somewhat annular zone of the yolk-sac wall is also brought into intimate relation with the maternal syncytium around the above-mentioned allantoic placental area, by means of a close approximation of its exceedingly thin ectodermal cells. This annular zone of yolk-sac wall corresponds to the embryonic vascular area, and at this stage the portion of the syncytium in relation to it is more highly vascular than the allantoic placental syncytium itself. This structural arrangement can hardly be considered other than a yolk-sac placental formation, functional at a time when the allantoic placenta is yet only being formed.

(B) Formation of the Functional Allantoic Placenta. —This is brought to pass through the gradual degeneration and resorption of the enlarged chorionic ectoderm cells over the placental area proper. These cells thus take no further part in placental formation. The allantoic capillaries can now directly reach the vascular surface of the allantoic placental syncytium, to which they become intimately attached, dipping down into the depressions in its surface, and forming in places a regular interlocking system. The fœtal and maternal blood-streams are now only separated by their thin endothelial walls, and perhaps a thin layer of syncytial protoplasm.

III. Parturition.

At birth not only is there no loss of maternal tissue (i.e. no decidua is formed), but the vesicular portion of the allantois remains persistently attached to the placental syncytium, and is gradually absorbed *in situ* along with the latter through the agency of maternal leucocytes.

The fœtus, whilst still connected with the placental area by

the lengthened allantoic stalk, passes to the exterior, not by way of the lateral vaginal canals, but by breaking through along a median track leading backwards from a posterior common portion of the two uteri.

Description of Stages.

Structure of the Non-pregnant Uterus.

The uterine wall is shown in transverse section in fig. 1. Externally is the fairly thick serosa (*s.*) continued on from the ligamentum latum. Internal to the serosa is the muscularis, composed of circularly running non-striate fibres (*c. m.*). The mucosa (*m.*) follows immediately on the muscularis, and is on the whole sharply marked off from the latter; occasionally, however, the terminal ends of the uterine glands may penetrate into the muscularis.

The mucosa varies considerably in thickness in different uteri, averaging about ·75 mm. Its free surface is thrown into irregular longitudinal folds. The matrix of the mucosa consists of fairly compact retiform connective tissue (*c. t.*), in which are embedded the uterine glands and blood-vessels.

The uterine glands (*gl.*) are very numerous, straight or greatly convoluted tubules, averaging ·045 mm. in diameter. They are lined by a low columnar epithelium, outside of which is a thin tunica propria derived from the surrounding connective tissue. They open freely into the uterine lumen.

The blood-vessels enter the mucosa from the circular muscularis. The majority of the superficial vessels of the mucosa are of the nature of capillaries, with only an adventitious layer of connective tissue surrounding the endothelium; in the deeper portions of the mucosa, however, vessels with distinct muscular walls also occur.

The lining epithelium of the uterus (*ep.*) consists of a layer of low columnar cells with a thickness of about ·015 mm., and continuous with the lining epithelium of the glands at the gland openings.

Stage A.—P. nasuta.

Both uteri were somewhat enlarged, and presented a congested appearance. In the right uterus an early blastocyst was found.

The wall of the blastocyst was separated on one side from the enclosing membrane (Selenka's "granulosa membran" [2], Caldwell's shell membrane [3]) by a space, and had a transverse diameter of ·525 mm.; while, including the investing membrane, the whole blastocyst had a diameter of ·675 mm. This blastocyst has not yet been examined in sections, but it probably nearly corresponds to a ten hours' blastocyst of Didelphys, which, according to Selenka (2), has a transverse diameter of about ·5 mm.

Microscopical examination of the right uterine wall shows that as a whole it has increased considerably in thickness as compared with the non-pregnant uterus. This increase is mainly due to the enlargement of the mucosa, which now averages 1·5 mm. in thickness.

The uterine glands are closely packed together, causing the interglandular connective tissue to appear greatly reduced. They have increased both in length and in transverse diameter, the latter now averaging ·075 mm., and their epithelial lining has undergone marked proliferation. It now consists of a high cylindrical epithelium with numerous small deeply staining nuclei basally situated. The meshes of the connective-tissue network are occupied by lymph coagulum, and numbers of somewhat enlarged capillaries are also present, but as yet in no great abundance.

The most important change in this uterus, however, concerns the lining uterine epithelium. Through the disappearance of the cell outlines between the cells, it has become transformed into a continuous protoplasmic layer or syncytium all over the surface of the mucosa (fig. 2, *syn.*), and at the same time it has increased somewhat in thickness, now measuring ·025 mm.

Along with this fusion of the cell bodies, the nuclei have

undergone active proliferation. They now form an irregular band occupying the mid region of the syncytium, and are so numerous as to frequently overlap even in very thin sections (fig. 2). They vary considerably in shape, mostly ovalish or elongated, and are evidently in a most active phase. Though I have not been able to make out undoubted mitotic figures in my preparations, there can be no doubt that marked proliferation of the syncytial nuclei has taken place.

Minot's description (4) of the early changes taking place in the uterine epithelium of the rabbit prior to its complete degeneration is equally applicable to Perameles. He says "the thickening [of the uterine epithelium] is due to the enlargement and fusion of the epithelial cells, and this enlargement of the cells is due to the proliferation of the nuclei, and to the growth of the protoplasm which begins later, and continues longer (as later stages show) than the multiplication of the nuclei." It may be pointed out, however, that the agreement in the two cases goes no further than the earliest stages. As we know from the researches of Minot (4), Duval (5), and others in the rabbit, this nucleated protoplasmic layer formed from the uterine epithelium soon degenerates and disappears; in Perameles, on the other hand, as will be abundantly evident further on in this paper, the syncytial layer derived from the uterine epithelium not only does not degenerate, but, increasing in size and becoming vascularised by maternal vessels, persists throughout the whole period of pregnancy, and takes a most essential part in placental formation.

Stage B.—P. obesula.

The left uterus was somewhat larger than the right, measuring 17 mm. in length by 11 mm. in breadth. It contained two blastodermic vesicles, with the "granulosa membran" of Selenka still in greater part persistent round them. The embryo measures about 7 mm. in length, and possesses at least fifteen mesodermal somites. It is characterised as follows:— Anterior end strongly flexed and enclosed in the large proamnion; medullary plate in anterior cerebral region still

unclosed, but just closed in trunk region; distinct sinus rhomboidalis enclosing primitive streak; fore-limb buds; median heart anlage; blood circulating; distinct auditory grooves.

Both uteri were examined microscopically, and were found to have undergone exactly the same changes. It may here be noted that such changes as have occurred are not limited to any special region of the mucosa, but occur uniformly all over it.

The general appearance of the uterine wall under a low power is shown in fig. 3. Owing to the enlargement of the uterus as a whole, the serosa and muscularis appear to be somewhat thinner than in the preceding stage. The mucosa is approximately of the same thickness as in that stage, but has altered considerably in appearance. The uterine glands (fig. 3, *gl.*) are now for the most part widely separated from each other, and the interglandular connective tissue appears greatly attenuated. It consists of a very delicate retiform tissue, and is permeated by abundance of lymph coagulum, while numerous leucocytes are also distributed through it.

The glands appear the same as in the preceding stage. The mucosa is now much more vascular than in Stage A. The syncytial lining of the uterus has undergone further enlargement and differentiation. The layer has an average thickness of ·035 mm., i. e. it is somewhat thicker than in Stage A. Further, its inner surface is now found divided up into a series of numerous close-set lobular projections of somewhat irregular size (fig. 4, *syn. l.*). The greater number of the syncytial nuclei are disposed in relation to these lobules, in many cases filling them completely, in other cases forming an irregular layer in the marginal protoplasm of the lobule. Scattered nuclei also occur in the superficial portion of the syncytium, but not abundantly. Like the syncytial protoplasm, the ovalish or rounded nuclei stain deeply. They now present the appearance of typical resting nuclei, a fact which suggests that the further enlargement of the syncytium is not to any great extent at least accompanied by active division of the nuclei.

Another highly significant fact in connection with the syncytium is that it is already vascularised. Both between the lobules and enclosed in the protoplasm of the syncytium itself small capillary vessels, with distinct nucleated endothelial walls and containing maternal blood-corpuscles, can be readily made out (fig. 4, *syn. c.*). These syncytial capillaries are derived from the capillaries of the mucosa, which are seen to pass up between the syncytial lobules, and from there to ramify out in the syncytium itself. That the capillaries actually penetrate into the syncytium by their own growth seems beyond question, but no doubt the subsequent gradual enlargement of the syncytium as a whole, and especially of its lobules, also aids in bringing about the enclosure of the capillaries.

We can only regard the formation of the syncytial lobules as the result of the enlargement and growth of the protoplasm, and it seems probable that the direct invasion of the syncytium by ingrowing capillaries may have been the inciting cause of this mode of growth.

This transformation of the uterine epithelium into a vascular syncytium is a highly distinctive and peculiar feature in the developmental history of the placentation of Perameles. Such a condition has hitherto never been met with in any other mammalian form, and is especially interesting in view of the wide-spread occurrence of degeneration of the uterine epithelium prior to placental formation in so many diverse Eutherian orders. The only form known to me which in the behaviour of its uterine epithelium offers any points of analogy with the above-described transformation of the epithelium in Perameles is Sorex. Hubrecht has shown that, in this Insectivore, modification of the uterine epithelium over the placental area, by way of proliferation, is the first and most important change "that takes place in the maternal tissues preparatory to the reception, fixation, and nutrition of the blastocyst" (7, p. 491). But when one compares the details of the proliferation in the two cases they are seen to be essentially different in character, though offering interesting analogies. In Sorex, following

Hubrecht's account, we have to do with a proliferation of cells from the under surface of the uterine epithelium. These proliferated cells eventually form crypts, between which vessels penetrate. The crypts, however, play only a temporary rôle in the formation of the placenta, and take no ultimate part in its development.

In Perameles, on the other hand, we have to do not with a proliferation of cells, but of nuclei in a continuous syncytial layer; and what is more important is the fact that here this transformed epithelium persists to form the maternal portion of the functional placenta.

Stages C and D.

General Account of the Fœtal Membranes.

Before proceeding to the detailed consideration of Stages C and D, it is advisable here to give a general account of the fœtal membranes, so far as they can be made out from these two stages.

In Perameles the fœtal membranes have the same general arrangement as in Phascolarctus, my two stages exhibiting characters corresponding to the stages described and figured by Caldwell (**12**) and Semon (**8**).

Owing to the mode of growth and the development of an exceedingly voluminous proamnion the embryo is found, at the stage when the amnion is complete, sunk down into the cavity of the yolk-sac, and partially surrounded by the upper portion of the yolk-sac wall (text fig., *y. spl.*), which is thus invaginated into the yolk-sac cavity. Semon distinguishes this invaginated portion of the yolk-sac wall (or briefly "yolk-sac splanchnopleure") simply as "inneres Blatt."

The space in which the embryo, enclosed in its amnion, lies is, of course, the extra-embryonic splanchnocœle (text fig., *cœ.*), and is closed externally by a discoidal area of true chorion.[1] It is with this discoidal area that the allantois fuses, and over it the allantoic placental connection is eventually established.

[1] We use the term chorion here in the sense specified by Minot ('Human Embryology,' p. 286), viz. the true chorion is that part of the extra-embryonic somatopleure which remains after separation of the amnion.

The periphery of this true chorionic area thus indicates the limit of extension of the cœlom, and beyond that limit chorion and invaginated yolk splanchnopleure alike merge into the

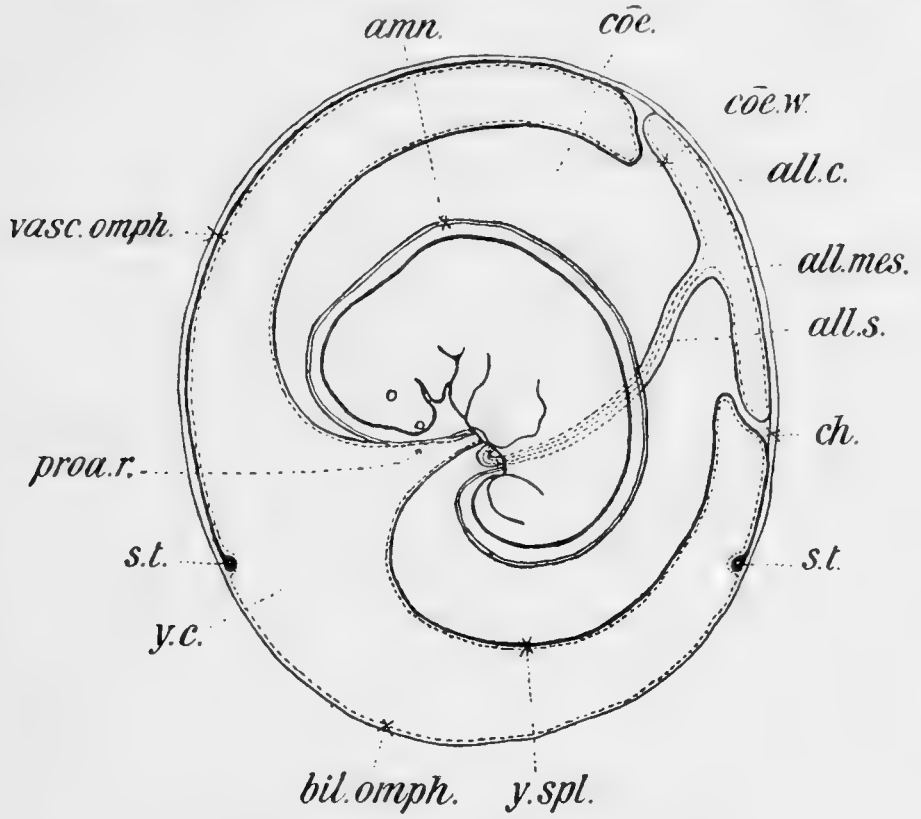

Diagram showing the arrangement of the fœtal membranes in Perameles. *amn.* Amnion. *all. c.* Allantoic cavity. *all. mes.* Allanto-chorionic mesenchyme. *all. s.* Allantoic stalk. *bil. omph.* Bilaminar omphalopleure. *ch.* Marginal zone of true chorion around the allanto-chorionic area. *cœ.* Extra-embryonic splanchnocœle. *cœ. w.* Inner or cœlomic wall of allantois. *proa. r.* Persistent remnant of proamnion. *s. t.* Sinus terminalis. *vasc. omph.* Vascular omphalopleure. *y. c.* Cavity of yolk-sac. *y. spl.* Invaginated yolk-sac splanchnopleure. The ectoderm is represented by a thin line, the entoderm by a dotted line, and the mesoderm by a thick line.

unsplit wall of the blastodermic vesicle or primitive yolk cavity (text fig., *vasc. omph.*). For this outer unsplit wall of the yolk-sac no very suitable name exists in the literature of embryology. Semon simply terms it "äusseres Blatt." If we restrict the use of the term chorion, as Minot has done, then such terms as "omphalo-chorion" (Fleischmann), "Dot-

tersackchorion seu pseudo-chorion" (Selenka), are open to objection. Hubrecht's term "omphaloidean diplotrophoblast" (**9**, p. 385) is also inadequate, since it is only properly applicable to the somatopleural constituents of the as yet unsplit yolk-sac wall. To avoid confusion it seems desirable to employ a distinctive term descriptive of this unsplit yolk-sac wall in its entire thickness and extent; and for this end I propose to adopt, at the suggestion of my friend Professor J. T. Wilson, the term "omphalopleure."

The term omphalopleure, then, signifies the whole of the wall of the blastodermic vesicle or primitive yolk-sac, beyond the region of extension of the splanchnocœle. The employment of the term omphalopleure will thus prevent the unnecessary use of the expression "blastodermic vesicle wall" in stages when the embryo is completely folded off, and one no longer wishes to speak of a blastodermic vesicle as such. According to the extension of the unsplit mesoderm the omphalopleure may be trilaminar or bilaminar in greater or lesser extent. Also a unilaminar condition may be temporarily found in a position corresponding to the lower pole of the blastodermic vesicle, prior to complete ventral extension of the yolk-sac entoderm.

In Marsupials the trilaminar portion of the omphalopleure is co-extensive with the vascular area, the sinus terminalis marking not only the margin of the vascular area, but also the peripheral limit of the unsplit mesoderm. We may thus refer to the trilaminar omphalopleure in Marsupials as the "vascular omphalopleure" (text fig., *vasc. omph.*). Beyond the sinus terminalis mesoderm is absent, and there the omphalopleure consists solely of ectoderm and entoderm (text fig., *bil. omph.*). We shall hereafter refer to this as the "bilaminar omphalopleure," which seems more expressive than Semon's term "Prokalymma" (**8**). We can thus distinguish in the outer wall limiting the whole spheroidal embryonic formation three areas of widely different structure: towards each pole a discoidal area,—the one limiting the extra-embryonic cœlom, and consisting of true chorion; the other limiting the yolk-sac

cavity, and consisting of bilaminar omphalopleure; between these and running round the mid region of the whole structure, a somewhat annular zone of vascular omphalopleure.

The allantois of Perameles consists of a long stalk (text fig., *all. s.*) and a terminal expanded and much-compressed vesicular portion. The stalk, leaving the embryo immediately behind the yolk-stalk, curves round its right side, and, extending through the extra-embryonic cœlom, expands at its distal end to form the flattened vesicular portion. In the stalk the allantoic cavity is reduced to a narrow compressed canal opening distally into the continuous cavity of the vesicular portion (*all. c.*). From the flattened form of this latter we may, for descriptive purposes, term that portion of its wall next the cœlom the inner or cœlomic wall (*cœ. w.*), and that turned towards the chorion the outer or placental wall.

In Stage C the mesoderm of the outer wall of the allantois is found fused with the somatic mesoderm of the discoidal chorionic area (*all. mes.*), the enlarged ectodermal cells of which are firmly adherent to the vascular maternal syncytium. For the chorion, after the allantois has fused with it, we shall employ the term "allanto-chorion" (Fleischmann). The allantoic vessels consist of a large vein and two smaller arteries. They extend unbranched in the stalk, and, in fact, constitute its greater bulk. At its distal end the arteries branch out on the inner wall of the vesicular portion of the allantois, while the vein is there formed by the union of two main factors which accompany the main arterial trunks. The latter branch in a dichotomous manner, each of the larger arterial branches being accompanied by a corresponding venous channel. This arrangement is, however, as Fleischmann (**10**) has pointed out for the cat (cf. his fig. 7, Taf. iv), confined to the larger trunks; the finer branchings do not thus correspond. These vessels ramifying on the inner wall of the allantois pass round the margin of the allantoic vesicle into the allanto-chorionic mesenchyme of the outer wall, and there form a rich capillary network corresponding to the so richly developed network described and figured by Semon (**8**) for Phascolarctus (cf. his

fig. 38, Taf. iv). Of the vitelline vessels unfortunately I can give only a very incomplete account. In opening up the uteri in both Stages C and D the vascular area was partly destroyed, so that I can only state the course of the main trunks. As in Æpyprymnus and Phascolarctus, according to Semon's account (8), the yolk-sac is supplied by a vitelline artery and a vitelline vein.

The vitelline artery on leaving the yolk-stalk runs obliquely backwards in the yolk splanchnopleure, and finally passes over into the vascular omphalopleure, where it bifurcates into a right and left trunk, which together constitute the circular sinus terminalis (text fig., *s. t.*). From the sinus smaller branches pass off into the vascular area. Whether the two trunks actually inosculate or are only connected by capillary anastomosis I am unable to state.

The vitelline vein is formed close to the base of the yolk-stalk by the union of two main factors which arise in the vascular omphalopleure by the union of tributaries coming from the capillary network of the vascular area. These two main vitelline trunks coming from opposite sides of the vascular area pass over from the vascular omphalopleure to the yolk splanchnopleure, and there run posteriorly over the left side of the head of the embryo. They gradually approximate as they approach the yolk-stalk, near the base of which they unite to form the single vitelline vein.

The last point to which we need refer in this general account concerns the existence of a persistent remnant of the proamnion somewhat similar to the "proamnion-rest" described by Semon (8) for Phascolarctus. As in that form, the persistent connection between the amnion and the here non-vascular yolk splanchnopleure forms a small pear-shaped area bounded laterally by the main factors of the vitelline vein, and extending from their point of union up to about the level of the eye on the left side of the embryo. A section through this proamniotic area is represented in fig. 14. It will be seen therefrom that both the ectoderm and entoderm over the area are considerably modified.

The ectoderm (fig. 14, *ect.*) is markedly thickened, and at the margins of the area forms cushions several cells in thickness. The superficial cells often project freely, and are club-like in form, with the nucleus lying in the freely projecting part of the cell. Apparently in Phascolarctus such a marked thickening of the ectoderm does not exist.

The entoderm presents a somewhat varied appearance in different sections. In the section drawn (fig. 14, *ent.*) it is a quite irregular layer of some thickness. Many of its cells are greatly enlarged and vesicular-looking, presenting quite a degenerate appearance. Even in places where the entoderm over the area does not differ greatly from the entoderm of the yolk splanchnopleure, one often meets with similar isolated enlarged and vesicular-looking cells. In Phascolarctus Semon describes the entoderm over the area as "stark verdickt und eigenthümlich gewulstet" (8, p. 31). At the edges of the area (fig. 14) the somatic mesoderm of the amnion (*amn.*) is continuous with the mesoderm of the splanchnopleure, so that the continuity of the extra-embryonic cœlom is here definitely interrupted. The mesoderm penetrates into the area for a short distance peripherally; but, contrary to what Semon describes for Phascolarctus, it does not form a continuous layer extending right through and separating the ectoderm of the amnion from the entoderm of the yolk-sac. In the centre of the area these layers may be either in close apposition or separated by a narrow cleft. This central portion of the area thus consists of true proamnion.

Stage C.—P. obesula.

The left uterus was very much larger than the somewhat enlarged right, and formed a large globular swelling containing a single embryo (fig. 5) with a crown-rump measurement of 7 mm. For details of the internal anatomy of this embryo see table (appendix).

This is a most important stage, since it shows the mode of attachment of the embryo. Microscopic examination of the

empty right uterus shows that it has undergone changes parallel with those undergone in the pregnant left, a vascular syncytium of some thickness having been formed all over the surface of the mucosa.

In this and the following stages I propose to describe separately (1) the changes in the uterus, and (2) the structural features of the fœtal membranes and their relations to the uterine wall.

I. Left Uterus.—The discoidal allantoic placental area is situated on the ant-mesometrial side of the uterus. The uterine surface is thrown into a series of irregularly longitudinal folds, this folding being especially marked in the allantoic placental area. One may emphasise this by stating that from edge to edge of the area in a straight line the breadth in its mid region is only about 4 mm., while following the folds the allantoic placental area has a breadth in sections of 13—14 mm.

The muscularis is apparently somewhat thicker than in the preceding stage.

So far as the corium is concerned no very sharp distinction can be drawn between the portion of it lying beneath the allantoic placental area and that outside the latter. Still for descriptive purposes it is convenient to speak of these two portions separately.

The corium beneath the allantoic placental area varies very greatly in thickness over its extent owing to the greatly folded nature of the surface. Measured from the bottom of a depression between two folds the corium may have a thickness of only ·6 mm., while in the region of a fold the thickness may reach as much as 2·7 mm. Outside the allantoic placental area the mucosa is not so markedly folded, and is on the whole thinner. The corium here varies in thickness from ·5 to 1·7 mm. This difference in thickness is mainly due to the expansion of the interglandular connective tissue below the allantoic placental area, so that here the corium has a less compact appearance, and the glands are on the whole more widely separated from each other than in the corium outside

this area. Abundance of lymph coagulum is found throughout the meshes of the delicate interglandular connective tissue.

The uterine glands have not essentially altered since Stage B. Some few of them, however, are now enormously enlarged, and lined by a low cubical epithelium.

In the allantoic placental area the gland openings are occluded by the chorionic ectoderm cells, which at this stage form an almost continuous layer firmly attached to the surface of this portion of the syncytium. In the region surrounding the allantoic placental area the gland openings are similarly occluded by the close contact of the vascular omphalopleure with the syncytial surface.

The corium is now very richly supplied with blood; the capillaries are numerous and greatly dilated.

Syncytium.—The syncytium in this stage is found to have undergone differentiation into three fairly well-defined regions corresponding to the three areas we have already distinguished in connection with the fœtal membranes, viz. the allantochorion, the vascular omphalopleure, and the bilaminar omphalopleure. The area of the syncytium to which the enlarged ectoderm cells of the allantochorion are attached we shall term the allantoic placental syncytium, as distinguished from that portion of the syncytium in relation to the omphalopleure.

(*a*) Allantoic Placental Syncytium.—This is at once to be distinguished from the remainder of the syncytium, not only by the fact that the chorionic ectoderm is at this stage firmly adherent to it, but also by the facts of its greater thickness, and the larger size and deeper staining qualities of its nuclei (figs. 6 and 7, *pl. syn.*). It has now an average thickness of 1 mm., i.e. it is just about three times as thick as the syncytium in Stage B.

The outer surface of the layer is not smooth, but wavy and irregular, while its inner surface is distinctly lobulated. The large and deeply staining ovalish or rounded nuclei are for the most part closely aggregated together in nest-like groups in

the deeper lobular zone of the syncytium, while scattered nuclei also occur in its superficial portion just as in Stage B (figs. 7 and 8, *pl. syn.*).

The syncytium is now much more vascular than in Stage B, numbers of enlarged capillaries with distinct endothelial walls occurring throughout the protoplasm. As in Stage B, the capillaries are found to enter the syncytium between the lobules. They pass mainly into its superficial zone in order there to ramify just beneath its surface, clothed at this stage by the chorionic ectoderm.

(*b*) Syncytium beyond Allantoic Placental Area.—The allantoic placental syncytium at its margin becomes greatly reduced in thickness, and continues on as the syncytium in contact with the vascular omphalopleure (fig. 6, *ex. syn.*). Here it has a thickness of about ·05 mm. It has essentially the same characters as the allantoic placental syncytium. The nuclei are, however, smaller, and stain somewhat less deeply. They are, as a rule, aggregated in nests in the lobules, though here and there they tend to become more irregularly distributed throughout the protoplasm.

What, however, specially characterises this portion of the syncytium in contact with the vascular area is the richness of its blood-supply. At this stage it is distinctly more vascular than the allantoic placental syncytium. The capillaries form a rich network just beneath and upon its irregular free surface (fig. 6, *syn. c.*). The significance of this fact will be pointed out later, in connection with the description of the vascular omphalopleure.

Beyond the sinus terminalis, where the syncytium is in contact with the bilaminar omphalopleure, it is on the whole just about half as thick as over the vascular area. Here, though the characteristic arrangement of the nuclei in nests is still to be seen, the nests are small and inconspicuous, and are often separated by fairly wide intervals, in which the nuclei are irregularly distributed in the protoplasm (fig. 6). The nuclei are similar in size and in staining properties to those of the syncytium in contact with the vascular area. The vas-

cularity of this region is considerable, but much less so than in the syncytial region in relation to the vascular omphalopleure.

II. Fœtal Membranes.—(*a*) Chorionic Ectoderm.—The ectodermal cells of the discoidal area of allanto-chorion are at this stage found firmly adherent to that portion of the syncytium we have already described under the term allantoic placental.

The chorionic ectoderm consists of a single layer of irregularly columnar or cubical cells, of large size and with large ovalish or rounded deeply staining nuclei (figs. 6, 7, and 8, *ch. ect.*). The layer is on the whole sharply marked off from the underlying syncytium by the fact that the protoplasm of the ectoderm cells stains much less deeply than the syncytial protoplasm. The outer ends of the ectoderm cells project quite irregularly, and are found to be accurately adapted to the irregular syncytial surface, dipping down into and filling up every little depression in its surface. The connection, then, between the chorionic ectoderm and the underlying syncytium is of the closest and most intimate character. Such close and accurate apposition could only have arisen through a mutual process of growth and enlargement, affecting both the ectoderm cells themselves and the underlying syncytium.

So far the above description of the chorionic ectoderm would apply to the whole of that layer in a stage slightly earlier than the one under consideration. In this latter, however, the ectoderm is not uniform in character over its whole extent, since over certain portions of it degenerative processes have already set in. Such a portion is represented in fig. 9. It will be there seen that the ectoderm no longer forms a continuous and uninterrupted layer of fairly regular cells, but is irregular and definitely interrupted on the right side of the figure, thus allowing the allantoic capillaries to come into direct contact with the vascular syncytium. A comparison of the chorionic ectoderm in fig. 8 (*ch. ect.*) with that shown in this fig. 9 will render at once apparent the marked changes which have taken place in the characters of the cells. Here they are

of a very varying size and shape, and in places, through the disappearance of the outlines between adjacent cells, large multinucleate cells have been formed. The protoplasm of these degenerating cells often stains just as deeply as that of the syncytium, rendering it difficult to determine accurately the limit between the two. In many of the ectoderm cells, shown in fig. 9, the nuclei are also seen to be in various stages of disintegration. Many of them stain only slightly; the nuclear membrane is becoming indistinct, while the chromatin is found broken up, and diffused in the form of small granules throughout the delicate nuclear reticulum. Eventually the position of the nucleus is only marked by a few straggling irregularly thickened remnants, which finally become diffused throughout the protoplasm, and lost to view.

It may be noted that degeneration does not take place in a uniform manner over any given area, but quite irregularly in patches, so that in a small portion of the allantoic placental area (as in fig. 9) various stages in the degenerative process are met with. In certain portions of the chorionic ectoderm where it still forms a continuous layer of fairly regular cells, and shows no signs of the degenerative process just described, I have found that the inner ends of the cells are greatly vacuolated, a fact which suggests that a process of vacuolation may also play a rôle in the retrogression of the chorionic ectoderm.

That the chorionic ectoderm is destined to disappear is abundantly evident from this stage alone, and I am inclined to believe that the allantoic capillaries, so closely related to its inner surface, are by no means the least active agents in effecting its removal. Of direct fusion of the degenerate ectoderm with the syncytium there can be no question. All the facts negative such a view.

The rôle of the ectoderm is apparently merely that of attaching the embryo to the previously prepared maternal syncytium. Once the allantoic capillaries have spread out on its inner surface, it degenerates and disappears in order to allow of closer proximity between the fœtal and maternal

capillaries, and thus takes no part in the constitution of the functional placenta.

(*b*) Allantois and Mesenchyme of Allanto-chorion.—The allantoic stalk is ovalish in cross-section (fig. 10), measuring in long diameter ·3 mm., and in short ·18 mm. In it run the three allantoic vessels, two smaller arteries (*all. a.*), and a larger vein (*all. v.*). The vessels are lined by a delicate endothelium, round which the mesoderm is condensed to form a thin sheath. Lying in the mesenchyme between the two arteries is the small canal of the stalk (*all. cl.*) lined by a layer of somewhat flattened entoderm. The stalk is covered externally by a layer of mesothelium, internal to which and forming the matrix of the stalk are branched mesenchyme cells.

The stalk enters the body of the embryo behind and to the right of the intestinal loop to which the yolk-stalk is attached, and has the usual relations; i.e. the stalk, now consisting of the entodermal canal and the two arteries, passes back in the median line attached to the inner surface of the ventral abdominal wall, and its canal finally opens into the cloaca. In the abdomen and close to the body of the embryo, the entodermal lining of the canal consists of a low cubical epithelium. At its distal end the stalk expands into the flattened vesicular portion of the allantois, the canal of the stalk being in direct continuity with the cavity of the vesicular portion (fig. 11). This latter is an exceedingly thin-walled sac, possessing a continuous cavity of very irregular form (fig. 7, *all. c.*). The inner or cœlomic wall of the allantois (fig. 7, *cœ. w.*) is fairly smooth and unfolded, but its outer wall has grown out into great hollow folds which enter the deep depressions of the uterine surface, and thus is of far greater extent than the inner wall.

The allantoic cavity is lined by a very thin layer of entoderm with small flattened or ovalish nuclei (fig. 7, *all. ent.*). The mesoderm covering the inner wall (fig. 7, *cœ. w.*) consists only of a thin mesothelial layer, except along the vessels. The mesoderm of the outer allantoic wall is now organically con-

tinuous with the somatic mesoderm of the chorion, forming the allanto-chorionic mesenchyme. This is, at this stage, an exceedingly thin layer, which becomes somewhat thicker at the margin of the allantoic placental area and around the larger allantoic capillaries (figs. 6, 7, and 8, *all. mes.*). The mesenchyme consists of small branched cells, the delicate processes of which anastomose with each other, and with the entodermal lining of the allantoic cavity. I find that the allantois of a late uterine embryo of Macropus dorsalis shows essentially the same structural features as the vesicular portion of the allantois of Perameles, only in the Macropus embryo the vessels are less marked; and there is, of course, no union with the chorion. In both cases the allantoic wall is characterised by its extreme tenuity.

In the inner or cœlomic wall of the allantois, between the mesothelium and the entoderm, run the main branches and factors of the allantoic arteries and vein. Corresponding to their characteristic mode of branching, one finds in sections the larger trunks in pairs, a smaller and slightly thicker walled arterial trunk accompanied by a larger venous channel. In the inner wall the vessels have distinct thin sheaths of condensed mesenchyme. In order to reach the outer surface of the allantois these vessels, ramifying in the inner wall, turn round the periphery of the flattened vesicle. Seeing the allantoic cavity is a continuous and uninterrupted one, there are no direct passages across the cavity by means of cellular bridges, as Hubrecht describes for the allantois of Erinaceus (9). The vessels of the inner wall gradually decrease in size by repeated branchings as they approach the periphery of the vesicle, their mesodermal investing sheath becomes reduced to a layer a single cell thick, and they pass round into the allanto-chorionic mesenchyme. There they again branch repeatedly, forming a network of small capillaries, with only endothelial walls. The ultimate branches of this capillary system come into very close contact with the inner surface of the chorionic ectoderm, and even in places where cells of the latter have disappeared, into contact with the vascular maternal

syncytium (fig. 9). The true chorion at the margin of the allantoic placental area yet remains to be mentioned (text fig., *ch.*). It will be seen from figs. 6 and 7, representing sections through the opposite margins of the allantoic placental area, that the allantois does not at this stage completely extend over the whole of the chorionic area, but leaves outside its periphery a narrow marginal zone of true chorion, which intervenes between the allanto-chorion and the vascular omphalopleure.

On the one side (fig. 6) the allantois does not even extend completely to the outer limit of attachment of the chorionic ectoderm, but leaves here a marginal attached portion of true chorion. On the other side (fig. 7) the margin of the allantois corresponds fairly accurately with the outer limit of attachment of the chorionic ectoderm, so that here only a narrow strip of free chorion remains. On both sides the ectoderm cells of this marginal chorionic zone become gradually reduced in size, and pass over into the thin ectoderm of the vascular omphalopleure. Closely adherent to the inner surface of this transitional chorionic ectoderm is the thin single layer of somatic mesoderm of the chorion (figs. 6 and 7, *som.*), continuous on the one side with the allanto-chorionic mesenchyme (*all. mes.*), and on the other with the mesoderm of the yolk splanchnopleure (*y. spl.*).

(*c*) Yolk Splanchnopleure.—The line along which the somatic mesoderm of the chorion is continuous with the mesoderm of the yolk splanchnopleure marks the outer limit of the splanchnocœle (figs. 6 and 7, *cœ.*), and thus also the commencement of the vascular omphalopleure (*vasc. omph.*). The mesoderm of the yolk splanchnopleure is a thin layer, carrying fairly numerous vessels, except in that portion of it included between the two main factors of the vitelline vein, which is, as Semon has pointed out (8), permanently non-vascular. Its entoderm is similar to that of the vascular omphalopleure.

Traced round to its connection with the embryo, the yolk splanchnopleure narrows to form the yolk-stalk, or vitelline duct, which opens into the gut at the apex of the intestinal loop.

(*d*) Vascular Omphalopleure and Yolk-sac Placenta.—The vascular omphalopleure includes, as already pointed out, the area of the yolk-sac wall between the periphery of the true chorion and the sinus terminalis, and is co-extensive with the vascular area. Its entoderm (figs. 6 and 7, *vasc. omph.*) consists of a fairly thick, somewhat flattened layer of varying width, with oval or rounded nuclei staining deeply like the protoplasm. The unsplit mesoderm is a thin and delicate layer carrying the very numerous capillaries of the vascular area.

The ectoderm is especially noteworthy on account of its extreme tenuity (fig. 6, *ect.*). It consists of a delicate thin layer of mostly flattened cells with small oval or fusiform nuclei. Here and there at intervals, cells of a somewhat triangular shape are found, with their outer pointed ends projecting beyond the level of the general surface of the ectoderm.

In Didelphys, according to the descriptions both of Osborn and Selenka, the ectoderm of the vascular omphalopleure is a much thicker layer than in Perameles. Osborn describes it as consisting of "elongated cells with amœbiform processes which are closely applied to the lining epithelium of the uterus" (**11**, p. 378; cf. also his fig. 4, Pl. xvii); and Selenka, describing the layer in a $7\frac{3}{4}$ days' embryo, says (**2**, p. 137), "Bei weitem der grösste Theil dieser Ektodermzellen dehnt sich aus und nimmt blasige Form an, unter gleichzeitiger Vergrösserung der Kerne (Taf. xxviii, fig. 5, *d.*). In diesem Felde von blasigen Zellen bemerkt man gegen Ende der Incubation vereinzelte Fleckchen kleiner nahezu kubischer Ektodermzellen (*c* und *a*) und hier und da sah ich an Schnitten sogar Andeutung von Zottenbildung (*b*) mit axialen Mesodermzellen." Again, in a 5—6 days' old Hypsiprymnus he describes the ectoderm of the vascular omphalopleure in the following words (**2**, p. 184):—"Doch zeigten sich die Ektodermzellen des Chorion auffallend vergrössert und gegen die Uterushöhle Zapfen-oder kuppelartig vorgewölbt (Tafel xxxii, Figur 2)." Caldwell, again, referring presumably to Phasco-

larctus, says (12, p. 657), "The whole vascular area is covered by flat cells of the subzonal membrane." In a uterine embryo of Macropus ruficollis at a stage slightly later than the one of Perameles under consideration, I find that the ectoderm of the vascular omphalopleure consists of a fairly thick flattened layer, which more nearly resembles the entoderm of the vascular omphalopleure of Perameles than the ectoderm of the same.

So far, then, as the forms mentioned are concerned, it appears that the extreme thinness of the ectoderm of the vascular omphalopleure in Perameles is an exceptional and noteworthy feature. A glance at fig. 6, which includes the whole of the extent of the vascular omphalopleure on one side, will show that, although the latter is separated in sections from the syncytium, there is an accurate and fairly close correspondence between the two, elevations in the one corresponding to depressions in the other, and vice versâ. The vascular omphalopleure, in other words, appears moulded to fit the irregular surface of the maternal syncytium, and without doubt during life the two surfaces were accurately apposed the one to the other, the above-mentioned projecting ectoderm cells even serving as an actual attachment. It has already been pointed out that the portion of the syncytium in relation to the vascular omphalopleure possesses a very rich network of maternal capillaries, on and just beneath its surface, and that at this stage it is even relatively much more vascular than the allantoic placental syncytium. The fœtal capillaries of the vascular area are thus only separated from the maternal by a thin and delicate ectodermal layer, plus a thin layer of syncytial protoplasm, the latter often absent indeed where the maternal capillaries actually reach the surface.

These facts point to the conclusion that, prior to the period of functional activity of the allantoic placenta, the placental function is subserved by the close contact of the vascular omphalopleure with the vascular maternal syncytium, an arrangement which we are therefore justified in designating as an actual yolk-sac placenta.

That the yolk-sac placenta is of high functional importance in the nutrition of the embryo at this stage is borne out not only by the fact that the vitelline vein is nearly three times as large as the allantoic, but also by the further fact that the larger proportion of the purified and food-laden blood coming from the vascular area passes directly to the heart. So far as one can judge from structure alone, this veritable yolk-sac placenta of Perameles appears to be more efficiently adapted for respiratory and nutritive functions than the arrangement found in other described Marsupials; e. g. in Macropods the ectoderm of the vascular omphalopleure is a comparatively thick layer, the uterine epithelium persists, though in a somewhat modified form, and the maternal capillaries existing below it are not very numerous, and nowhere directly project on the free surface.

(*e*) Bilaminar Omphalopleure.—Beyond the sinus terminalis (fig. 6, *s. t.*) mesoderm is absent, the wall consisting here solely of ectoderm and entoderm, for the most part in close contact with each other.

The entoderm is, on the whole, slightly thicker than that of the vascular omphalopleure. It consists of a layer of cells of varying size and shape, so that its inner contour is somewhat irregular (fig. 6, *bil. omph.*, and fig. 12, *ent.*). In places the entoderm cells present a vacuolated appearance.

The ectoderm differs markedly from that of the vascular omphalopleure. It is a very much thicker layer, the cells are large, rich in protoplasm, and vary greatly in form and size. Their outer ends project more or less freely in a quite irregular manner, so that the free surface of the layer presents a roughened irregular appearance (fig. 6, *bil. omph.*, and fig. 12, *ect.*).

Like Semon, I see no evidence in sections of the existence at this stage of "pseudopodia-like" processes of these ectoderm cells, such as Caldwell (12) describes as serving to attach the blastodermic vesicle to the uterus.

The significance of the persistence of this bilaminar portion of the omphalopleure for a longer or shorter period in dif-

ferent mammals has been already discussed by Semon (8). He has come to the conclusion that it has an important physiological meaning; in his own words (p. 55), "hier erfolgt eben der Durchtritt der von der Mutter gelieferten Nahrungsstoffe in das Innere des Dottersacks, von wo aus weiterhin die Aufnahme und Uebergabe an das Blut durch die Entodermzellen der gefässhaltigen Zone ausgeführt wird." We may, however, point out that in Perameles only a small proportion of the uterine secretion need take this indirect way of reaching the vessels of the vascular area. By far the greater proportion of the secretion, no doubt, passes directly through the thin ectoderm of the vascular omphalopleure into the yolk-sac vessels.

Stage D.—P. obesula.

Upon the examination of this stage was based the preliminary account already published (1). It is specially important since it shows the allantoic placenta well developed. Both uteri were greatly enlarged; the left contained two embryos, measuring respectively 8 and 8·25 mm., while the right contained a single embryo measuring 8·75 mm. from crown to rump. For the structural characteristics of this latter embryo see table (appendix).

These three embryos all present substantially the same features of placental connection. Fig. 13 represents the larger of the three embryos attached to the placental area of the uterine wall, and still partially enclosed in its membranes. The dissection from which this figure was made was prepared by opening up the uterus by a ventral longitudinal incision, which also involved the closely adherent omphalopleural wall. In the figure, then, we see the inner surface of the yolk-sac wall lying on the inner surface of the uterus, which has been spread out flat.

In the middle of the figure lies the embryo seen through the amnion and the yolk-sac splanchnopleure (*y. spl.*). At the back of the embryo and partly concealed by the body of the latter is the allantoic placental area (*pl. a.*), also seen through

the yolk splanchnopleure. The allantoic stalk with its three vessels (*all. a.* and *all. v.*) is seen to emerge from under the embryo's right side, and at its distal end the vessels spread out on what is the inner wall of the vesicular portion of the allantois. The distribution and mode of branching of the allantoic vessels is clearly shown. As already described, the vein (*all. v.*) is formed by the union of two factors which accompany the corresponding arteries (*all. a.*).

The placental area is discoid in shape and, from the presence of a prominent rim on the side visible, appears somewhat depressed below the general surface of the uterus. In sections it is found to commence a little behind the anterior margin of the flexed head end of the embryo, and to terminate some distance behind the level of its curved posterior end. Its length, in the direction of the long axis of the embryo, is thus about 9 mm., while in its middle region, following the folds, it has a breadth of about 12 mm. transversely to the long axis of the embryo.

The flattened allantoic stalk has an approximate length of 8 mm.

The course of the vitelline vessels is also shown in the figure. Leaving the yolk-stalk, the artery (*vit. a.*) passes obliquely backwards, traversing the yolk splanchnopleure (*y. spl.*) to reach the vascular omphalopleure (*vasc. omph.*), and there it almost immediately divides into two right and left branches, which form the sinus terminalis (*s. t.*), a portion of which is visible. The area outside the sinus (below) in the figure is part of the bilaminar omphalopleure (*bil. omph.*). From the yolk-stalk the two factors (*vit. v.*) of the vitelline vein pass anteriorly in the yolk splanchnopleure, and gradually diverging from each other, they pass over into the vascular omphalopleure, where they are formed by the union of lesser factors coming from the capillary system of the vascular area.

I. Uterus.—The serosa, muscularis, and corium are essentially the same as in Stage C.

(*a*) Allantoic Placental Syncytium.—This presents the same general features as the corresponding area in Stage C.

but is somewhat thicker (averaging ·12 mm.) than in that stage. It is also much more highly vascular (cf., e. g., fig. 15 with fig. 7). The capillaries entering between the syncytial lobules ramify in great abundance at and just beneath the surface, where they form a rich network. These superficial capillaries are large, and vary considerably in diameter, averaging about ·01 mm., some attaining a width of ·028 mm. The surface of the syncytium is here by no means smooth, but owing to the bulging of the capillaries on the surface it presents an irregularly ridged structure (figs. 16 and 17, *pl. syn.*).

(*b*) Syncytium beyond Allantoic Placental Area.—That portion of it in contact with the vascular omphalopleure forms a fairly uniform layer, averaging ·09 mm. in diameter. It is thus nearly double as thick as the corresponding portion of the syncytium in Stage C, and is also rather more vascular. These facts are significant in view of what has been said above on the high functional importance of the yolk-sac placenta prior to the complete formation of the allantoic.

The superficial capillaries of this portion of the syncytium are markedly developed; many of them lie at the surface (fig. 24, *syn. c.*), which here presents a wavy contour, but is not ridged, as is the allantoic placental syncytium.

That area of the syncytium in relation with the bilaminar omphalopleure presents the same features as were described for the corresponding region in Stage C. It is, however, somewhat thicker than in that stage.

II. Fœtal Membranes.—(*a*) Chorionic Ectoderm.—The chorionic ectoderm has now almost completely disappeared. It is still, indeed, recognisable as a continuous layer of cells at the margin of the allantoic placental area (fig. 15, *ch. ect.*), but over the remainder of the latter it is represented only by more or less isolated degenerating cells (figs. 19—21, *ch. ect'.*).

The persistent marginal zone of ectoderm (fig. 15, *ch. ect.*) is narrow but of very variable width. Its most peripheral cells, adjoining the ectoderm of the vascular omphalopleure, are the least altered, but the remainder are irregular, and

vary both in size and shape. A comparison of fig. 15 with fig. 7 shows at a glance the marked change in the character of the marginal chorionic ectoderm in this stage; for the process of degeneration and absorption which had set in, in the central part of the placental area in Stage C, has now extended nearly to the margin.

In connection with the disappearance of the chorionic ectoderm at this stage it may be noted that the straggling isolated cells of it which yet persist have become greatly hypertrophied. In some cases they are multinucleated (figs. 20 and 21, *ch. ect'.*), or the single nucleus is also hypertrophied and vesicular (figs. 15 and 19, *ch. ect'.*). Fig. 20 is worthy of remark as showing how such isolated and hypertrophied cells may be gradually undermined by the ingrowth below them of an allantoic capillary; the area of contact of the larger cell shown in the figure with the syncytium is in this way greatly reduced, while the smaller cell is entirely separated from the syncytium, and awaits its resorption in isolation in the allanto-chorionic mesenchyme.

(*b*) Allantois and Allantoic Placenta.—In its general features the allantois is essentially similar to that of Stage C. The allantoic stalk with its vessels now, however, is just about half as thick again as that of the preceding stage (cf. fig. 23 with fig. 10), thus showing that the allantoic circulation has increased considerably in volume. In correlation with this increase in the blood-supply, the capillary network (fig. 22, *all. cap.*) in the allanto-chorionic mesenchyme is now much more richly developed than in Stage C.

Now that the chorionic ectoderm has almost wholly disappeared, the capillaries are able to attach themselves closely to the syncytial surface. We have already laid emphasis on the characters of the allantoic placental portion of the maternal syncytium, and have pointed out that its surface is both highly irregular and very vascular, and in certain patches exhibits an irregular system of interlacing vascular ridges separated by depressions. With this irregularly ridged and highly vascular surface the allantoic capillaries are in most intimate contact;

so close, indeed, is the attachment that the walls of the capillaries appear as if united with the syncytial protoplasm. The capillaries dip down into and accurately fill up the depressions between the vascular ridges, so that there is here and there formed an actually interlocking system of vascular projections of the syncytial and allantoic surfaces respectively (figs. 16 and 17). As already indicated, this interlocking does not occur uniformly all over the placental area, but varies in its degree of perfection in different places (cf., e. g., figs. 15, 18, and 19 with fig. 17). The interlocking here referred to represents the highest state of placental differentiation realised in Perameles.

It is thus evident that in this placental differentiation fœtal and maternal elements take an approximately equal share. In the functional organ so produced, it will be noted that the fœtal and maternal blood-streams are separated from each other only by the thickness of two endothelial walls, with at most the addition of a thin layer of syncytial protoplasm. We may here point out that in Perameles, contrary to what obtains in most other mammals,[1] the uterine glands of the placental area do not degenerate, but persist throughout the whole period of pregnancy. In this stage some of the gland openings in the placental area are still occluded by persistent portions of the chorionic ectoderm in a more or less degenerate condition; others of the openings, however, are obstructed by allantoic capillaries extending right over them (fig. 18, *gl.*). It may be that nutritive substances derived from the uterine secretion of such glands are directly absorbed by the allantoic capillaries occluding their openings.

(*c*) Yolk Splanchnopleure.—This is essentially as in Stage C. We may mention, however, that the yolk-sac cavity no longer stands in open communication with the gut, as was the case in that stage.

(*d*) Vascular Omphalopleure and Yolk-sac Placenta.

[1] Strahl (**15**) and Vernhout (**14**) describe a similar persistence of the uterine glands in the mole. According to Vernhout they are invaded by the "plasmodiotrophoblast" shortly before parturition.

—The vascular omphalopleure is on the whole similar to that of Stage C. Here and there, however, the flattened cells of the entoderm give place to larger somewhat cubical cells with rounded free ends. In the protoplasm of some of these larger cells there occur vacuoles. Over the sinus terminalis and the larger vessels of the vascular area the entoderm cells are also markedly enlarged, and much more so than in Stage C. The entoderm cells in these positions are now somewhat club-shaped in form, with their enlarged ends projecting freely and containing the ovalish or rounded nuclei (fig. 24, *ent.* over *s. t.*). Selenka has already described a similar condition of the entoderm cells of this region in Didelphys. He says (**2**, p. 138), "Die Entodermzellen des Chorion verändern gleichfalls vielfach ihre Gestalt während der letzten zwei Tage des Foetallebens. Sie werden cylindrisch oder birnförmig, zumal in der Nähe der grösseren Blutgefässe. Streckenweise behalten sie aber ihre frühere abgeplattete Form bei oder nehmen mehr oder wenig an Volumen zu."

The unsplit mesoderm of the vascular omphalopleure is exactly as in Stage C.

The ectoderm is, as in that stage, an exceedingly delicate layer of greatly attenuated cells (fig. 24, *ect.*). In my preparations of this stage, not only is the vascular omphalopleure very evidently stamped with the contour of the highly vascular syncytial surface, but in places the two are in most intimate attachment, thus affording support for the belief already expressed that we have here an actual yolk-sac placental connection.

In this stage, then, we regard the yolk-sac placenta as being in functional activity along with the allantoic, though now it has diminished considerably in importance, as the examination of the fœtal circulation shows. The vitelline vein in this stage is both absolutely and relatively smaller than in Stage C, and now most of its blood has to pass through the capillary system of the liver before reaching the inferior vena cava. These facts, taken in conjunction with the already mentioned greater size of the allantoic trunks in this stage as compared with the

preceding, show conclusively that with the advent of the allantoic placenta the yolk-sac circulation is giving place to the allantoic. The latter, indeed, is now the predominant one; and we may add that just as, in the preceding stage, most of the blood coming from the yolk-sac placenta passed directly to the heart, so now most of the blood coming from the allantoic placenta passes by way of the left allantoic vein and the ductus venosus Arantii directly into the inferior vena cava.

The question whether the yolk-sac placenta remains functional, though in a diminished degree, throughout the whole period of intra-uterine life of the embryo; or whether, as seems likely from comparison with other placental mammalian forms, it soon after this stage gives entire place to the later appearing allantoic placenta, can only be definitely decided when further material is available. As tending to support the latter alternative, it may be pointed out here that in the next (post-partum) stage, while the allantois was still found adherent to the syncytium of the placental area, no portion of the omphalopleure was to be found in the uterine cavity. Further, the syncytium outside the allantoic placental area no longer showed a richly vascular surface, but was rapidly retrogressing, and indeed was already partly covered by the regenerating uterine epithelium. The syncytium of the allantoic placental area, on the other hand, though in process of absorption, had not altered to such a marked degree.

These facts render it probable that the omphalopleure breaks up and disappears some time before the end of intra-uterine life (cf. also next section).

Unfortunately I am unable to give any details as to the relative dimensions of the vascular area in this and the preceding stage. It may, however, be mentioned that the vessels of the vascular area in this stage are apparently not nearly so richly developed as in a Macropod embryo of about the same developmental stage.

(*e*) Bilaminar Omphalopleure.—This presents features tending to suggest that it is even now in process of degeneration. The ectoderm has on the whole become greatly flattened

and attenuated. This is especially noticeable close to the sinus terminalis (fig. 24, *bil. omph.*). Further out one meets with scattered projecting cells of large size and of irregular form, the protoplasm and nuclei of which stain deeply.

The entoderm has also become considerably thinner in places. Where it has not undergone attenuation the cell protoplasm is often found to be greatly vacuolated, with irregular deeply staining nuclei. Here and there, also, unaltered entoderm cells are met with either singly or in groups.

Stage E.—P. nasuta (post-partum).

The material available for this most important stage consisted of the genital organs (less the cloaca) of a female P. nasuta, together with two newly born young from the pouch.

The new-born young (fig. 36) had a crown-rump measurement of 14 mm., and a head length of 6 mm. For the details of their external characters and internal anatomy see table (appendix).

Both uteri were considerably enlarged: the left, the larger of the two, measured 17 mm. in length by 9 mm. in breadth; the right had a length of 16·5 mm. and a breadth of 8·25 mm.

When the uteri were opened up it was found that parturition had been recently accomplished, and that in each uterus the flattened vesicular allantois with its stalk attached was still adherent over the placental area (fig. 25, *pl. a.*). This latter formed a fairly sharply circumscribed ovalish area, bounded by an almost continuous ridge, and differed from the rest of the irregularly ridged uterine surface by its closer texture. It was situated on the dorso-mesial inner surface of the uterus, i. e. ant-mesometrially. The area measured 9 mm. in length by about 5 mm. in breadth (i. e. without following the folds). The allantoic vessels ramifying in the inner wall of the allantois could not, in surface view, be very definitely made out. Apart from the adherent allantois no other portions of the fœtal membranes were encountered in the uterus.

The left uterus alone was submitted to microscopic examination.

I. Uterus.—The serosa and muscularis are of about the same thickness as in the non-gravid uterus. The muscularis is penetrated by numerous large vessels.

As in previous stages, the whole mucosa is folded, the folds being especially marked in the placental area.

Corium beneath Allantoic Placental Area.—This portion of the corium now differs markedly in appearance and character from the remainder, being as a whole much denser and more compact-looking; and its component parts—inter-glandular connective tissue, uterine glands, and blood-vessels—have all undergone important modifications.

In the preceding stages we have seen that the connective tissue of the whole corium consisted of a very delicate retiform tissue. Now, however, in this region the connective-tissue cells have not only increased in number, but also very greatly in size. From the large, usually rounded, deeply staining cell bodies less deeply staining processes pass off, which anastomose with similar processes of adjacent cells to form a much coarser and closer network than that seen in preceding stages, or even in the corium outside this region in the present stage (fig. 26, *c. t'.*).

There can be no doubt that we have here to do with a process of proliferation of the connective-tissue cells beneath the placental area, accompanied by their subsequent hypertrophy.

This proliferation and overgrowth of the connective tissue in Perameles offers interesting points of comparison with the formation of the decidual cells in the pregnant human uterus, which arise, as Minot has rendered certain, by the direct proliferation and enlargement of the anastomosing connective-tissue cells of the mucosa (4, p. 419).

Numbers of polynuclear leucocytes occur throughout the proliferated connective tissue, especially in its superficial portions immediately beneath the syncytium.

Many of the uterine glands of the placental area, and especially their peripheral portions, are now in process of marked degenerative change. Various stages in the degenera-

tive process are met with, from the first signs of alteration of the gland epithelium to the almost complete obliteration of the gland lumen, by an accumulation of cellular débris, derived from the completely disintegrated epithelial lining of the gland (fig. 26, *d. gl.*).

The deeper portions of the glands adjacent to the muscularis are on the whole less altered. The gland epithelium here still forms a distinct deeply staining layer, but it is somewhat thinner, and not quite so regular as the gland epithelium of former stages. The gland secretion not being able to pass away, owing to the degeneration and occlusion of the peripheral ends of the glands, is here often found in the lumen as a deeply staining coagulum. The mouths of the glands still open freely on the placental area. The glands would thus appear to retain their function throughout the whole period of intra-uterine development of the fœtus.

The blood-vessels also show considerable alteration. The vessel walls appear greatly thickened, and the endothelial cells have increased in size (fig. 26, *m. v.*) and proliferated, thus markedly diminishing the lumen, and in some cases occluding it completely (fig. 26, *m. v'.*). In other more advanced cases the whole vessel is found to have undergone fibrous degeneration, and appears quite solid in section (fig. 26, *m. v''.*). In the coagulated blood in certain of the vessels, polynuclear leucocytes are found to occur.

Corium beyond Allantoic Placental Area.—Here the corium has not undergone such marked change as that of the placental area proper.

Though in parts the connective-tissue cells have undergone a considerable amount of proliferation, yet the tissue as a whole presents the same open and loose appearance as in the preceding stages.

The glands, too, present a more normal appearance, and even where degeneration of their epithelium occurs it has not advanced to such an extent as beneath the placental area.

The blood-vessels of this region show the same essential

alterations as in the placental region. Here, however, the vessels are not nearly so numerous as in the latter.

Allantoic Placental Syncytium.—Following the folds of the mucosa in section, this portion of the syncytium has a breadth of about 9 mm. in the mid region of the placental area; it varies in thickness from ·15 to ·28 mm., and is thus slightly thicker on the average than in Stage D.

The syncytium here is now in active process of absorption and retrogressive metamorphosis.

The syncytial protoplasm is coarsely granular, and numerous irregular spaces and clefts occur throughout its extent (figs. 27 and 28, *sp.*). Its nuclei are much less numerous, and no longer form such distinct nest-like groups in the syncytial lobules (fig. 29). They vary very greatly in size and in shape, being often quite irregular, and occur in all stages of retrogressive change. Many of them are vacuolated and greatly hypertrophied (fig. 27).

Throughout the protoplasm great numbers of leucocytes occur. The majority of these are of the polynuclear variety, possessing oval or rounded slightly staining cell bodies, in which numbers of small deeply staining nuclei lie (fig. 27, *p. leuc.*).

It seems certain that these polynuclear leucocytes are the active agents in the absorption and removal of the degenerating syncytium, and accordingly it may be observed that the leucocytes greatly predominate in the more degenerate areas.

The placental syncytium is still vascular (figs. 28 and 29), though the superficial capillaries are now not nearly so prominent as in Stage D.

The persistence even in this advanced stage, of comparatively unaltered portions of the syncytium, shows that no essential alteration takes place in the latter from Stage D up to the time of birth, and that the features peculiar to the present stage are solely characteristic of post-partum metamorphosis.

Syncytium beyond Allantoic Placental Area.—This portion of the syncytium has undergone more marked alteration in character than the placental portion. On its deeper

surface in many places it is no longer sharply marked off from the underlying connective tissue, but there is a gradual transition from the one to the other (fig. 30, *ex. syn'.*).

The small and often deeply-staining nuclei are irregularly distributed throughout the altered protoplasm, which stains deeply, and is often vacuolated. The maternal capillaries are now very greatly reduced both in size and in number. They occur quite irregularly in the protoplasm, and no longer form a superficial network (fig. 30, *syn. c.*).

Polynuclear leucocytes occur in numbers in the connective tissue immediately below the syncytium, but only sparingly in the syncytium itself.

In regions where disintegration of the syncytium is well marked, i. e. where its remains are practically incorporated with the underlying connective tissue, regeneration of the uterine epithelium has already commenced. Here, as in the human uterus, the uterine epithelium is regenerated by the growth of the gland epithelium at the openings of the uterine glands. In fig. 30 the opening of such a gland is shown, with its lining epithelium spreading out over the degenerate syncytial surface (*ex. cyn'.*) to form the thin and somewhat irregular uterine epithelium (*r. ep.*), better seen in fig. 31. The regenerative process does not take place uniformly all over the surface of the syncytium in this region, but in patches, and is apparently conditioned by the stage of degeneration of the syncytium.

As we have already pointed out in connection with Stage D, it seems probable that the much more degenerate condition of this region of the syncytium, as compared with the placental, is to be correlated with the presumed early retrogression of the entire omphalopleure.

II. Fœtal Membranes.—Allantois.—The allantois is the only portion of the fœtal membranes which is found intact and persistent in the uterus. In its general relations it is essentially as in the preceding stage. Its outer wall closely follows the folds of the mucosa, and is about three times as extensive as the inner. In parts the outer wall is still closely

adherent to the irregular surface of the syncytium, while in others it has become separated from the latter (figs. 28 and 29).

The allantoic cavity (fig. 28, *all. c.*) is distinct and continuous, but its entodermal lining is no longer distinguishable. It contains here and there an irregular cellular detritus. The walls of the allantois have altered considerably in character. The inner (cœlomic) wall (fig. 28, *cœ. w.*) is somewhat thicker than the outer (*all. mes'.*), and is now composed of a dense mesodermal layer, carrying embedded in it in pairs the branches and factors of the allantoic arteries and vein.

The allanto-chorionic mesenchyme of the outer (placental) wall (*all. mes'.*) has also become quite compact in appearance. On its outer surface the allantoic capillaries project (figs. 28 and 29, *all. cap.*). They now contain enucleated fœtal blood-cells, and can still in places be seen to fit in and adhere to corresponding depressions in the syncytial surface (fig. 29).

It will be apparent from figs. 28 and 29 that no essential change has taken place in the constitution of the placenta in the period intervening between Stage D and the time of birth.

The question whether or not the allantois is resorbed in *situ* is at once settled positively by the occurrence in the outer allantoic wall, and to a lesser degree in the inner as well, of numbers of polynuclear leucocytes similar to those already described as existing so abundantly in the placental syncytium (figs. 32—34, *p. leuc.*).

There is not the least doubt but that these leucocytes migrate from the syncytium into the allantoic walls. In sections the leucocytes are found not only at the surface of the syncytium, but actually in the spaces which exist here and there between the syncytium and the outer allantoic wall, and they are even to be seen just in process of entering the latter.

Absorption of the allantois has, however, not yet actively begun, still it is breaking up in portions of its extent (cf. fig. 28), and the cellular detritus in the allantoic cavity can only have arisen through disintegration of its walls.

And if, as we have seen, the fœtal portion of the placenta is

not expelled at birth, but absorbed in situ, it is obvious from the nature of the case there cannot be any shedding of maternal tissue, i. e. no decidua is formed.

The case of Perameles is thus in most striking agreement with the condition in the mole, where, according to the observations of Hubrecht[1] (**9** and **13**), "no afterbirth is shed, although the animal has a discoid placenta;" and he has further pointed out that "not only is the mole not deciduate, but that even embryonic tissue is left behind against the uterine surface, and is gradually resorbed in situ" (**13**, p. 117).

It is thus obvious, as Professor Hubrecht has pointed out to me (in litt.) that the term non-deciduate as long ago used by Huxley is altogether inadequate and misleading as applied to the post-partum conditions obtaining in Talpa and Perameles. In these two forms there is not only no complete separation of maternal and embryonic structures at birth (Adeciduata), but no maternal tissue is lost (Deciduata); on the contrary, fœtal tissue is actually resorbed by the mother. For such a condition Professor Hubrecht suggests the term Contra-deciduata.

The discovery of the contra-deciduate character of the placenta of Perameles thus affords welcome testimony to the rightness of Hubrecht's opinion, based on a consideration of Talpa alone, that this contra-deciduate condition is "not a secondary modification which has arisen among mammals that were already frankly deciduate, but [is], on the contrary, a more primitive developmental phase" (**13**, p. 118), a view with which I am in complete agreement.

Possible Vestiges of other Fœtal Membranes.—In connection with the margin of the allantois there are, in some sections, appearances which I can only interpret as greatly altered remnants of the walls of the yolk-sac. These remnants vary very greatly in their detailed relations and in their extent,

[1] Later confirmed by Vernhout (**14**).

and are often entirely absent in the sections. It is unnecessary to enter into details.

I would simply refer to figs. 32 and 33, and point out that from the knob-like projection (*a*) attached to the margin of the allantois (*all. m.*) there arise two cell layers, one from each side. The one (*c*) may possibly be a vestige of the yolk-sac splanchnopleure, while the other (*b*) may similarly be a vestige of the vascular omphalopleure.

Whether this be so or not the fact remains, that besides these structures no other traces of the yolk-sac were found in the uterus, and this fact, taken in conjunction with the already described greater degeneration of the syncytium outside the placental area as compared with that of the latter, renders it almost certain that the omphalopleure breaks up and disappears some time previous to parturition.

Finally I may direct attention to the cells marked *ch. ect''*. in figs. 32 and 34, as they may possibly represent persistent marginal chorionic ectoderm cells.

Parturition.

In fig. 25 the genital organs of this stage are shown partially dissected. The left uterus (*l. ut.*) has been opened by a ventral longitudinal incision, so as to expose the placental area (*pl. a.*). The vaginal cæca (*v. cæc.*) and the bladder (*bl.*) are pushed over to the left side, and the allantoic stalk (*all. s.*) arising from near the centre of the inner wall of the adherent allantois has been traced posteriorly. It was found to pass backwards through a posterior common portion of the two uteri (common uterine canal) into what sections show to be a median cleft-like passage in the connective tissue lying between the two lateral vaginal canals. Through this median passage, or median pseudo-vaginal passage, as we may term it, it is obvious that delivery must have taken place.

At the time of making this dissection I was unaware of the existence of any median passage in Perameles. Owen (**16**, p. 683), in his short description of the female genital organs of P. obesula, makes no mention of such; and, indeed, in

my own dissections of non-gravid genital organs I had discovered no such median passage. I was therefore considerably surprised to find the allantoic stalk extending straight back into the connective tissue between the lateral vaginal canals, and not into one of the latter, which I had believed must serve for the passage of the young at birth in spite of the narrowness of their communications with the uterine cavities. The novel features revealed in the dissection were, however, further elucidated by series of transverse sections across the urino-genital strand[1] (fig. 25, *u. s.*), which demonstrated the existence of a slit-like passage enclosing the allantoic stalks, one from each uterus.

On investigation the stalks could be traced down the median pseudo-vaginal passage from the centre of the inner wall of the allantois for a distance of about 3 cm. They did not extend quite to the extreme posterior end of the urino-genital strand shown in fig. 25, but this is no doubt to be accounted for by tearing of the stalks in the process of removal of the genital organs. In part of their course they were found to be looped upon themselves.

In Stage D the allantoic stalk of the larger embryo measured only about 8 mm. in length, so that shortly prior to or during parturition a very considerable lengthening of the stalks must take place. The stalk no doubt becomes severed from the embryo only at the moment of birth, leaving merely an insignificant portion (in length about ·5 mm.) attached at the navel of the latter (fig. 36). Similarly, in Erinaceus, Hubrecht (**9**, p. 347) has shown that "by far the longer portion" of the strand formed by the lengthened-out allantoic vessels at parturition remains attached to the afterbirth, which, though eventually shed, is found in the uterus shortly after delivery.

A section of the mid region of the urino-genital strand of

[1] This name is applied to the elongated mass of connective tissue in which are embedded the lateral vaginal canals and urethra. It is united to its surroundings by more areolar connective tissue, and is of very considerable length.

this stage is shown in fig. 35. It will be there seen that the median pseudo-vaginal passage (*med. p.*) is simply a cleft-like space in the central connective tissue of the strand, lying dorsally to the urethra (*ur.*) and between the lateral vaginal canals (*vag. l.*). Its walls are entirely formed by the connective-tissue core of the strand, and they exhibit no histological differentiation into coats, muscular or other. The passage is of somewhat varying outline, with a greatest long diameter of about 1·2 mm., and a short diameter of ·6 mm. In this cleft together with the allantoic stalks there occur masses of coagulated blood (*c. bl.*), especially abundant along the dorsal portion of the passage, where indeed the clot in certain sections forms a definite ovalish mass almost as large as the allantoic stalks, and partially separated from the rest of the passage by an imperfect fibrous septum. This clot, however, is continuous anteriorly and posteriorly with that present in the main subdivision of the channel, and also with the extravasated blood so abundantly present in the surrounding connective tissue (fig. 35, *c. bl.*). The allantoic stalks (fig. 35, *all. s.*) are somewhat oval in outline, and measure ·3 mm. by ·2 mm. in diameter. They are now in process of histological degeneration. In the centre of each the cells appear clear and vesicular, and the nuclei are for the most part quite degenerate; marginally they stain very deeply. The allantoic vessels are either empty or are partly occupied by degenerating, mainly enucleated, fœtal blood-cells, together with a granular deeply staining detritus. Their endothelial lining has disappeared, and their mesodermal wall is enucleated and fibrous-looking. In some sections the allantoic canal can be indistinctly made out, but no longer with an entodermal lining.

Direct observations of the parturition phenomena in Marsupials are by no means numerous. I know of only three accounts:—(1) Owen (**16**, p. 721) quotes from a paper by Rennger to the effect that in Didelphys azaræ the young "in gestation make the circuit of the lateral canals in which they are found to be deprived of their fœtal envelopes;" (2)

Osborn (11, p. 377) records finding in Didelphys virginiana "the fœtal membranes . . . crowded into the uterine orifices of the vaginæ, which indicates that they had been detached from the embryo in the uterus itself;" (3) Stirling (17) furnishes a valuable account of the parturition in Macropus robustus [Osphranter erubescens]. He has shown that in this form the young one passes out through the median vaginal canal; and that while the ventral portion of the yolk-sac remains in the uterus, interdigitating with the folds of the mucosa, its dorsal portion, remaining attached to the fœtus, becomes, as the latter passes down the median vaginal canal, drawn out into a long stalk carrying the three vitelline vessels. The two forms, wallaroo and bandicoot, thus agree in giving birth to the young through a median channel; but the median canal of the one with its definite walls is by no means homologous with the median cleft-like passage of the other; for while the former is morphologically continuous with the lateral vaginal canals, and is a true epithelially lined tube, the latter has no connection whatever with the lateral canals, at no time possesses an epithelial lining, and in fact is non-existent prior to the first parturition.

It may further be pointed out that in the behaviour of their fœtal membranes at parturition the two forms exhibit an interesting parallel and contrast. In the wallaroo, while the extra-embryonic allantois has disappeared at birth, the yolk-sac remains persistent in the uterus, and is drawn out into a long cord, which remains connected with the embryo in its passage outwards. In the bandicoot, on the other hand, it is the allantois which similarly remains attached to the embryo by its stalk during its course down the median passage, and which persists in the uterus, while the yolk-sac has entirely disappeared. This parallel behaviour of non-homologous structures, by means of which nutriment is conveyed to the fœtus, tends to suggest that the passage of the young outwards is a quite gradual one.

The discovery of this unique mode of parturition in Pera-

meles led to a re-investigation, by means of serial sections of the structure of the female genital organs, especially with reference to the question of the existence or otherwise of such a median pseudo-vaginal passage in virginal and non-gravid genital organs. The results of this investigation will be set forth in detail elsewhere. Suffice it here to state that in the virginal genital organs the two uteri do not open into each other posteriorly, and there is no trace of a median vaginal passage or of any epithelial or other track, which might indicate the site of a future passage of any kind whatever.

In the non-gravid organs of animals with large pouch-young, on the other hand, the median pseudo-vaginal cleft is found to exist, but it neither stands in open communication with the common uterine canal nor does it open into the cloaca. As in the post-partum stage, the passage is wholly destitute of any epithelial lining or any other specialised wall.

As to the mode of formation of this median passage in the first instance I am unable to come to any definite conclusion. It has just been stated that in the virgin the uteri do not communicate with each other posteriorly, and no median passage exists. The latter is, then, evidently formed either just before or at the first act of parturition. That the embryo should in its passage out literally bore its way through the connective tissue seems to me improbable, but at least it would seem as if the hindrance to the exit of the fœtus offered by the narrow opening of the uterus into the lateral vaginal canals was actually greater than the resistent power of the tissue between the posterior ends of the uteri, and that rupture of the latter must occur. That some such rupture does occur is evidenced not only by the appearance of the false passage, but also by the pretty extensive extravasations of blood found both in and surrounding the track followed by the fœtus during its egress, i. e. the median pseudo-vaginal passage.

It is evident that the detailed character of the phenomena of parturition, and above all the nature of the causes producing the extraordinary condition above described, can only be definitely ascertained by the examination of the genital organs

of a female immediately prior to the commencement of the first parturition. But whatever may be the precise mode of formation of the passage, this most remarkable method of getting rid of the young would seem to be without parallel in the whole mammalian class.

New-born Young.—Here[1] I need only point out that, so far as my observations (cf. appendix) go, the new-born Perameles does not appear to differ to any very great extent, in its degree of development, from the new-born young of undoubted non-placental Marsupials, e. g. Didelphys.

Stage F.—P. obesula (post-partum).

The material for this stage consisted of the genital organs (less the cloaca) of a female with two pouch-young measuring from crown to rump 22 mm. The left uterus, the larger of the two, measured 15 mm. in length by 6 mm. in breath.

Microscopical examination shows that the uterus has now almost completely regained the resting condition.

The mucosa, however, is just about half as thick as that of the resting uterus. The epithelium of the uterine glands has been laid down anew, and now consists of a low cubical epithelium with fairly large ovalish nuclei. The gland lumen is nearly always occupied by a finely granular coagulum, which may contain cellular constituents. The interglandular connective tissue is in parts fairly open in appearance, consisting of a network of anastomosing cells; in other parts it is quite dense and compact owing to the presence of great numbers of young connective-tissue cells.

The syncytium and allantois have completely disappeared, and the uterine epithelium now forms a continuous layer all over the surface of the mucosa. It consists of a low layer of cubical cells with rounded closely packed nuclei in a single row.

Over the greater portion of its extent the uterine surface is

[1] I hope later to return to this question, and also to consider therewith the question of the "critical period" in Perameles (J. Beard, 'On Certain Problems of Vertebrate Embryology,' Jena, 1896).

comparatively smooth and flat; but in a certain section on the ant-mesometrial side of the uterus it is markedly folded and very irregular in contour, owing to the presence of irregular projections of the uterine epithelium. This folded area no doubt represents the former allantoic placental area. The projecting portions of the uterine epithelium just mentioned are apparently eventually shed off into the uterine lumen, for the lumen at this stage contains a detritus consisting of maternal red blood-corpuscles, together with cellular elements evidently derived from these irregular projections. The presence of these projections is readily intelligible when one remembers how irregular was the surface of the allantoic placental syncytium.

Genital Organs.—In this stage the lumen of the common uterine canal is still continuous with the median pseudo-vaginal passage. This latter, about its mid region, measures ·7 mm. in long and ·12 mm. in short diameter. It now appears lined by a very delicate layer of connective-tissue endothelium, outside which the tissue is very compact and vascular, but the extravasated blood present in it in the preceding stage has now disappeared. In its middle portion the passage contains an irregular detritus consisting of red blood-corpuscles and cellular elements.

Anteriorly, just behind the point of opening of the common uterine canal into the passage, portions of allantoic stalks are found still persistent in a degenerate condition, but with the positions of the allantoic vascular trunks still recognisable. The stalks are three in number,[1]—a larger one measuring in diameter ·4 mm. by 3· mm., and two smaller ones with a diameter of ·2 mm. each. In the region where the remains of the allantoic stalks are found, the lumen of the passage is almost completely obliterated, since the stalks are not only closely surrounded externally by a loose layer derived from the surrounding connective tissue, but are separated from

[1] The genital organs reached me with only two young. It may be that the larger and more degenerate stalk here described has persisted from a previous parturition.

each other by delicate partitions derived from the latter. The stalks now present quite a reticular appearance; the larger one stains less deeply than the other two, and has undergone marked fibrous degeneration. The nuclei are few in number, and stain deeply and homogeneously. The lumina of the allantoic vessels are occupied more or less completely by loose branching cells.

In two other sets of genital organs, one from a P. obesula with pouch-young measuring 4 cm., I have found similar persistent remains of allantoic stalks in the upper portion of the pseudo-vaginal passage in various stages of degeneration and absorption. It is not necessary here to describe the appearances in detail. Suffice it to say that the enucleated stalks, closely invested by a connective-tissue sheath, undergo marked fibroid degeneration, and eventually become invaded and broken up by the ingrowth of the surrounding connective tissue.

I may point out here that the existence of these remains of the allantoic stalks, blocking up the pseudo-vaginal passage, shows conclusively that the vesicular portion of the allantois must be absorbed *in utero*, a view already maintained on account of the presence of maternal leucocytes in it.

Concluding Remarks.

Before concluding this paper we may briefly inquire what conclusions may legitimately be drawn from the fact of the occurrence of an allantoic placenta among the Metatheria.

Has the allantoic placenta of Perameles been independently evolved within the limits of the Marsupial order or is it directly and genetically related to that of Eutheria through the common ancestry of the Meta- and Eu-theria from an earlier diphyodont protoplacental stock? In a previous paper (**18**) in this Journal, on the tooth development of Perameles, by Professor J. T. Wilson and myself, we incidentally touched upon this question, and expressed our preference for the latter of these two views; and I may here at once say that a much fuller knowledge of the details of the placentation process in

Perameles has in no whit served to weaken our previously expressed opinion.

In view of the present very incomplete state of our knowledge regarding the condition of the fœtal membranes in other Australian polyprotodont Marsupials, especially in Dasyurus and Myrmecobius, and even of the precise uterine changes in Phascolarctus and other Diprotodonts whose fœtal membranes have been examined, it is impossible to decide finally between these two views, which alone seem to us worthy of consideration. In the concluding section of the paper just referred to we presented in brief form a case in favour of the second alternative. And in this summing up we dealt with the bearings upon the case of facts relating to the dentition, the placentation, and the mammary function. Here it is proposed rather to treat shortly of those facts and considerations which, in the opinion of the writer, tend to negative the first alternative.

From the preceding account of the placentation phenomena in Perameles I think we may justly conclude that the processes of utero-gestation in that form are fundamentally the same as those occurring in the more generalised Eutherians. Such differences in detail as exist are, in my opinion, to be regarded either as evidences of primitiveness of type on the part of the Perameles placenta, or as physiological adaptations such as Hubrecht has pointed out we may expect to find in different types of placentation, in view of "the great youth of the placenta as compared with the other chief components of the organisation of a mammal" (**9**, p. 388).

I wish here more especially to lay emphasis on my conviction that it is just as impossible to draw a hard and fast line between the placentation phenomena as they occur in Perameles and in the lower Eutherians, as it is to arbitrarily mark off from each other the various types of placental formation occurring among the Eutheria themselves.

Now it seems on a priori grounds exceedingly improbable that an allantoic placenta should have been twice independently acquired, and in such a fundamentally similar manner

within the limits of the mammalian class. Such would, in our opinion, be a most remarkable instance of parallelism.

It is true that in the existence of a vascular syncytium formed from the uterine epithelium, the placenta of Perameles exhibits a modification of structural arrangement of a kind occurring in no other mammal. But it cannot be held that the existence of even such a unique modification gives support to the view of the genetic independence of the placenta of Perameles, any more than the existence of manifold and even more marked structural differences in the types of placentation occurring among the Eutheria themselves witnesses to their essential morphological diversity. In each of these cases alike we find the real, or at least by far the most probable, explanation in a differentiation of truly homologous parts due primarily to physiological adaptation.

It is no doubt a tempting and easy solution of the problem to regard the allantoic placenta of Perameles as a direct and natural advance upon such a condition of fœtal membranes as occurs in Phascolarctus (alone, so far as is yet known, among marsupials), where, as Caldwell (**12**) and Semon (**8**) have shown, the vesicular allantois reaches and fuses with the discoidal area of true chorion, develops a rich respiratory surface, but forms no union with the uterine wall. And certainly, if the placenta of Perameles be an independent acquisition within the marsupial order, Phascolarctus would seem to present the more primitive type of arrangement of fœtal membranes. As Semon (**8**) has pointed out, "wir haben uns nur vorzustellen, dass beim Phascolarctus—Typus im Bereich der Athemfläche der Allantois eine innige Vereinigung der Keimblasenwand mit den mütterlichen Geweben eintrat um auf den Urtypus der Eihüllenanordnung der Placentalier zu kommen." At the same time he makes the further important qualification, "Deshalb, weil sich bei Phascolarctus in der Anordnung seiner Eihüllen primitivere Zustände erhalten haben als bei den meisten anderen Marsupialieren, halte ich ihn natürlich nicht überhaupt für ein besonders primitives Beutelthier, oder gar für den Stammvater der übrigen." But,

indeed, the evidence on all hands goes to show that the Diprotodontia represent a comparatively recent offshoot from a primitive polyprotodont stem. And we are entirely unable to accept the derivation of the Eutheria from a Perameles type through a Phascolarctus type, as suggested by Semon (**21**, p. 310). For it must ever be borne in mind that on the strength of the evidence derived from a study of dentition the whole marsupial order constitutes a well-marked natural group, exhibiting like characteristics of degeneracy from the typical and original mammalian condition. And, in this group, Phascolarctus is distinguished not by less, but by an even greater degree of retrogressive dental modification than Perameles. It therefore seems unlikely that the former should have retained unmodified more primitive embryonal nutritive arrangements than the latter. So far, indeed, as the decision of this problem can be shown to depend upon the question of the primitiveness of the general structural organisation exhibited by Phascolarctus and Perameles respectively, it can hardly be denied that the evidence at our disposal is strongly in favour of the latter to be regarded as representing a more archaic marsupial type.

It is, of course, possible that in the remote past the immediate promammalian (?) ancestors of the protoplacental stock may have exhibited a condition of the fœtal membranes somewhat resembling that of Phascolarctus; but we are unable to avoid the conviction that as in dentition, so in its embryonic appendages, Phascolarctus has shared largely in the general marsupial decadence. And the fact that in all non-placental marsupials, with the single exception of Phascolarctus, so far as is known, the allantois never reaches the chorion, but remains buried in the extra-embryonic splanchnocœle as a rudimentary structure, with no respiratory function, we consider as indirect evidence in favour of our view. For, as Selenka and Semon have pointed out, this condition is certainly to be regarded as a secondary one; and if this be so, then the admitted existence of such a process of late secondary reduction renders our view of still earlier cœnogenetic sim-

plification, to say the least, not wholly improbable. Thus, in our view, it is unnecessary to trace the placental ancestry of Eutheria back into the marsupial group. The occurrence there of a true allantoic placenta, and its absence in the majority of members of the order, do no doubt, at first sight, suggest that in this group we must find the first beginnings of the organ. But we believe that the explanation is to be found in the fact that marsupials are, after all, a markedly specialised group, and that in it conditions have obtained producing placental disappearance, just as conditions (probably identical in character) have determined the degeneration of other early nutritional arrangements, i. e. the milk-teeth. We therefore fall back upon the view that the Metatheria and Eutheria are the divergent branches of a common ancestral stock, which was not only diphyodont but also placental.

We may next inquire whether the facts and conclusions detailed in the present paper have any bearing upon the question of the condition of the fœtal membranes in these primitive Placentalia.

We believe that the facts of placentation in Perameles most strikingly confirm and support the opinion of Balfour (**19**), that in the primitive types of Placentalia both the allantoic and yolk-sac vessels may have been concerned in maintaining a placental circulation. We have insisted on the fundamental similarity of the placentation of Perameles with that of the more generalised Eutheria; and if we select Erinaceus as representing a fairly generalised Eutherian type, we find that here, according to Hubrecht's account (**9**), just as in Perameles, an extensive yolk-sac (omphalopleural) placental connection is developed at an early stage, only to be replaced later by the formation of the definitive allantoic placenta, through the union of a large vesicular and vascular allantois with the non-vascular chorion. Now if we leave aside the trophoblastic differentiation in the one and the formation of a maternal syncytium in the other, the type of placentation occurring in these two generalised Metatherian and Eutherian forms is an essentially similar one. This fact can in our

opinion only be regarded as conclusive in favour of the view that from such a condition of fœtal membranes as is common to these two types, Perameles and Erinaceus, phylogenetic speculation on the placenta must start. We would, therefore, attach very special phylogenetic importance to the non-separation in marsupials of the vascular omphalopleure into yolk-sac splanchnopleure and somatopleural chorion. This non-separation, ensuring, as Semon (**8**) has pointed out, the retention of the vessels of the vascular area in a superficial position, eminently suited for the performance of nutritive and respiratory functions, we can only regard as a physiological adaptation, and as probably the first to have been adopted on the initiation of uterine development in the common ancestors of the Metatheria and Eutheria. This condition is not "probably a purely marsupial modification," as Minot would have us believe (**6**, p. 129), for it is undoubtedly also manifested in the occurrence in certain lowly Eutherians of a temporary yolk-sac placenta preceding the formation of the definitive allantoic one. As Hubrecht has shown in Erinaceus (**9**), it is only after the allantoic placenta has taken the place of the omphalopleural that the mesoderm of the vascular omphalopleure splits into splanchnic and somatic layers, and this delaying of the splitting process Hubrecht (**20**) attributes to the vital importance of the yolk-sac placenta.

It is not necessary to dilate on the significance of the discoid form of the allantoic placenta of Perameles. "On the Balfourian hypothesis," as Professor G. B. Howes has pointed out, the view that the discoidal type of allantoic placenta is the most primitive "is by far the most natural one warranted by the facts."[1]

University of Sydney, N.S.W.;
April 16th, 1897.

[1] 'Nature,' vol. xl, p. 420.

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EXPLANATION OF PLATES 29—33,

Illustrating Mr. Jas. P. Hill's paper on "The Placentation of Perameles."

("Contributions to the Embryology of the Marsupialia," I.)

All sections drawn were outlined by means of Zeiss's camera lucida.

List of Common Reference Letters.

all. a. Allantoic artery. *all. c.* Allantoic cavity. *all. cap.* Allantoic capillary. *all. cl.* Allantoic canal. *all. ent.* Allantoic entoderm. *all. mes.* Allantochorionic mesenchyme. *all. v.* Allantoic vein. *bil. omph.* Bilaminar omphalopleure. *c. m.* Circular musculature. *cæ.* Extra-embryonic splanchnocœle. *cæ. w.* Inner (cœlomic) wall of allantois. *ch. ect.* Chorionic ectoderm. *ch. ect'.* Isolated chorionic ectoderm cell. *c. t.* Interglandular connective tissue. *ect.* Ectoderm. *ent.* Entoderm. *ex. syn.* Syncytium beyond allantoic placental area. *gl.* Uterine gland. *p. leuc.* Polynuclear leucocytes. *pl. a.* Allantoic placental area. *pl. syn.* Syncytium of allantoic placental area. *som.* Somatic mesoderm of chorion. *s. t.* Sinus terminalis. *syn.* Syncytium. *syn. c.* Capillary of syncytium. *syn. l.* Syncytial lobule. *vasc. omph.* Vascular

omphalopleure. *vit. a.* Vitelline artery. *vit. v.* Vitelline vein. *y. spl.* Invaginated yolk splanchnopleure.

N.B.—Unless otherwise stated, sections are transverse.

Fig. 1.—Wall of non-gravid uterus, Perameles. *s.* Serosa. *m.* Mucosa. *ep.* Uterine epithelium. × 55.

Stage A.

Fig. 2.—Portion of the syncytium, showing the numerous nuclei in a continuous protoplasmic layer. × 740.

Stage B.

Fig. 3.—Portion of left uterine wall (serosa not shown). *m. c.* Capillary of corium. × 55.

Fig. 4.—Portion of the uterine syncytium, showing the lobular character of its deeper surface (*syn. l.*), the arrangement of the nuclei, and the presence in it of maternal capillaries (*syn. c.*). *l.* Leucocytes. *m. c.* Capillary of corium. × 40.

Stage C.

Fig. 5.—7 mm. embryo, P. nasuta. *all. s.* Allantoic stalk. *y. s.* Yolk-stalk. × about 8.

Fig. 6.—Section passing through the margin of the allantoic placental area, and including the whole breadth of the vascular omphalopleure (*vasc. omph.*) on one side, and a portion of the bilaminar omphalopleure (*bil. omph.*), together with the adjacent syncytium (*ex. syn.*). *y. s.* Cavity of yolk-sac. × 118.

Fig. 7.—Section of marginal portion of the allantoic placental area. × 140.

Fig. 8.—Portion of allantoic placental area centrally from Fig. 7, showing the chorionic ectoderm (*ch. ect.*) as a continuous layer of enlarged cells adherent to the syncytium (*pl. syn.*), as in Fig. 7. × 140.

Fig. 9.—Portion of the central region of the allantoic placental area, showing degeneration of the chorionic ectoderm (*ch. ect.*) × 230.

Fig. 10.—Section of allantoic stalk. × 140.

Fig. 11.—Section passing through opening of allantoic canal (*o. all. cl.*) into vesicular portion of allantois. × 118.

Fig. 12.—Portion of the bilaminar omphalopleure in section. × 220.

Stage D.

Fig. 13.—Right uterus opened up, showing embryo still partially enclosed in its membranes and in relation to the allantoic placental area (*pl. a.*). For description see text (pp. 411, 412). × about $6\frac{1}{2}$.

Fig. 14.—Section through the persistent portion of the proamnion. *amn.* amnion. × 140.

Fig. 15.—Section through the marginal portion of the allantoic placental area (cf. with Fig. 7). × 150.

Fig. 16.—Portion of the central region of the allantoic placental area (cf. with Fig. 8). × 150.

Fig. 17.—Portion of the allantoic placental area, showing the interlocking of the allantoic capillaries (*all. cap.*) with the vascular ridges of the syncytium. × 325.

Fig. 18.—Section passing through a gland opening in the allantoic placental area, occluded by an allantoic capillary (*all. cap.*). × 230.

Figs. 19 and 20.—Portions of allantoic placental area, showing isolated and greatly enlarged chorionic ectoderm cells (*ch. ect'.*). × 230.

Fig. 21.—Portion of allantoic placental area, showing an isolated multinuclear chorionic ectoderm cell (*ch. ect'.*) × 230.

Fig. 22.—Horizontal section of allantoic placental area, to show the network formed by the allantoic capillaries (*all. cap.*). × 225.

Fig. 23.—Section of allantoic stalk (cf. with Fig. 10). × 140.

Fig. 24.—Section passing through the sinus terminalis (*s. t.*), and including the adjacent portions of the vascular (*vasc. omph.*) and bilaminar omphalopleure (*bil. omph.*), together with the syncytium (*ex. syn.*) in contact therewith. × 240.

Stage E.

Fig. 25.—Genital organs from the ventral aspect. The left uterus (*l. ut.*) has been opened up, exposing the allantoic placental area (*pl. a.*), and the allantoic stalk (*all. s.*) has been traced back into the median pseudo-vaginal passage (*med. p.*). *bl.* Bladder. *f. t.* Fallopian tube. *r. ut.* Right uterus. *u. s.* Urino-genital strand. *v. cæc.* Vaginal cæca. × 2.

Fig. 26.—Portion of the corium of the mucosa beneath the allantoic placental area, showing the alterations in the connective tissue, uterine glands, and blood-vessels. *c. t'.* Hypertrophied interglandular connective tissue. *d. gl.* Gland with its lumen occupied by the disorganised epithelium. *m. v.* Vessel of corium with thickened walls. *m. v'.* Vessel with its lumen filled up by the proliferated endothelium. *m. v''.* Vessel with its lumen completely obliterated. *t. p'.* Thickened tunica propria around the uterine glands. × 325.

Fig. 27.—Portion of allantoic placental syncytium in section, to show degenerative and absorptive change. *p. leuc.* Polynuclear leucocytes. *sp.* Spaces in syncytial protoplasm. × 550.

Fig. 28.—Section through the marginal portion of the allantoic placental area (cf. with Figs. 7 and 15). *all. mes'.* Outer (placental) wall of allantois. *sp.* Spaces in syncytial protoplasm. *syn. o.* Lamellar outgrowth of the marginal portion of the placental syncytium. × 80.

Fig. 29.—Portion of the allantoic placental area, showing the syncytium (*pl. syn.*) and the outer (placental) wall of allantois (*all mes'.*). × 230.

Fig. 30.—Portion of the greatly degenerate syncytium outside the allantoic placental area, showing the regeneration of the uterine epithelium. *d.* Detritus occupying gland lumen. *ex. syn'.* Degenerating syncytium. *gl. ep.* Gland epithelium. *gl. o.* Opening of gland into uterine lumen. *r. ep.* Regenerating uterine epithelium. × 230.

Fig. 31.—Superficial portion of the syncytium (*ex. syn'.*) and the regenerated uterine epithelium (*r. ep.*). × 520.

Figs. 32, 33, and 34.—Sections illustrating certain appearances found at the margin of the allantoic placental area (cf. text, pp. 424, 425). *all. m.* Margin of allantois. *all. mes'.* Outer (placental) wall of allantois. *ch. ect''.* Persistent chorionic ectoderm cells (?). *syn. o.* Lamellar outgrowth from margin of allantoic placental syncytium. All × 230.

Fig. 35.—Section through the urino-genital strand of Fig. 25, showing the two allantoic stalks (*all. s.*) in the median pseudo-vaginal passage (*med. p.*). *c. bl.* Blood coagulum. *ur.* Urethra. *vag. l.* Lateral vaginal canal. × 18.

Fig. 36.—P. nasuta, new-born young. × nearly 8.

APPENDIX.

Table of Comparison of the Organisation of the Embryos of Stages C—E.

	P. nasuta, Stage C. 7 mm. from crown to rump.	P. obesula, Stage D. 8·75 mm. from crown to rump.	P. nasuta, new-born. Stage E. 14 mm. crown to rump. Head length 6 mm.
Form of body	Marked cervical flexure. Facial region definitely established. External nares formed. Distinct hyomandibular groove and precervical sinus (Fig. 6).	Head still strongly bent, neck protuberance prominent. External auditory meatus and triangular ear pinna. Snout now more marked. Precervical sinus closed.	Head raised but bent at right angles with trunk. Lips fused to form "Saugmund." Prominent snout. Positions of eyes and ear pinnæ just recognisable, covered by epitrichium. Remains of allantoic stalk at navel (Fig. 36).
Limbs	In fore-limb, 5 digits indicated, the 3rd the largest. Hind limb still a flattened bud. In both, plantar surface still directed mesially.	In the fore-limb the 5 digits are now distinct, 1st and 5th quite small, 3rd the largest. Limb now flexed at the elbow. Plantar surface directed somewhat dorsally. Digits of hind limb still united, paddle-like, plantar surface mesially directed.	In fore-limb slender recurved claws on 2nd, 3rd, and 4th digits, the third digit the largest. In hind limb digits all indicated, but not free from each other; the 4th the largest.
Notochord and vertebral column	Notochord unconstricted, invested by a continuous mesenchymatous sheath.	Cartilaginous centra and neural arches laid down.	Marked cartilaginous centra with transverse processes and neural arches, the latter not yet united above spinal cord.

	P. nasuta, Stage C. 7 mm. from crown to rump.	P. obesula, Stage D. 8·75 mm. from crown to rump.	P. nasuta, new-born. Stage E. 14 mm. crown to rump. Head length 6 mm.
Nervous system	Distinct hemisphere anlagen. Dorsal and ventral spinal nerve-roots united. Spinal cord reaches tip of tail, but is here rudimentary. Anterior commissure of spinal cord distinct. Anterior and posterior white columns laid down. Lateral columns very thin. Hypophysis still connected with roof of mouth by a stalk with a narrow lumen.	Mesial hemisphere wall thickened in hippocampal region but still no fissura arcuata. Hypophysis no longer communicates with the oral cavity. Indications of "Sprossen."	Marked fissura arcuata along mesial hemisphere wall. Corpora striata developing. Hypophysis dorso-ventrally compressed. "Sprossen" not very marked.
Eye	Optic stalk still open. No pigment in outer wall of optic cup. Lens with cavity. Mesoderm has penetrated into optic cup.	Optic stalk still with a lumen. Outer wall of optic cup pigmented. Ovalish lens cavity. No differentiation of the retina into layers.	Retina deeply pigmented, no differentiation into layers. Eyelids united and covered by epitrichium.
Ear	Auditory vesicle surrounded by condensed mesenchyme Long ductus endolymphaticus. Evaginations for semicircular canals.	Anterior vertical semicircular canal now formed. Utriculus and sacculus still in wide communication. Periotic capsule condensed mesenchyme.	Semicircular canals formed, but perilymphatic spaces not yet differentiated. Cartilaginous periotic.
Nose	Slit-like nasal sac in open communication with mouth.	Nasal cavities still open directly into mouth. Shallow-grooved anlagen of the organs of Jacobson. Solid anlagen of lachrymal ducts.	Adult form nearly established. Turbinal projections arising. Jacobson's organ formed, and its cartilage laid down. Lachrymal duct opens into nose.
Mouth	Palate unformed.	Palate unclosed. Lens-shaped Zahnleisten anteriorly. Taste-buds differentiating on tongue.	Palate formed, and invests larynx posteriorly. Teeth anlagen. Tongue grooved, and with distinct taste-buds.

	P. nasuta, Stage C. 7 mm. from crown to rump.	P. obesula, Stage D. 8·75 mm. from crown to rump.	P. nasuta, new-born. Stage E. 14 mm. crown to rump. Head length 6 mm.
Alimentary canal, &c.	Œsophagus open. Separate dorsal and ventral pancreas anlagen. Cloaca not open to exterior. Lateral thymus anlagen. Yolk-sac cavity opens into gut.	Cloaca just opened to exterior, still remains of cloacal membrane. Yolk-sac no longer opens into gut. Thymus anlagen united mesially, but free posteriorly; they now reach the pericardium. Lungs now lobed, the right with a ventro-mesial lobe. Bronchi have branched out to form secondary bronchi. Diaphragm still incomplete close to the mesial line.	Pancreas completely formed. Thymus anlagen approximated in their mid-regions. Lungs with numerous simple alveoli. Cartilaginous rings round trachea. Diaphragm complete.
Heart and vessels	Distinct septum superius and septum spurium. Commencing division of the ventricular cavity indicated by an internal fold and external groove. Transversely expanded sinus venosus, opening into right auricular division. Truncus aortæ undivided. Two anterior dorsal aortæ. Yolk-sac circulation predominant.	Ventricular septum (septum inferius) almost reaches the cushions of the auriculo-ventricular ostium, likewise the septum superius, the auricles only communicating below its concave edge. Sinus venosus less extensive. Inferior vena cava established. Allantoic circulation predominant.	Adult circulation. Position of allantoic arteries still recognisable in urachus.
Urino-genital system	Mesonephros of considerable size, tubules convoluted and with distinct glomeruli. Wolffian ducts open into cloaca together with allantoic canal. Distinct supra-renal anlagen. Genital-leisten small.	Mesonephros now of large size. Peritoneal funnels of the Mullerian ducts laid down. Anlagen of ureters as short outgrowths from the posterior ends of the Wolffian ducts, surrounded by condensed mesenchyme. Genital-leisten distinct, indifferent (?).	Mesonephros still of great size. Mullerian ducts laid down for part of their course. Permanent kidney definitely established and of some size, with tubuli contorti. Ureters open into cloaca mesially to Wolffian ducts. Projecting supra-renal anlagen. Prominent genital-leisten, ♂ (?).

	P. nasuta, Stage C. 7 mm. from crown to rump.	P. obesula, Stage D. 8·75 mm. from crown to rump.	P. nasuta, new-born. Stage E. 14 mm. crown to rump. Head length 6 mm.
Skin and skeleton	Skeleton in the mesenchymatous stage.	Nucleated epitrichial layer on epidermis. No trace of hair, claw anlagen on 3rd and 4th fore digits. Skeleton cartilaginous.	Thick epitrichial layer on epidermis. Well-marked hair anlagen on snout and cheeks. No ossification in cartilaginous skeleton, which is now fully formed. Upper and lower jaw ossifications.

Comparing the above three embryos with the tables and figures in Keibel's 'Normentafel zur Entwickelungsgeschichte des Schweines (Sus scrofa domesticus),' Jena, 1897, they correspond, as nearly as one can judge, as follows :

Embryo C to No. 85, Fig. 24.
Embryo D barely to No. 91, Fig. 28.
Embryo E to No. 93.

On the Green Pigment of the Intestinal Wall of the Annelid Chætopterus.

By

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With Plates 34—37.

I. Introduction.

In the year 1864 I obtained specimens of Chætopterus variopedatus, Renier (at that time called C. insignis by Baird), when collecting at Herm in the Channel Islands.

My specimens preserved in alcohol gave to the spirit a strong blackish-brown coloration, and the fluid was observed to have a deep red fluorescence. I showed the coloured fluid to Professor Stokes, of Cambridge, where in 1864 I was an undergraduate, and he rapidly examined it with a direct-vision spectroscope. He pointed out to me the remarkable absorption bands which the fluid caused in the spectrum of light passed through it, and expressed the opinion that these were similar to if not identical with those caused by some solutions of chlorophyll. In view of the fact that the colouring matter was soluble in alcohol and caused a red fluorescence, as well as a banded absorption spectrum resembling that of chlorophyll, Professor Stokes was of the opinion that the source of the colour was probably to be found in chlorophyll swallowed by the marine worm which had been immersed in the spirit.

A year or two later I acquired a Sorby-Browning spectroscope of my own, and became familiar with the absorption spectra of chlorophyll, of hæmoglobin, of turacin, and many

other organic pigments. I satisfied myself that the pigment derived from Chætopterus was in no way connected with the lodgment of particles of green vegetable matter in its alimentary canal, but was due to a strong and abundant blackish-green substance formed in the actual walls of the middle portion of the alimentary tract of the Chætopterus. I supposed that this substance, on account of its solubility, fluorescence, and banded absorption spectrum, must be considered as a 'variety' or 'species' of "chlorophyll."

In 1872 I obtained at Naples specimens of the Gephyrean Bonellia viridis, and was extremely interested to find that they—like Chætopterus—imparted a strong greenish colour to the alcohol in which they were placed, accompanied by fluorescence.

This coloured solution I found also gave a very powerful series of absorption bands when examined with the spectroscope, and I erroneously concluded that this colouring matter too—from Bonellia—must be considered as a "chlorophylloid" substance.

In the first and second editions of the English translation of Sach's 'Botany' (Oxford, second edition, 1882, p. 767) a note is published in which, on my authority, it is stated that "a chlorophylloid substance" occurs in the intestinal wall of Chætopterus and in the integument of Bonellia.

I had observed that the absorption spectrum of the colouring matter of Bonellia, though resembling that of chlorophyll, yet differed considerably from it, and I proposed to myself to make a more careful examination both of it and of the green pigment from Chætopterus, with the use of adequate measuring apparatus and scale in fixing the position of the bands in their absorption spectra.

I was prevented from carrying out my purpose by the loss of my Chætopterus material and the difficulty of obtaining more, and by the pressure of other pieces of work.

It fortunately occurred to me in 1875 to ask my friend Mr. H. C. Sorby, F.R.S., to undertake the investigation of the colouring matter of Bonellia, of which I had a certain quantity.

The result was the interesting and important paper by Mr. Sorby published in this Journal in 1875, wherein he describes the acid, neutral, and alkaline conditions of this pigment, characterises it spectroscopically, and gives to it the name "Bonellein." I shall take the liberty of changing that name in the present paper to "Bonellin."

Bonellin was subsequently studied by Krukenberg ('Vergl. Physiol. Studien,' ii, 1882), and by Schenck (Sitzb. k. Akad. Wiss. Wien,' 72, ii), who did not add anything material to the observations published by Sorby. Krukenberg, indeed, fell into some errors on the subject.

After Sorby's demonstration of the independent and peculiar nature of Bonellin, I was more than ever anxious to re-examine the pigment of Chætopterus, but more than twenty years have elapsed before I have carried out my intention.

In the meantime my friend and fellow-student Moseley provided himself with a micro-spectroscope as part of his equipment when he served as naturalist on the "Challenger" Expedition, and he published on his return in this Journal (vol. xvii, 1877) a very interesting account of a number of colouring matters obtained from marine animals and examined by him with the spectroscope. One of the most remarkable of these was a pigment from the integument of species of Pentacrinus, which occurs in these animals in such abundance that the spirit in which they are first preserved becomes deeply impregnated with it, and will yield the pigment as a powder on evaporation.

Moseley gave the name Purple Pentacrinin to this substance. He showed that its solution in alcohol was fluorescent, and, like Bonellin, could be changed in colour accordingly as the fluid was rendered alkaline or acid. It gave a very definite set of three absorption bands, the position of which were fixed and recorded in a spectrum map by Moseley. The colour transmitted by the acid solution is "an intense pink," that by the alkaline is bluish green, whilst the fluorescence is red. A second equally striking colouring matter was called Antedonin by Moseley, and found by him in deep-sea species of Antedon

(not in the European forms), and also in a Holothurian. This pigment also shows an acid and an alkaline condition, and can be converted from one to the other an indefinite number of times. It is, however, soluble in distilled water, as well as in alcohol.[1]

The Bonellin of Sorby and the Pentacrinin of Moseley indicate the existence of a group of tegumentary pigments in marine animals, which whilst they resemble chlorophyll in their solubility in alcohol, non-solubility in water, in the fluorescence of their solutions, and in their banded absorption spectra, yet differ from chlorophyll or any known derivative of that substance in the exact position and number of their absorption bands, and in their relative stability when exposed to sunlight, but most characteristically in the fact that they undergo a striking change of colour and in the position of their absorption bands, accordingly as the solution is rendered acid or alkaline, whilst the change from the acid to the alkaline state and back again can be effected an indefinite number of times without destruction of the pigment.

I may state at once that the pigment from the intestine of Chætopterus appears to be one of this class of bodies, and I propose to speak of it as Chætopterin.

Whilst I do not propose on the present occasion to attempt a review or classification of animal pigments, I think it appropriate to point out that there are other green tegumentary pigments among Gephyrea, Chætopoda, and Arthropoda which have properties very different from those of the Bonellin group.

I shall only refer to three of these, and but briefly. In the present number of this Journal Professor Herdman describes a new green-coloured Thalassema, which he has been kind enough to dedicate to me. At first one would naturally be inclined to suppose that the green pigment of Professor Herdman's Th. Lankesteri must be identical in nature with

[1] The Holothuria nigra of the Cornish coast imparts a magnificent flame colour to the alcohol in which it is placed, and the solution has a brilliant green fluorescence. The absorption spectrum has not been studied but it gives, I believe, no detached bands.

that of the allied Bonellia viridis. It is, however, quite unlike it. In actual tint Th. Lankesteri is of a much brighter green than Bonellia viridis—tending to what is called apple-green, whilst Bonellia is rather to be described as chrome-green. The colour of Th. Lankesteri is exactly the same as that of Hamingia arctica. I am able to state this as I am, I believe, the only person who has seen and recorded by a coloured drawing this Gephyræan in a living condition. I dredged it in company with the Rev. Dr. Norman, F.R.S., and Professor Bourne, F.R.S., of Madras, at the mouth of Lervik Harbour, Stordoe, Norway, in 1882. Moreover, the green pigment of both Thalassema Lankesteri and Hamingia arctica differs from that of Bonellia in that it is not soluble in alcohol.[1] According to Professor Herdman's observations, the pigment of Th. Lankesteri is slightly soluble in formol. Whether this signifies more than that the water holding the formaldehyde in solution takes up the pigment, and would do so even were the formaldehyde not present, seems doubtful. I have no observation as to solubility in water in reference to the green pigment of Hamingia, but it appears to me highly probable that it is identical with that of Thalassema Lankesteri. In addition to their want of solubility in alcohol, these two pigments differ from that of Bonellia in not yielding a series of detached absorption bands. I determined this in the case of Hamingia, and Professor Herdman has done so for his new Thalassema. Thalassemin does not change its colour when acted on by dilute acids, whilst Bonellin is changed to a rich violet tint.

A second instance of green tegumentary pigments differing from the Bonellin group is presented by Idotea viridis, the Isopod crustacean. The pigment is in this case insoluble in water, alcohol, or benzine. It is of a brilliant grass-green colour, and is not improbably similar in character and origin to the green pigment situated in the skin of some Lepidopterous larvæ, and other adult leaf-frequenting insects. It is time

[1] See Professor Herdman's paper, and accompanying notes by Professors Sherrington and Noël Paton, and Miss Newbigin.

that an effort was made to arrive at a further knowledge of these insoluble pigments.

A third case to which I wish to allude is the green coloration of the blood of some Lepidopterous larvæ. My friend and colleague Professor Poulton has shown ('Proc. Roy. Soc.,' 1885, and vol. liv, 1893) strong reason to suppose that the green colour of the blood in these larvæ is determined by the presence of chlorophyll or of etiolin in the food consumed by them. He has shown that the blood gives an acid reaction; the suggestion made by him is that the green chlorophyll or its immediate antecedent passes through the wall of the alimentary canal from the digested food into the blood in a modified state, which he calls metachlorophyll. Such a passage seems, on the other hand, to be impossible without a very radical change in the chlorophyll, which is itself neither diffusible nor soluble in watery media. It is most desirable that the study of the green pigment in the blood and skin of Lepidopterous larvæ should be carried further. One circumstance which induces me to allude to it here is that Professor Baldwin Spencer, of Melbourne, in his fine memoir on Pentastoma published in this Journal in 1893, vol. xxxiv, p. 31, made a suggestion similar to that made by Professor Poulton in regard to the passage of chlorophyll through the intestinal wall. Professor Spencer found that the perivisceral fluid of Pentastoma was coloured blood-red by hæmoglobin, and he supposes that the hæmoglobin has passed from the cavity of the gut of the Pentastoma through its wall into the perivisceral fluid. Some of the Nematoid parasites of birds have a blood-red perivisceral fluid which has not been examined spectroscopically. Possibly it also is due to hæmoglobin, and might throw further light on the question. It is a noteworthy fact that the suggestion should be made from two separate sources, that non-diffusible substances like chlorophyll and hæmoglobin pass through the wall of the alimentary canal into the blood fluid unchanged or but little changed. There is evidently something here worthy of further investigation. With regard to the supposed occurrence of chlorophyll in the blood of Lepidopterous larvæ, Professor Poulton's spectro-

scopic observations seem to prove conclusively that the green pigment present in the blood is not chlorophyll. In order to prove that it is a direct derivative of the chlorophyll taken in with food into the alimentary canal, it seems to be necessary to study the derivatives of chlorophyll, and to show that by chemical processes a substance can be produced from chlorophyll having the absorption spectrum of Poulton's metachlorophyll, which it has not; having the power of resisting the destructive action of light, which it has not; capable of diffusing through a living membrane, and of existing in solution in an acid albuminous fluid, which it is not; and lastly of changing to an opaque blackish brown pigment when simply exposed to oxygen gas, which it is not.

II. Description of Chætopterin: its Mode of Occurrence and Optical Properties.

Mode of Occurrence.—Dr. Blaxland Benham has kindly furnished me with an account of the mode of occurrence of the intestinal pigment of Chætopterus variopedatus from observations made by him at my suggestion in Mr. Hornell's laboratory in Jersey in the summer of 1896, and on material preserved by him in formol and brought to Oxford. The drawings in Plate 34 are by Dr. Benham, who has also recorded the spectra of Chætopterin and has carried out similar observations on Bonellin in my laboratory at Oxford. I am greatly indebted to him for his assistance in preparing this account of the two pigments.

The body of this strange-looking Chætopod is divided into three regions, as shown in fig. 1. The dark green, almost black-looking pigment is confined to the intestinal epithelium of the middle region. It gives the whole of the inner surface a black appearance, and can be seen through the transparent tissues of the body-wall.

In a transverse section its disposition is seen to coincide with that of the entire epithelial layer of the intestine, as shown in fig. 2. In order to observe satisfactorily its natural

position, the use of alcohol must be avoided since the pigment is dissolved by that preservative. It is, however, insoluble in formol.

Claparède, in his 'Annélides Sedentaires,' has described the pigmentation of the epithelium of this region of the intestine. A careful examination, by teazing fresh material and also by sections of material preserved in formol, shows, according to Benham's observations, that the pigment occurs solely in the form of spherical corpuscles varying in size (fig. 4), and embedded in the protoplasm of the epithelial cells (fig. 3). These granules are not dissolved by alcohol entirely, but a colourless, oily-looking stroma, quite structureless and translucent, of the same shape as the original coloured granule, is left in the cell-body.

Claparède speaks of the pigment in the intestinal wall as "hepatic" pigment. Joyeux-Laffuie ('Archives de Zoologie Expérim.,' 1890) gives a detailed account of the distribution of the pigment-bearing cells in the intestinal wall; he figures the cells and terms them "cellules biliaires." Dr. Benham distinguishes the elongated ciliated cells which contain the green granules from other associated "gland-cells," of which there appear to be two varieties.

It is evident that the terms "hepatic pigment" and "bile-cells" are not open to the same objection when applied to these cells of the enteric epithelium, as when applied, according to the custom of writers of forty years ago, to the brown-coloured tunic of the earthworm's intestine, now often called the "chloragogenous" tunic or cells. The pigmented cells of the intestine of Chætopterus are really of enteric origin, as is the hepatic gland in Vertebrates, whilst on the other hand the chloragogenous tunic is part of the cœlomic epithelium.

It is impossible to suppose, in view of the fact that Chætopterus lives buried in the sand in a large parchment-like tube, that the intestinal pigment can have any function as pigment. On the other hand, it is not unlikely that it may eventually be shown that this green fluorescent "Chætopterin" is really representative of the biliverdin of Vertebrate bile.

An extremely interesting comparison suggests itself with the "entero-chlorophyll" described by Dr. MacMunn as occurring in corpuscles in the livers (gastric glands) of Mollusca and in other Invertebrata ('Proc. Roy. Soc.,' vol. xxxv, p. 370). Dr. MacMunn was probably ill-advised in using the term "chlorophyll" in connection with the substance discovered by him. I have no doubt that he was led by my own erroneous classification[1] of several green pigments in animals under the chlorophyll group. It is not possible to come to a conclusion from a comparison of the absorption spectrum assigned by Dr. MacMunn to his entero-chlorophyll with that of Chætopterin, since Dr. MacMunn's pigment is very difficult to obtain free from admixture with other substances. On the other hand, Chætopterin can be obtained in a fairly pure condition, so far as the admixture of other pigments is concerned, by isolating the mid-region of the body of Chætopterus and preparing the alcoholic solution from that region only. It is true that even so the solution contains fatty matters and other impurities, and that we have not yet obtained Chætopterin either as a pure thoroughly cleansed powder or in the crystalline condition.

An investigation of the chemical properties of Chætopterin has been undertaken at my request by Miss Newbigin in Professor Noël Paton's laboratory, and there is reason to hope that before long we shall obtain this body in a chemically pure state, and learn something as to its chemical constitution and properties, which cannot fail to throw light on its physiological significance and possible relationship to MacMunn's entero-chlorophyll.

The determination of the characters of a body occurring in such definite form in the enteric epithelium of one of the simpler forms of animal life cannot but lead to a better understanding of the physiology of the alimentary canal, and of the

[1] This error of course did not include the green pigments of Spongilla, Hydra, and such ciliate Protozoa as Stentor. In them there is no doubt that the green pigment is chlorophyll, and that it occurs, as in plants, in self-propagating corpuscles.

internal chemical activities of the cells, upon a knowledge of which a true physiology must be based.

Colour and Absorption Spectra of Chætopterin.—The freshly-prepared alcoholic solution of Chætopterin (as obtained from a fresh specimen of the mid-region of the animal's body) is of a blackish green colour by transmitted light (see Pl. 35, fig. 6), and shows a powerful red fluorescence, resembling in colour that of an alcoholic solution of chlorophyll. This solution is found to be neutral in reaction. When examined with the spectroscope it shows four detached absorption bands, the position and intensity of which are represented in Dr. Benham's drawing (Pl. 34, fig. 5, uppermost spectrum), but are more exactly shown in the valuable observations kindly made for me by Professor Engelmann, and recorded in the two charts on Pl. 36.

When the neutral solution of Chætopterin is rendered acid by a very slight addition of HCl, it assumes a fine indigo-blue colour, as shown in Pl. 35, fig. 7. The absorption spectrum is still four-banded, but the position of all the bands is shifted, notably of the two in the blue. Dr. Benham's drawing in Pl. 34, fig. 5, shows this; the exact position and intensity of the absorption is shown by the two dotted lines in Professor Engelmann's chart, Pl. 36. Professor Engelmann finds in a sufficiently thin layer of the coloured liquid a faint "fifth" band at wave length 500, indicated by a dip and rapid rise in the curve traced by the upper dotted line of his chart. In a layer of greater thickness (recorded by the lower dotted line) the differentiation of this band from the absorption on either side of it is (as in Dr. Benham's drawing) inappreciable, excepting by the most careful measurement and comparison.

The acidulated solution may now be rendered alkaline by addition of KHO or NaHO, when it assumes a bright lemon-green colour (Pl. 35, fig. 8). The alkaline solution still exhibits four detached absorption bands, but they are very much in the same position as those of the neutral solution. The difference in the colour of the neutral and the alkaline solution is due, as is shown very clearly by Professor Engel-

mann's chart (Pl. 36), not to a difference in the position of the points of maximum absorption, but to a difference in the position of the points of maximum luminosity, and consequently of the area and graduation of the absorption around its maxima. This is very difficult to represent or record by shaded drawings, but is given with absolute precision by Professor Engelmann's beautiful method of observation and record, of which I will give some explanation below. The alkaline solution can be rendered again neutral or acid, and the process reversed and repeated indefinitely.

III. Colour and Absorption Spectra of Bonellin.

I had been anxious to compare the absorption spectra of Bonellin and Chætopterin for myself, and after I obtained a supply of the latter was unable for some time to procure the former.

I heard, however, in 1896 that Bonellia was flourishing in the beautiful healthy tanks of the Laboratoire Arago, erected and directed by Professor Henri de Lacaze Duthiers at Banyuls-sur-Mer, near Perpignan. I accordingly wrote to that distinguished zoologist, stating my desire to examine living specimens of Bonellia in Oxford. With a kindness and courtesy for which he is universally known and beloved, Professor de Lacaze Duthiers sent to me from Banyuls, by express parcels-service, two bottles of sea water, containing each a magnificent specimen of Bonellia viridis, which arrived in Oxford in a perfect condition of living vigour. I was thus able to examine again the pigment Bonellin, and to satisfy myself as to the position in which it occurs in the body of Bonellia. My best thanks are due to Professor de Lacaze Duthiers, and are here recorded, for his great kindness.

Greef has already correctly described the mode of occurrence of the green pigment of Bonellia. It is distributed in the superficial ectodermic epithelium in the form of very fine granules which give the ectodermic cells a grass-green appearance. It also occurs as fine granules in clusters of subepidermic cells apparently belonging to the connective tissue.

From the specimens received in Oxford, after examination of the histological relations of the pigment, Dr. Benham prepared an alcoholic solution which we proceeded to study by means of the spectroscope, and the application of acids and alkalies. A portion of the pigment in alcoholic solution was sent by me in the spring of 1897 to Professor Engelmann, together with the solution of Chætopterin. I am thus able to give here a very accurate record of the absorption spectra of Bonellin (Plate 37), for the purpose of comparison with those of Chætopterin. It will be seen at once that the two bodies differ entirely from one another in colour and absorption phenomena, whilst agreeing in solubility, fluorescence, and in the exhibition of neutral, acid, and alkaline conditions.

The appearance of the absorption bands of alkaline and acidulated alcoholic solutions of Bonellin, as seen with the Sorby-Browning micro-spectroscope, are drawn by Dr. Benham in Plate 35, fig. 5. The freshly prepared solution of Bonellin is alkaline, of a deep chrome-green colour. It is in this condition that the pigment appears to exist in the skin of the animal (Plate 35, fig. 11). When neutralised the solution assumes a greyish-blue colour (Plate 35, fig. 9). The addition of a small quantity of acid to the neutral solution, changes the colour to a splendid violet (Plate 35, fig. 10). The absorption spectra of these three conditions of Bonellin have been described by Sorby and after him by Krukenberg and by Schenck (who erroneously regarded Bonellin as a form of chlorophyll).

It will be found that the statements of these authors (cited on p. 449) are at variance in minor details with one another, and also with what I now place on record as the result of the observations of Professor Engelmann.

The carefully neutralised alcoholic solution of Bonellin exhibits but four marked or isolated maxima of absorption (absorption bands), as shown by Professor Engelmann's chart (Plate 37). These are as different in position as they well can be from the four bands of Chætopterin. There is no need to refer any further to a possible relationship between these two bodies.

The acid Bonellin also exhibits four, and only four absorption bands, but these do not coincide with any of the four bands of the neutral solution. The alkaline solution presents a six-banded spectrum, and of these six it is remarkable that the strongest, viz. the first and the sixth, coincide in position with those of neutral Bonellin; whilst the remaining four are similar to those of acid Bonellin, but all shifted a little towards the red end of the spectrum.

I am not prepared to discuss here either Sorby's slight divergences from Engelmann's record or Krukenberg's theory of Bonellin and Bonellidin. My principal object is to show how widely Bonellin differs from Chætopterin (though resembling it in general characters), and to present an accurate record of the absorption phenomena of neutral, acid, and alkaline alcoholic solutions of the pigment as obtained from fresh specimens of Bonellia.

It now only remains for me to give some explanation of the method of observation and record of absorption spectra—introduced by Professor Engelmann,—without which the reader will not properly understand the value of Plates 36 and 37.

IV. Measurements of the Absorption Spectra of Chætopterin and Bonellin by Professor Engelmann.

Professor Engelmann kindly offered last year (1896), when I was on a visit to Utrecht, to apply his beautiful instrument for the measurement of the absorption of the luminous spectrum by coloured media to Chætopterin and Bonellin. I was very glad to avail myself of his kind offer, in order to procure a more accurate record of the position and intensity of the absorption bands given by those pigments than is possible with the ordinary micro-spectroscope. The charts forwarded to me by Professor Engelmann as a result of his examination of the solutions which I sent to him are reproduced in Plates 36 and 37.

The instrument used by Professor Engelmann is described by him in the 'Archives de Microscopie.' It is applied to the body of an ordinary microscope, and consists in an arrange-

ment by which the light from a powerful incandescent lamp is passed through two parallel slits, A and B, giving in the field of view of the eye-piece spectroscope two spectra exactly parallel to one another, and of exactly equal intensity of light. A diaphragm is made to traverse the field by the turning of a screw, so as to present for observation a narrow band only of the juxtaposed spectra. The exact wave length of this strip is given by a scale introduced. Thus a width of the spectrum corresponding to a range of only some two or three millionths of a millimetre in wave length and of measured position in the spectrum can be examined, whilst there is on either side absolute darkness. The portion of the strip of light thus studied belonging to the light coming through slit A can be compared, as to the amount of light present, with the identical representative portion of the spectrum belonging to slit B. Under the conditions so far stated, the intensity of illumination (amount of light) of each half of the strip (that belonging to spectrum of slit A, and that belonging to spectrum of slit B) are exactly and sensibly equal. If now there be placed in front of slit A a coloured transparent body, some of the light passing through that slit will be stopped. Suppose the travelling eye-piece diaphragm is adjusted so as to present to the observer a strip of each spectrum corresponding to a wave length which is partially absorbed by the coloured medium introduced before slit A, then the portion of the strip belonging to slit A will be sensibly dimmer than that belonging to slit B.

Now by a micrometer screw Engelmann can reduce the width of slit B until the amount of light coming through that slit is no greater than the amount coming through slit A, obscured as it is by the coloured medium. The amount of movement of the screw required to bring the light of slit B down to the intensity of that of slit A furnishes the measurement of the absorption due to the coloured medium for the wave length under observation (isolated by the travelling eye-piece diaphragm slit).

The micrometer screw is standardised so as to give readings in

percentages of the total amount of light passing through the slit when not obscured, and having an aperture of ·2 millimetre.

The percentage is then written off on the chart by a dot corresponding to the wave-length (right or left of vertical lines of the chart), the percentage itself being given by the higher or lower position of the dot in relation to the horizontal lines.

The following is the report kindly furnished to me by Prof. Engelmann, together with the table of measurements and the charts given in Plates 36 and 37. It will be seen that by the present method it is easy to calculate the form of absorption curve for a greater thickness of solution from the observation of that of a less, and vice versâ, and that this has enabled Prof. Engelmann to apply a satisfactory test to the accuracy of his observations, which it must be remembered depend upon a very delicate comparative judgment of the light intensity of the two adjacent strips of spectrum, one of which is gradually darkened by the turning of the micrometer screw until it is judged to be exactly equal in light intensity to the other.

UTRECHT; 25th April, 1897.

. . . At last I am able to send you the results of the quantitative colour-analysis of your Chætopterin and Bonellin. You will find them in the accompanying tables, and in graphic form on the four charts of curves.

The measurements were carried out with my micro-spectrophotometer ('Zeitschr. f. wissen. Mikroskopie,' Sept., 1888; 'Archives de Microsc.,' xxiii, 1888, p. 82, pl. iv; 'Onderzochingen gedann in het physiol. laborat. des Utrechtsche Hoogeschool.,' 3, xi, 1889, pp. 39—49) with the use of an Auer's incandescent lamp as the source of light. The slit width of the spectrum apparatus was in all cases 0·2 mm. The light intensity thus given without absorption is taken as = 100 for all wave lengths. Measurements were made of the intensity of the light for a large number of λ, after passage through a plane-parallel layer of coloured solution of 5 and also of 2 mm. in thickness.

The measure of this intensity was in every case the width of

the comparison slit, by which, for a given wave length, an apparently equal luminosity in the absorbed and in the comparison slits was given. The apparatus allows one easily to read off a slit-width as small as 0·0005 mm. Every measurement was five times repeated. The number set down on the records is in each case the mean. Their probable error is in general barely more than 1 per cent. of the measured value; only in the outer red and violet, and with very powerful absorption, is the error greater on account of the diminished intensity of light. The course of the absorption curve (as drawn on the charts) will then in all essentials faithfully represent the fact.

An objective test for the criticism of the trustworthiness of the measurement results is afforded by the comparison of the numbers arrived at when two different thicknesses of the absorbing layer are used. If for any wave length the intensity of the original light is weakened to $\frac{100}{x}$ per cent, by passage through a layer of the thickness 1, then for the same wave length the intensity ($= i$) after passage through a layer of the thickness n is given by the formula $i_n = \frac{100}{x^n}$. Accordingly, if we have measured the course of the absorption for a known thickness of layer, we can reckon it also for every other possible thickness of layer. I have carried out the calculation for neutral Bonellin and neutral Chætopterin, and inserted the calculated values in thick type in the tables. The agreement between calculation and observation is amply sufficient, especially when we consider that important alterations in the light intensity value must be brought about by minute changes in the position and breadth of the spectrum strip, the average light intensity of which is being determined, in those parts of the spectrum where there are sharp alterations in the absorption. The greatest care was given on this account to the exact position and borders of the spectrum strip, and every time it was carefully determined whether the wave length scale was exactly in its proper position. The breadth of the spectrum strips, separately

examined as to light intensity, was on the average equal to $\frac{1}{2}$·001 μ wave length. For example, for determining the intensity at $\lambda = 600$ $\mu\mu$, the spectrum strip lying between wave lengths 0·597 μ and about 0·630 μ was isolated by means of the ocular screw diaphragm slit (accordingly the rest of the spectrum shut off). In the outermost red, where the dispersion is too small, spectrum strips of 0·01—0·02 breadth were isolated.

The coloured solutions were examined in small glass chambers of known height, which I had prepared for the purpose by Zeiss. They are to be recommended also for merely qualitative spectroscopic observations on coloured solutions, since one can work with a very small quantity of fluid (a few cubic millimetres). I intend soon to describe them and explain their use more fully. You can get them from Zeiss.

As you will observe, I have analysed a neutral as well as an acid and alkaline solution. All three show characteristic differences, and indeed the colours also appear different to the eye. It is a pity that neither Bonellin nor Chætopterin have been prepared in a chemically pure state, and perhaps cannot be. If they were, one could make exact determinations upon the (clearly very great) influence of the solvent upon the concentration of the solution.

CHÆTOPTERIN.

λ = Wave lengths in $\mu\mu$ ($1 = 0{\cdot}001\ \mu$).

i_n = Intensity of the perpendicularly falling light passing through a plane-parallel layer of n mm. thickness, in percentages of the original light intensity.

a. Neutral alcoholic solution.					*b* = *a*, made acid by HCl.				*c* = *b*, made alkaline by NaHO.			
λ.	i_5.	i_2.	(i_2 calculated from i_5).		λ.	i_5.	i_2.		λ.	i_5.	i_2.	
700	62·5	87·7	(**83·0**)		700	50·4	79·0		700	57·0	75·0	
680	44·2	73·5	(**72·0**)		680	45·0	74 0		680	39·0	65·0	
670	22·5	54·5	(**54·0**)		670	30·0	65·0		670	18·0	52 0	
655	2·4	18·7	(**23·0**)	I. Min.	650	3·5	28·0	I. Min.	655	2·0	19·0	I. Min.
640	11·2	42·0	(**41·5**)		640	7·0	34·0		640	15·0	43·0	
625	30·6	57·5	(**61·5**)		625	29·0	59·0		620	23·0	54·5	
600	14·5	47·3	(**46·0**)	II. Min.	620	27·5	54·0		610	21·5	52·0	
									600	15·0	45·0	II. Min.
580	20·1	52·0	(**52·0**)		615	23·0	51·0		590	18·0	50·0	
570	21·0	53·7	(**53·0**)		597	15·0	44·5	II. Min.	575	17·5	47·5	
560	17·6	50·7	(**49·6**)		575	18·5	50·0		560	14·0	42·2	
535	12·8	43·2	(**44·0**)	III. Min.	560	14·0	44·0	III. Min.	550	13·5	41·0	
					550	16·0	46·0					
520	15·8	48·2	(**48·0**)		540	17·0	48·0		540	11·0	40·0	III. Min.
500	10·9	42·2	(**41·4**)	IV. Min.	533	15·0	44·0	IV. Min.	520	13·5	44·5	
					520	17·0	46·0					
480	14·3	45·4	(**45·7**)		510	17·5	48·5		510	8·5	37·5	
					500	16·5	47·5	V.? Min.				
460	9·8	39·2	(**39·6**)		490	17·0	50·0		500	5·5	32·5	IV. Min.
440	1·6	15·5	(**19·0**)		480	16·0	49·0		480	9·0	36·5	
					470	12·0	—					
420	0·7	9·5	(**12·0**)		460	9·5	45·0		460	4·5	22·0	
					450	3·5	—					
					440	?	24·0		440	2·0	13·0	
					(too little light)							
					420	?	15·0		420	?	5·0	

BONELLIN.

λ = Wave lengths in $\mu\mu$ ($1 = 0{\cdot}001\ \mu$).

i_n = Intensity of the perpendicularly falling light passing through a plane-parallel layer of n mm. thickness, in percentages of the original light intensity.

a. Neutral alcoholic solution.					*b* = *a*, made acid by HCl.			*c* = *b*, made alkaline by NaHO.		
λ.	i_5.	i_2.	(i_2 calculated from i_5.)		λ.	i_5.		λ.	i_5.	
700	74·2	89·8	(**88·8**)		700	80·5		700	77·7	
680	69·6	86·5	(**86·5**)		680	73·5		680	69·7	
655	60·8	83·2	(**82·0**)		645	64·9		650	49·7	
635	1·7	18·0	(**19·6**)	I. Min.	630	48·9		635	10·6	I. Min.
615	47·5	72·2	(**74·2**)		620	16·7		625	23·3	
605	50·4	75·2	(**76 0**)		613	6·5	I. Min.	614	20·3	II. Min.
595	53·7	78·2	(**78·0**)		600	36·9		605	35·2	
585	50·9	75·8	(**76·3**)	II. Min.	590	43·4		595	40·5	
560	62·3	80·3	(**82·9**)		570	38·2	II. Min.	585	37·0	III. Min.
540	53·9	78·0	(**78·2**)		552	39·3		565	39·0	
520	50·5	75·8	(**76·1**)	III. Min.	545	37·3	III. Min.	550	37·3	IV. Min.
510	52·0	77·7	(**77·0**)		535	39·8		540	37·7	
490	23·1	56·8	(**55·6**)	IV. Min.	515	35·6	IV. Min.	530	36·0	
470	45·8	71·0	(**73 2**)		500	38·3		520	33·8	V. Min.
					480	49·9				
450	49·6	73·0	(**75·5**)		460	52·6		510	36·7	
					440	52·1				
430	50·2	73·5	(**75·8**)		433	47·1		490	30·7	VI. Min.
					430	43·3				
					425	40·7		470	45·8	
					420	37·7	V. Min.	450	55·0	

EXPLANATION OF PLATES 34—37,

Illustrating Professor Ray Lankester's Memoir on "The Green Pigment of the Intestinal Wall of the Annelid Chætopterus."

PLATE 34.

Fig. 1.—Chætopterus variopedatus, drawn of the natural size, as seen when removed from its tube. *A.* Anterior or cephalic region. *B.* Mid-region (in which the dark pigment occurs). *C.* Posterior region. *a.* Pinnule (modified notopodium of the 11th segment). *b.* Median sucker formed by modification of the pair of notopodia of the 12th segment. *c.* "Fans" formed by fusion of right and left notopodia in segments 13, 14, 15. *d.* Neuropodia of segments 12, 13, 14, 15. *e.* Right nephridiopore of the 15th segment. *f.* Notopodial cirrhi of posterior segments. *g.* Neuropodia of posterior segments. *h.* Right phosphorescent gland at the base of the right pinnule. *int.* Pigment of the intestinal wall showing through the integument.

Fig. 2.—Transverse section of the body of Chætopterus variopedatus taken at the point marked *int.* in fig. 1. (From a drawing by Dr. Benham.) *a.* Dorsal musculature forming a median crest or ridge. *b.* Transparent integument. *c.* Connective tissue holding the gut-wall to the body-wall. *d.* Cavity of the gut. *e.* Green pigmented epithelium of the gut. *f.* A nephridium in section. *g.* Ventral musculature. *h.* Nerve-cords.

Fig. 3.—Epithelial cells of the gut to show the position of the green granules. From a section made from a specimen preserved in formol so as to avoid the solution of the green granules which occurs when alcohol is employed. *a.* Free surface of the epithelium. *b.* Branched base of an epithelial cell. *c.* Oval nucleus.

Fig. 4.—Some of the green granules detached from the epithelial cells and more highly magnified, showing their varied size and spherical form. (Drawn by Dr. Benham.)

Fig. 5.—Absorption spectra of Chætopterin and Bonellin as seen with Sorby's micro-spectroscope. (Drawn by Dr. Benham.) Besides the shading or absorption in the form of bands of greater or less breadth, the position of the chief Frauenhoffer lines is indicated, and the whole spectrum is divided into thirty-five spaces, the divisions between which correspond to wave lengths ranging from 400 millionths of a millimetre on the right (blue end) to 750

millionths of a millimetre on the left (red end)—a division line being ruled at every point corresponding to the position of a difference in wave length of 10 millionths of a millimetre. In this drawing the dispersion of the spectrum as actually seen is represented, the intervals corresponding to 10 millionths of a millimetre of wave-length becoming increasingly larger as we pass from the red (wave lengths of 750—650 millionths) to the blue and violet (wave lengths of 500 to 400 millionths). But in the charts given in the next two plates, for which I am indebted to Professor Engelmann, the intervals occupied by wave lengths differing by ten millionths of a millimetre are laid down without reference to dispersion at equal distances from one another. The charts in fact correspond to a pure spectrum, whilst the drawing, fig. 5, represents the appearance given by a prism of small dispersion.

PLATE 35.

FIGS. 6—8.—Representation of the colour of the neutral, acid, and alkaline alcoholic solutions of Chætopterin, as seen by transmitted light.

FIGS. 9—11.—Representation of the colour of the neutral, acid, and alkaline alcoholic solutions of Bonellin, as seen by transmitted light.

PLATE 36.

Charts prepared by Professor Engelmann showing the intensity of absorption in different parts of the spectrum of acidulated, alkaline, and neutral alcoholic solutions of Chætopterin. The horizontal lines in the charts—numbered in ten groups of ten—correspond to 100 units of light intensity. The round dots indicate the successive parts of the spectrum observed and measured. The position of the dot in vertical displacement records the percentage of light transmitted. Thus the highest horizontal = 100 per cent., the lowest 0 per cent. or complete absorption. The successive "dot points" of observation are joined by oblique lines, giving thus a continuous but irregular curve of absorption. The vertical lines as shown by lettering on the chart correspond to millionths of a millimetre of wave length. The position of the chief solar lines is also indicated by strong vertical lines on the charts. The upper chart has the record of four distinct solutions. The two records in dotted lines are those relating to experiments with an acidulated solution—in the one case the light was passed through a thickness of the solution amounting to 5 millimetres, in the other case only 2 millimetres were used (of the same solution). [It is not possible in our present knowledge of Chætopterin to say what percentage of pure Chætopterin was present in the alcoholic solution.] The unbroken black lines are the records of similar experiments with an alkaline alcoholic solution of Chætopterin. In

the lower charts two records are given of the absorption of two different thicknesses (respectively 2 millimetres and 5 millimetres) of a carefully neutralised alcoholic solution of Chætopterin. (For further details and the comparison of the observed absorption of the smaller thickness of solution with the theoretical value calculated from that given by the greater thickness, the reader is referred to the text.)

PLATE 37.

Charts prepared by Dr. Engelmann of the observed absorption of the spectrum by acidulated, alkaline, and neutralised alcoholic solutions of Bonellin. Two thicknesses of each solution were made use of, and their absorption recorded. See explanation of Plate 36 and the fuller statements in the text.

Materials for a Monograph of the Ascons.—I. On the Origin and Growth of the Triradiate and Quadriradiate Spicules in the Family Clathrinidæ.

By

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With Plates 38—42.

Contents.

Preface.

The Ascons are a group of calcareous sponges which present many points of interest and importance to the zoologist. As sponges they occupy the lowest systematic position, for though the possession of a distinct skeleton is an advance upon the condition found in the so-called Myxospongiæ—supposing these not to be degenerate in this respect,—yet in almost all other points of organisation the Ascons show a far more generalised condition than is found in any other class of sponges. Regarded also not merely as sponges, but from the wider standpoint of the Metazoa generally, the Ascons claim our attention as extremely primitive types, characterised by a simplicity of structure scarcely equalled in any other form of animal life above the Protozoa. Not only, therefore, are many problems of sponge morphology and embryology to be found reduced to their lowest terms in the Ascon type, but it might be expected further that a careful study of the group would shed much light upon biological questions of still wider significance, since many facts of organisation and development which, in other animals, lie deep and are difficult to approach, are in these forms almost upon the surface, as it were.

For these reasons I have devoted myself for some time past to studies upon the structure, development, and classification of the Ascons, in the hope that a complete knowledge, so far as this is possible, of the group might form a material contribution to zoology. It might, however, appear to some that there would be but little of importance to discover in the group which was not already known, seeing how voluminous is the literature of the Ascons, and how many authors have published studies and monographs upon them even in quite recent times. So far is the subject from being exhausted, however, that the most elementary facts in their organisation still remain unknown or misrepresented, and it is not too much to say that the Ascons offer a field which, so far as results go, is almost unexplored. If this statement seems to many exaggerated, I can only hope to justify it by bringing forward

new facts and observations which will, I trust, bear me out in what I have said.

Studies upon a group of animals naturally fall under four main heads—systematic, structural, developmental, and physiological, including under the last-named category rather more than is meant by the term in our universities and medical schools. The first of these lines of study is independent of time and place, and can be conducted in any laboratory or museum with material fresh or preserved; the second and fourth can also be carried on at any time, given a good laboratory and aquarium and abundance of healthily living specimens; the third alone, that is the embryology, must be studied at the right season as well as in the right place, and the observer must be prepared when the season comes to lay aside other work for the time and devote himself to it. Thus, although I have been occupied for some time past with the histology of Ascons, the embryology has always been regarded by me as having a first claim on my time, and in consequence my histological studies have been disjointed, and are still very far from completion. At present my embryological material is nearly complete, and I hope before long to commence the publication of studies upon it; but as some time must elapse before a large quantity of material, comprising the whole developmental cycle of a number of species, can be worked through, it seemed best to bring out at once an account of some histological points in which my results approach to finality. The formation of the spicules so characteristic of these sponges has long engrossed my attention, and I trust that the descriptions and figures to follow will be held to substantiate my claim to have made out the main points in the morphology of the triradiate and quadriradiate spicules in the genera characterised by having the rays of the triradiate systems meeting at equal angles,—that is to say, the genera for which in my recent revision of the system I have used the names Clathrina, Gray, and Ascandra, Haeckel.

Even with regard to the development of the spicules I am well aware that I still have much to do. On the one hand, I

am not in a position as yet to give a detailed account either of the monaxon spicules in Clathrina, or of the three- and four-rayed spicules in Leucosolenia; on the other hand, many minute details in the development of these spicules, such as their exact relations to the secreting cells, and so forth—all such details, in fact, as require thin sections for their elucidation—remain for the present to be studied. But thin sections of the developing spicules and their formative cells are, as a matter of fact, very difficult to obtain. In the adult sponge new spicules are formed amongst and between older ones, which are often, and indeed usually, present in such masses as to make it hopeless to obtain thin sections without previous decalcification, which, by destroying the spicules, defeats the purpose in view, while thick sections are scarcely more instructive for the minuter points than are surface views. In the embryo, however, where stages can be obtained containing only minute spicules, thin sections of material not decalcified can be made without much difficulty. I have preferred, therefore, to let the questions of minute detail stand over for the present until I can return to them in the course of my embryological studies, and rather to bring forward my observations as they are than to wait until I can make them exhaustive. In the present memoir I propose to deal with the formation of the equiangular triradiates and quadriradiates rather from the morphological than from the cytological point of view,—that is to say, the number and arrangement of the cells which build them up, and their origin within the sponge. These are questions which for the most part can be studied satisfactorily only in surface views.

Before, however, proceeding to describe the results of my observations, I feel it my pleasant duty to say a few words of thanks and acknowledgment to those who have assisted me in my work and made it a possibility for me. In the first place I must acknowledge my indebtedness to two "pious founders," one of whom has been dead more than six hundred years, while the other, I am glad to say, is alive and well. But for my election to a fellowship on the Foundation of Walter de

Merton, it would, perhaps, have been impossible for me to have engaged in these researches at all. To Professor de Lacaze-Duthiers, on the other hand, founder of the marine laboratories of Roscoff and Banyuls, I am under the greatest obligation for his generous hospitality. On the frequent occasions on which I have visited these stations I have been made entirely free of all their resources, not only of laboratory and aquarium, but even of lodging, and have been treated with that uniform kindness and consideration which in France is always shown, without regard to race or nation, to those whom the fame of French scientific institutes attracts as visitors and students. In this way I have had at my disposal a rich and varied fauna, especially at Banyuls, the value of which can scarcely be estimated.

I must further express my warm thanks to Professor Ray Lankester for his kindness in giving me a place in his laboratory at Oxford, which has been, so to speak, my permanent head-quarters, where the greater part of my studies have been conducted. I am also indebted to him for much help and kind encouragement in his double capacity, both of Professor in the University and Editor of this Journal. Finally, I have to tender my thanks and gratitude to Professor R. Hertwig, of Munich, for the kindness and hospitality which he extended to me during a three months' stay at Munich last year, making me free of his laboratory and helping me in every way.

To each and all of those I have mentioned, whether of the thirteenth or nineteenth centuries, I wish to acknowledge my obligations and express my sincere thanks.

I. Introductory.

Before approaching the subject of spicule development a few words must be said, first, as to the technique employed, and secondly, upon the structure and histology of the sponge. In order to understand properly the origin of the spicule-forming cells, and to distinguish these cells from other tissue elements, it is necessary to be acquainted with the forms and

characteristics of the cells composing the wall of the sponge. I propose, therefore, to describe the histology in sufficient detail for the aim in view, reserving fuller treatment of the various issues for another paper.

(*a*) Methods of Investigation.—For studying the development of the spicules, and the general arrangement and characters of the cell elements composing the body-wall of an Ascon, no very elaborate histological technique is required; in fact, almost everything can be made out from surface views of pieces of the body-wall fixed with osmic acid, stained with picrocarmine, and laid out flat in glycerine. There is, however, one precaution, the importance of which cannot be too strongly emphasised: the sponges should not be brought back to the laboratory and there preserved, but should be fixed quite fresh from their habitat immediately they are found, care being taken to select fully expanded specimens.

My method of operating is to carry with me, when seeking for the specimens, different tubes containing osmic acid solution, distilled water, and picrocarmine, or other reagents as may be required. I take 1 per cent. osmic solution, and dilute it with an equal volume of sea water. The picrocarmine employed is either Ranvier's or Weigert's, both obtained from the well-known firm of Grübler at Leipzig. Ascons are very easy to preserve, even in liquids of very feeble penetrating powers, on account of the numerous cavities and channels, resulting from their structure as systems of thin-walled tubes. Hence quite large specimens can be fixed whole in osmic without fear. After being five to ten minutes in osmic the specimens are rinsed with water and placed in the picrocarmine. In this stain they can be brought back to the laboratory, but should not stay in it more than two hours; in general one hour is sufficient. The specimens are then washed with distilled water and transferred to glycerine or alcohol, according as surface preparations or sections are required. As a rule I put one half of each specimen so preserved into glycerine, the other half into alcohol.

This method preserves the cells, so far as shape, appearance,

and cytoplasm are concerned, most excellently, and has the great advantage of not acting upon the spicules or corroding them in any way; though should it be required to remove the spicules, the specimens can easily be decalcified by adding a little hydrochloric acid either to the glycerine or to the alcohol, without the stain being in the least affected thereby. The nucleus is shown up very distinctly in the cells, and as a rule nothing else but the nucleus is stained by the picrocarmine. But in the case of granular nuclei the finer structure of the framework and chromatin elements is not well shown by this method, which gives an evenly stained nucleus, exhibiting only traces of granulation; and hence for nuclear studies this method requires to be supplemented by others. Vesicular nuclei, on the other hand, are very well shown. The difficulty with which we have to contend in Ascon histology is that all methods which show good nuclear detail, require fixing fluids which are more or less strongly acid, and such fluids act, of course, upon the spicules. The best preservative I have found for the nuclear structures in these forms is Flemming's fluid, but its application causes the spicules to dissolve rapidly, with abundant evolution of gas, as the result of which the tissues may be mechanically injured. Moreover the collar-cells are not nearly so well preserved by Flemming's fluid as by the osmic-picrocarmine method, at least as regards collar and flagellum. Absolute alcohol is a preserving agent much employed for sponges, but I have not found it very good for Ascons. The general structure of the cells is not so well preserved by the method already mentioned, while the nuclei often have their structure altered and deformed by it. It has, however, the advantage of preserving the spicules.

Since I propose in the present memoir to discuss only the general histological structure as seen in preparations made by the osmic-picrocarmine method, and to leave the discussion of finer cytological details for a subsequent paper, I will not consider further here the action of the various fixatives and stains.

Sponges preserved with osmic and picrocarmine are often more or less macerated, especially if the osmic has been

allowed to act too long. The collar-cells become detached from their position, and are found loose in the tubes. When this process has gone too far the elements of the dermal layer may become displaced also. For surface views slight maceration is not always harmful, since it makes it easier to remove the collar-cells, but it is certainly to be avoided in material for sections. Hence care should be taken not to let the osmic acid act any longer than is absolutely necessary for the fixation of the cells. For studying the dermal layer in surface views it is generally desirable to remove the collar-cells as far as possible. This can be effected by brushing the gastral surface of the body-wall gently with a fine paint-brush when the specimens are in glycerine. In the species without quadriradiate spicules the collar-cells can be removed easily in this way without damaging any other elements. In the species with gastral rays to the spicules it is not so easy to clear away all the collar-cells, and the cells on the gastral rays are also brushed off in the process.

As a medium for mounting the pieces of the body-wall for surface views glycerine is greatly to be preferred to Canada balsam; cells can be focussed much more clearly at different depths, and the refraction of the spicules interferes less with the distinctness of the vision. Glycerine has, however, the disadvantage that it acts slowly upon the spicules. After a time they become corroded, and finally dissolved. The time this takes varies greatly in different preparations; I have some two years old which are only slightly corroded, while others go quicker. As a rule the corrosion does not become marked for at least six months, so that plenty of time is allowed for studying the preparations, though it should not be deferred too long. As a matter of fact this corrosion takes place also, as a rule, in preparations mounted in Canada balsam, though much more slowly. It is very little use, I have found, trying to isolate the elements of the dermal layer. Good pores and epithelial cells may sometimes be obtained in this way, but the spicule cells almost invariably become separated from their spicules.

(*b*) Anatomy and Histology of the Genus Clathrina.—The young sponge, as is well known, has the form for which Haeckel proposed the term Olynthus, a form shaped like an open vase or sac, fixed at the blind extremity, and opening at the other by a large aperture termed the osculum. The wall of this organism is pierced by numerous pores, by which water can pass from the exterior into the large central space, or gastral cavity so-called. The inner and outer surfaces of the body-wall may therefore be conveniently distinguished as the gastral and dermal surfaces respectively.

The body-wall is composed of two layers, an outer dermal layer and an inner gastral layer. The former makes up the greater part of the wall, consisting mainly of a structureless jelly; it contains the skeleton, the pores, and the nutritive wandering cells, and is covered at all surfaces where it is exposed by a flat contractile epithelium. The gastral layer is uniform in composition, and consists of the characteristic collar-cells; it lines the interior of the so-called gastral cavity, but, besides being interrupted at the pores, does not reach the extreme margin of the osculum. There is thus formed a rim or collar surrounding the oscular opening, composed of the dermal layer alone, and covered on both faces by the flat contractile epithelium. For this region I propose the distinctive term "oscular rim."

The olynthus is only a transitory stage in the life-history of a calcareous sponge, and in the Ascons, in which the gastral layer remains continuous and not restricted to "ciliated chambers," the complicated form by which the full-grown specimens are characterised is attained by growth at two points. The olynthus increases in length and becomes tubular by growth at the oscular rim, and at the same time the surface of the wall is increased by the formation of blind outgrowths or diverticula from the sides of the tube. These diverticula continue to grow in length, and repeatedly branch and anastomose until a dense network of tubes, from which new oscula may arise, is formed surrounding the original osculum of the olynthus, which in its turn may have multi-

plied by constriction and subsequent fission of the tube in a longitudinal direction. Thus arises the sponge form typical of the Ascons, consisting of a dense network of hollow tubes converging towards, and opening by, one or more oscula; and we obtain either the large erect oscular tubes with comparatively small basal network characteristic of the genus Leucosolenia, or the short and often insignificant oscula, acting as vents for a greatly developed network of tubes, characteristic of the genus Clathrina, according as growth preponderates at the oscular rim, or at the ends of the diverticula.

Such being the general structure of the sponge, we may now consider in more detail the composition of the body-wall, and more especially of the dermal layer. The gastral layer is uniform throughout, and consists only of collar-cells. For the purposes of the present investigation it is not necessary to discuss the details of the structure of the collar-cells, since they take no share whatever in the formation of the spicules. Only one point may be mentioned, sufficiently obvious when attention is called to it, but which, if passed over in silence, might lead to the impression that my figures or preparations were at fault. The wall of the sponge is the wall of a hollow cylinder, and is possessed of a certain thickness; hence the gastral layer, which occupies a more internal position, has a less extended surface than the more externally placed dermal layer. As a consequence when the wall is laid out flat the gastral surface is stretched, and the collar-cells tend to separate slightly from one another here and there, producing cracks and spaces, as can be seen in Pl. 41, fig. 39, and Pl. 42, fig. 50. These spaces are not natural, but are the inevitable result of flattening out a curved surface. In their natural position the collar-cells are in close contact, their cell limits forming in surface view a network of polygonal areas.

The dermal layer in the genus Clathrina is sharply differentiated into an external contractile or neuro-muscular layer, and a more internal connective-tissue layer, scattered about in which are the pore-cells and the wandering cells. The connective-tissue layer consists of spicules and their

formative cells, suspended in a structureless jelly, which makes up the greater part of the body-wall. Before we can discuss the relations of the connective-tissue layer and the origin of its cells it is necessary to be acquainted with the other elements of the dermal layer, namely, the neuro-muscular layer, the pores, and the wandering cells, in at least sufficient detail to enable us to distinguish them from one another, and so to trace their history.

The Dermal Epithelium (Neuro-muscular Layer; "Ectoderm" of Authors).—In the first place let me repeat the statement that the dermal layer, wherever exposed, is covered by flattened epithelium, at least in the normal and expanded condition of the sponge. I wish to lay especial stress upon this, even at the risk of offending by repetition, since reference has been made to me as one of those who deny the existence of the flat epithelium, although, as a matter of fact, I have never done so, but, on the contrary, have repeatedly affirmed its presence. Thus in 1892 (2, p. 490, foot-note), when criticising Bidder's statement that the external epithelium—or ectoderm, as we all called it then—consisted in Ascons of mushroom-shaped cells, I stated such cells to be of rare occurrence in freshly preserved material of Clathrina clathrus, "the predominant form of the ectoderm being flattened epithelium." Again, in the same year (3, p. 181) I began my description of the "ectoderm" of the same species with the following statement:—"This layer, the contractile layer of the sponge, consists in the expanded state of flattened non-ciliated cells." In the face of these very definite statements I am unable to understand how Lendenfeld is able to say (1894 [1], p. 161), "Neuerlich haben. . . Bidder und Minchin die Behauptung aufgestellt, . . . dass die Spongien überhaupt kein Plattenepithel besässen." And again in the same year (1894 [2], p. 508) he refers to me as one who, in company with Bidder, has opposed Schulze's views as to the nature of the flat epithelium. It is evident that von Lendenfeld has taken very little trouble to understand the views that he criticises. To clear up any misconceptions which this careless reviewer

may have been the means of spreading, I think it necessary to reaffirm the views which I have held ever since I began to study the histology of the Ascons, and which I still hold more strongly than ever; namely, that in the normal expanded condition of the sponge the dermal layer is limited at its free surfaces by a flat epithelium, of the usual type of epithelia to which this term is applied; that this flat epithelium, in the family Clathrinidæ at least, is the contractile or neuro-muscular layer of the sponge; and that when completely contracted the epithelial cells alter in form, becoming mush-room-shaped everywhere,—that is to say, except in the interior of the oscular rim.

The cells of the dermal flat epithelium have a very characteristic appearance and structure in all the species. Seen in surface view (Pl. 38, figs. 1, 3, and 10; Pl. 39, figs. 16 and 20; Pl. 41, fig. 40, &c.), the nuclei are seen scattered about, each in the centre of a patch of very granular protoplasm, in which the nucleus is sometimes almost hidden. Cell outlines can often be made out as very delicate bright lines dividing the surface into polygonal areas, but it is very difficult to discern them as a rule, on account of the refraction of the underlying spicules. The nucleus of the epithelial cell is spherical and relatively large, usually causing the surface to bulge out, as seen in sections (Pl. 41, fig. 41). It contains a fine and more or less even reticular framework, with chromatin at the nodes, and usually a small nucleolus placed somewhat excentrically. Stained with picrocarmine after fixation with osmic acid, it appears evenly stained or finely granular, with a darker spot representing the nucleolus. Its absolute size varies in different species, as is the case with the nuclei of all the other tissue elements; Clathrina contorta is especially remarkable for the large size of its cells, and the nuclei of its flat epithelium are nearly twice as large as those of coriacea, as may be seen by comparing Pl. 41, fig. 40, and Pl. 39, fig. 16. The relative size of the nuclei of the various tissue elements in each species is a much more constant character, and especially the proportion between the

nuclei of the flat epithelium and those of the collar-cells; speaking generally, the diameter of the latter is about four fifths of that of the former (compare Pl. 41, figs. 39 and 40).

The cytoplasm of the epithelial cells is packed with granules of a very characteristic kind, which easily distinguish the flattened epithelium and its derivatives, the pore-cells and the spicule-cells, from either the collar-cells or the wandering cells. As a convenient type in which to study the granulation of the epithelial cells, we may take Clathrina coriacea (figs. 1—16). Seen in osmic-picrocarmine-glycerine preparations these granules appear of rounded but rather irregular outline, and vary both in size and depth of colour. Some are pale, while others appear darker and even quite black. If one of the paler granules be carefully focussed, it appears to have a dark border surrounding a clear central spot. On slightly raising the focus the clear central spot disappears, and at a still higher focus a dark central spot is seen surrounded by a clear area. The darker granules, of which there are usually several in each cell, do not show these changes distinctly, but appear simply black. In this species the granules have, in preparations of the kind mentioned, a yellowish or yellowish-brown shimmer, and impart a similar tint to the protoplasm of the whole cell. In general the granules appear opaque and dull,—in short, they may be described as having an almost chitinous appearance. In life they are the elements to which the sponge owes its colour, whatever it may be. If the sponge is white, which is more usual, the granules appear black in transmitted light, dull white in reflected light. In the numerous colour varieties of C. coriacea the granules appear red, orange, yellow, lilac, or whatever may be the colour of the sponge, in reflected light; and in Clathrina clathrus they similarly have a constant lemon-yellow colour.

The distribution of the granules varies slightly with the condition of the cell, though they are always more or less concentrated round the nucleus. When the cell is fully expanded the nucleus is superficial, with very few granules or none at all over it, that is to say external to it, and the granules are

spread out in the cell, extending in irregular groups and rows up to its extreme limits. When the cell is contracted, even slightly, the nucleus lies much more internally, and is covered, or even hidden, by the granules which are aggregated in the centre of the cell. In the latter condition the epithelium as a whole presents in surface views the appearance of compact granular masses of protoplasm, each with a nucleus situated more deeply, scattered about at considerable intervals one from another.

The protoplasm of the epithelial cells, apart from the granulations already described, is clear and very difficult to make out in surface views when the cell is very expanded, except just round the nucleus. It appears to have a distinct alveolar or reticular structure. The characteristic yellowish tint of the cells is similarly most distinct when they are more or less contracted and their protoplasm concentrated; when they are expanded and spread out they appear simply greyish.

The appearance of the epithelial cells and the relative quantity of their granules vary slightly in the different species which I have been able to examine, though in all the general characteristics are such as have been described above.

Clathrina coriacea is a species remarkable for having its dermal epithelium very granular, and the leathery appearance from which it derives its name is due partly to this circumstance, partly to the very contracted condition in which it is usually found when exposed at low tide, the condition in which it usually figures in collections. In contorta (Pl. 41, fig. 40) the granules are relatively smaller and fewer, and tend to be oval or slightly elongated in form, with occasionally a larger, more rounded one amongst them. The number of the larger granules varies; usually there are two or three in each cell. The yellowish-brown tint is very distinct in this species. In blanca and clathrus the granules are smaller and the cells appear greyish, the yellowish tint being scarcely noticeable except when contracted. C. cerebrum has epithelial cells rather different in appearance from those hitherto described. In this form the protoplasm is remarkably vacuo-

lated, and the granules, which are small, are lodged at the nodes, giving the cells a characteristic marbled appearance, with a yellowish-brown tint, rather more brownish than in the other species. The epithelial cells of reticulum are very similar to those of cerebrum, except that the granules are larger and the vacuolation less marked, giving a more normal appearance.

Finally I have examined at Banyuls a species apparently new, with all three kinds of spicules, triradiate, quadriradiate, and monaxon. I propose to refer to this species as Clathrina, sp. dub., in the present memoir. The epithelial cells of this species are very similar in their characters to those of coriacea, but rather less granular (Pl. 39, figs. 17—20).

Thus the dermal epithelium shows not inconsiderable variations in its general appearance in different species, but in all the forms its main characteristics are similar—flattened granular cells of a type easily recognised. One small point remains for notice, as it may at first sight seem strange in the figures. The flat epithelium forms the external covering of the dermal layer, and the spicules are placed of course internally to it, lodged in the structureless jelly which makes up the greater part of the dermal layer. In some species, where the wall contains a great number of large spicules, those placed more externally often cause the wall to bulge out to such an extent that the spicules sometimes become almost enveloped in the epithelium, which is seen to be tucked in under them. Thus in Pl. 39, fig. 19, a surface view of Clathrina, sp. dub., from the inner or gastral side, the cells of flat epithelium often appear on the upper side of the spicules placed most deeply, although, being external, they should really appear below all the spicules. Other epithelial cells are seen here and there in side view. Similarly in fig. 20, seen from the dermal aspect, some of the epithelial cells appear almost hidden under the spicules. These appearances are not difficult to understand, and need not detain us further.

The Pores.—The true nature of the pores was first described by Bidder (1891, p. 631) and myself (1892, p. 266,

fig. 21) independently as consisting each of a single perforated cell, placing the internal gastral cavity in communication with the outer world through the body-wall. We differed, however, in our views as to the origin of these pore-cells, Bidder regarding them as derived from metamorphosed "endodermal" collar-cells, while I described them as arising from immigrated cells of the "ectodermal" flat epithelium. More recently Dendy (1893, p. 214) has thrown doubt on these statements, and is inclined to regard the pores as "inter-cellular and not intra-cellular in nature," "simply gaps between cells." It is evident, therefore, that Dendy has never studied the pores in surface views of material suitably preserved and mounted, for a single glance at such a preparation would be sufficient, I think, to dispel his doubts for ever. On the other hand, so long as the matter is only studied in series of sections, chopped up in paraffin from material thrown into alcohol and stained in borax carmine, it will be possible to doubt anything.

No fact in the histology of Ascons is more easily demonstrable, even to the tyro in these matters, than that each pore is, in the expanded condition, a single perforated granular cell, of a peculiar and easily recognisable type. Compare especially figs. 10, Pl. 38, and 39, Pl. 41 ; also figs. 1, Pl. 38, 18 and 18*a*, 19 and 19*a*, and 20, Pl. 39, and figs. 49, 50, and 52, Pl. 42. Seen in surface view the pore has two openings, a smaller external or dermal aperture (*derm. ap.*) on a level with the flat epithelium, and a larger internal or gastral aperture (*gastr. ap.*) on a level with the collar-cells. The dermal opening perforates a thin membrane which is usually more or less free from granules, and stretches like a tympanum across the cavity of the pore-cell. The gastral opening is surrounded by a thick and granular wall, smooth on the inner side, but running out into points and processes on the outer side—a configuration due, as can be seen at once from fig. 39 on Pl. 41, to the pressure of the adjacent collar-cells. The projecting points fit into the interstices between the collar-cells, each depression between two projections being the impression made

by the body of one such cell. At one side of the pore is lodged the nucleus in a thickening of the wall of the pore. Sometimes the protoplasm surrounding the nucleus is clear, but more often it is so full of granules as to obscure the view of the nucleus (figs. 18, 19, 39). In its position the nucleus is usually situated rather towards the gastral aperture, nearly on a level with the collar-cells.

The minuter characters of the pore-cells are such as to leave but little doubt, even if there were no further evidence obtainable, as to their histological affinities. In all respects the pore-cells resemble the cells of the flat epithelium, but with the characters of the latter exaggerated. The nucleus is similar in structure, but slightly larger, and usually paler in its staining reactions (osmic and picrocarmine). The granules are identical in character with those of the epithelium, but are present in much greater number—the cell, as a whole, being much bigger,—and attain, both on the average and in individual instances, a much greater size. This is less noticeable in coriacea, with its very granular epithelium, than in such a species as contorta, where the epithelial cells have fewer and smaller granules (compare Pl. 41, figs. 39 and 40). In consequence of the great increase in the size and number of the granules, the yellowish-brown colour which they impart to the cells in osmic-picro-carmine-glycerine preparations is very distinct, and picks them out at once from the rest of the tissue elements, even in those species in which this tint is scarcely discernible in the epithelial cells. In life also the granules of the pore-cells show the peculiarities described above for the epithelial cells, being dull white when the sponge is white, and of the same colour as the sponge when it is coloured. In short, apart from the especial functions and consequent peculiar form of the pore-cells, or porocytes, as they may be termed generally, we may say that while, on the one hand, all their characters show them to belong to the same category as the epithelial cells, on the other hand, those characters are modified to an extent and with a constancy sufficient to enable us

to regard the pore-cells as constituting a distinct class of cell-elements.

If the pore-cells always remained expanded, it is inconceivable that any misconceptions should have arisen as to their true nature. But unfortunately, perhaps, for the student of sponge literature, the pore-cells, like the epithelium from which they originally sprang, are very contractile. On the least provocation the pores close up, and then present an appearance which has led to much confusion. The external aperture seems to be the part first affected, the opening disappearing by contraction of the delicate membrane in which it is situated, like the closing of an iris diaphragm. Hence pore-cells occur commonly which show a widely open gastral aperture, but no trace of the dermal opening (Pl. 42, fig. 50). Next, the gastral opening narrows itself, and finally closes up, and the result is a large compact granular cell, of a type very familiar to all students of Ascon histology, since in all specimens not completely expanded these cells are the largest and most conspicuous elements in the sponge. It is, in fact, far easier to obtain preparations showing the pores closed than to obtain them with the pores open. In order to show the open pores, as in the preparations represented in Pl. 38, fig. 10, and Pl. 41, fig. 39, it is necessary to preserve fully expanded specimens of the sponge as soon as they are found. If, on the other hand, the sponge be brought back to the laboratory and there preserved, every pore will be found, as a rule, completely closed, and no longer recognisable as a pore except by a comparison with preparations showing pore-cells in the expanded state. Pl. 41, fig. 38, though showing pore-cells which have not as yet acquired an opening, may be taken as representing the characteristic appearance of the closed pores, and fig. 39 also shows one such cell. It is hardly necessary to state that in all their cytological characters these cells agree with what has been said above for the pore-cells, and differ from them only in form; their compactness makes their yellowish-brown tint even more obvious than is the case when expanded.

The closed pore-cells are a very obvious feature of contracted Ascons, and with increased contraction of the sponge the pore-cells go through a remarkable series of movements. Situated at first in the dermal layer on a level with the inner ends of the collar-cells, they push their way inwards between the latter. If the contraction goes still further, as it does commonly in the species without gastral rays, the collar-cells are forced one over the other, and form a layer two or three cells deep; during these changes, or before, the porocytes migrate completely inwards, and form a granular epithelium covering over the collar-cells, and lining the gastral cavity. Finally in the extreme state of contraction the tubes become solid, filled completely by the collar-cells, in the centre of which the pore-cells are to be found forming, as it were, a granular axis to the tube. As the sponge expands again all these movements are gone through in reversed order.

The contracted porocytes have attracted considerable attention, as might have been expected, from those who have studied calcareous sponges, and unfortunately they have also received very different interpretations. They seem to have been first clearly described by Metschnikoff (1879) in Clathrina clathrus and primordialis in specimens which, as is evident from the figures and descriptions given of the dermal epithelium, were in a state of complete contraction. Metschnikoff described with his usual accuracy the appearance of these cells, chiefly as seen in the living condition; their large size and compact form, their densely granular protoplasm, and the colour of their granules, which were yellow in clathrus, dark brown by transmitted light in primordialis, like the perfectly similar granules in the "ectoderm" (l. c. pp. 360, 361, pl. xxii, figs. 1, 2, 5, and 8). Nevertheless Metschnikoff regarded them as mesoderm cells, which is the more remarkable since he represented them quite correctly lying between, not under, the collar-cells (l. c. fig. 2). He believed them to give rise to the triradiate spicules, or rather he confused the cell masses which form the spicules with these cells, which, as we shall see, was an error.

Since Metschnikoff's description of these cells they do not seem to have been much noticed until comparatively recent times. In the eighties we can refer only to Carter, who observed them in coriacea (1884, p. 20, and pp. 21, 22), and described their granules as "spherical, translucent, and glairy, glistening from refraction of light, of a faint yellow tinge," appearing, "when in situ among the spicules and spongozoa, to be loosely grouped round a delicate nucleated cell respectively, the 'Kern' of Haeckel." This description betrays a strange confusion between cell and nucleus, as well as a complete misapprehension on the part of the author as to the meaning of the word "Kern." The author gives some microchemical reactions observed, and mentions that in the colour varieties of the sponges they are the seat of the colours; the possibility is suggested "that they grow into the larger cells of the protoplasm (the 'Kerne') from which they appear to be derived, when they may fulfil other offices." It is evident that the author's description includes dermal epithelium as well as porocytes. Dendy figured and described porocytes in "Leucosolenia" (Clathrina) cavata (1891 [2], pp. 18, 19, and pl. vi, figs. 4 and 5), and considered them, as well as those seen by Carter, as symbiotic algæ. Bidder (1891) was the first to recognise their true nature as closed pores in clathrus and primordialis, though I cannot follow him in the account he gives of their origin as "metamorphosed collar-cells," and am doubtful about the excretory function which he ascribed to them, a view which he developed further in later contributions (1892, &c.). Bidder proposed for these elements the technical term "Metschnikoff's cells," and pointed out correctly that their granules only differed from those in the cells of the dermal epithelium in being of larger size.

In the same year Lendenfeld figured and described these cells in "Ascetta" spinosa (1891, fig. 22, p. 205), and apparently also in primordialis (l. c., fig. 23, pp. 201, 202) and clathrus (p. 212). In the case of spinosa he interpreted these cells as "parasites or symbiontes of vegetable nature," as Dendy had done before him. In the case of pri-

mordialis, Lendenfeld took the singular view that the cells in question were the mother-cells of the choanocytes, apparently for the sole reason that they were continuous with some sort of coagulum which appeared in his preparations between the collar-cells. Indeed, although I formerly believed that von Lendenfeld's supposed "Kragenmutterzellen" were in reality the closed pores, I should be more inclined now to regard them simply as parts of the coagulum which he figures, had he not more recently (1894 [2], p. 508) asserted definitely that the cells which Bidder and myself identify as closed pores are the same as those which he regards as the mother-cells of the choanocytes. In that case the statement which he twice repeats (1891, p. 201, and 1894 [2], p. 508), that the protoplasm of these cells agrees in its characters with that of the collar-cells, is absolutely astounding, even to those who are acquainted with his writings on sponge histology; for if there are in the sponge body, cells which differ more than others, in every character in which cells can differ, from the collar-cells, it is the porocytes (compare my Pl. 41, fig. 39). Looking again at his figures, I am inclined to think that in spinosa (l. c., fig. 22, *a*) he has represented true porocytes, which he identifies as symbiotic vegetable bodies, but that the structures which in primordialis he has figured as "Kragenmutterzellen" (l. c., fig. 23) are simply broken-down cell remnants in a very badly preserved preparation. The question is, in fact, impossible to decide, for it is enough to glance at the figures to which reference has been made to see that they are utterly untrue to nature, and represent, if anything, the cell elements in an advanced stage of putrescence and disintegration. It is nothing short of ludicrous that such figures, and others in the same memoir (figs. 34—37, for instance, showing myriads of absolutely non-existent connective-tissue cells) should ever have appeared in a serious scientific journal as representing the histology of these sponges; "histology" of this kind would certainly not pass muster in any other group of animals. I think, therefore, that it would be an entire waste of time to attempt to discuss further what von Lendenfeld may or may

not have seen, or what his views may have been at one time or another, as to the nature of these or other cell elements.

The last author who, so far as I am aware, has described the porocytes is Topsent (1892). In the same year (1) I had published some observations far from complete upon the histology of "Leucosolenia" coriacea, amongst which I had figured and described the contracted porocytes in section, but had wrongly interpreted them as the amœbocytes or "cellules digestives pigmentées" of Topsent. In criticising my observations Topsent rightly pointed out my error, and gave a good and accurate description of the porocytes, but without himself arriving at a true understanding of their nature. He terms them "cellules sphéruleuses du mésoderme," in distinction to the amœbocytes or "cellules granuleuses du mésoderme," and considers that they represent reserves of nutriment. He was guided to this interpretation from the reactions of their granules. It is by no means impossible that the cells in question should combine the functions of storing reserve nutriment with that of acting as pores, but I certainly cannot agree with Topsent in regarding this as their sole function. I feel quite convinced that if he had compared them in the expanded state with the contracted condition in which he figures them, he would have agreed with me in my interpretation of them as closed pore-cells simply; for that his drawings do represent contracted material is shown by internal evidence. The appearance of the "ectoderme" in his figs. A and B as compact masses of granules at considerable distances apart is exactly the appearance it shows when contracted. Moreover in the surface view A not a single pore is shown; but a piece of this size, if expanded, would contain at least half a dozen pores (compare Pl. 38, fig. 10). Topsent remarks, in his criticism on my work, that I had only been moderately struck ("médiocrement frappé") by these very conspicuous cells; but that is simply because my material was all preserved immediately when found, and as a result the pores were found expanded, and described by me as such, except in one specimen which was contracted when found, as

of course often occurs, and in which, after cutting sections, I saw and figured the contracted pore-cells, though without myself recognising their true nature. Had Topsent made and studied similar preparations he would have been rather surprised, I think, to have found his "cellules sphéruleuses" either completely absent or comparatively rare; here and there of course one finds a contracted pore in a specimen which, as a whole, is expanded, and one generally also finds, without difficulty, every transition between expansion and contraction. It is possible that then Topsent would have been just as "moderately struck" by these cells as I was myself.

I have gone into the question of these porocytes rather fully; but for this the importance of these porocytes, as we shall see, from the point of view of spicule formation, as well as the great confusion and diversity of opinion in the literature, must be my excuse. It is, indeed, a tangled web to unwind when we try to introduce harmony into the existing descriptions of these structures, nor can we expect that it should be otherwise until spongiologists have more generally recognised the excessive contractility of the species of Clathrina, and the ease and rapidity with which they alter in appearance.

Looking generally at the matter, we see that contracted pore-cells, however interpreted, have been seen in a considerable number of species, more particularly in those which are characterised by a very granular dermal layer, or, like clathrus, by the granules being coloured. Thus Metschnikoff saw them in clathrus and primordialis, but overlooked them in blanca, where they are much less conspicuous. Primordialis is a species which resembles coriacea in being very granular; and in the latter Carter, Topsent, and myself noticed these cells. Dendy observed them in cavata, a species which his figures show to possess a very granular dermal layer, and the only species in which Lendenfeld has seen them beyond doubt is spinosa, a species which I have myself had opportunity of studying at Banyals, and which very closely resembles contorta, if it is not identical with it. The only distinctive feature of contorta is the possession of

monaxon spicules, and these, when present, vary very much in size and number. To Bidder, however, belongs the credit of having been the first to recognise the true nature of these cells as contracted pores. In addition to the species already enumerated, I can vouch for their occurrence in blanca, cerebrum, reticulum, contorta, and "Clathrina, sp. dub.," and since in all these species the pores have similar characteristics and contract in the same way, I have but little doubt these usually very conspicuous cells—"Körnerzellen," "cellules sphéruleuses," "yellow granules," as they have been so variously termed—will be found in any species of Clathrina where they are sought for under suitable conditions.

Origin of the Porocytes.—We have now considered in some detail the structure and appearance of the pore-cells, both when expanded and functioning as pores, and in the contracted condition in which they most often figure in the literature. The porocytes do not, however, form a separate layer, growing and multiplying amongst themselves, but each porocyte arises separately and independently from the dermal flat epithelium. I have already described in a former communication (1892 [3]) the more usual method by which the pores arise, either at any point during the general growth and increase in size of the tubes or at the ends of the blind diverticula, namely, by immigration of a cell of the flat epithelium. Without going fully into the matter at present, I will merely refer to Pl. 39, figs. 15 and 16, *por. c.*, which represent two of these future porocytes, in process of immigration. It will be seen that the cells in question only differ from the rest of the flat epithelium in their larger size and more numerous granules, and in the possession of rather larger nuclei. Each cell shows a portion still on the surface, and therefore visible at the higher focus, which has the granules spread out, and is without definite limits, and a deeper portion, visible at a lower focus, which has already come into contact with the collar-cells, and in consequence has a sharp outline showing the characteristic bays and points. My reason for figuring these two cells is that they show a feature often to be observed, namely, the

presence of vacuoles, containing bodies which, to judge from their appearance, consist of calcareous matter. The near proximity to one of them (fig. 15) of what seems to be a much corroded fragment of a spicule, has led me to the belief that these porocytes may exercise the additional function of removing, or rather absorbing, pieces of broken spicules. In so far as they perform this office they might be termed "scleroclasts." I make this suggestion with all caution, but there seems to me no inherent improbability in the porocytes, especially at an early stage in their differentiation, exerting a scleroclastic function.

A very important growing point in the sponge, as has been said, is the oscular rim; and here, too, porocytes are formed in great numbers, and in a manner differing in details from that which I have mentioned above as the typical method. The oscular rim is, in fact, a region where the origin of porocytes is so easy to study that it is a marvel to me that it should not have been described long ago. For reasons which will be apparent when we come to study the formation of the fourth ray of the quadriradiates I propose to go into the matter rather fully, and as a type I will take contorta, where the facts are very plain.

If a piece of the oscular rim of contorta be laid out flat and examined from the dermal surface, flat epithelium of the normal type is seen (Pl. 41, fig. 40). If, on the other hand, the oscular rim be examined from the gastral view, especially rather near the limit of the collar-cells, an epithelium is seen which at first sight appears very different (Pl. 41, fig. 38). The cells are packed with granules which obscure and often hide the large pale nucleus, and the whole cell has a most pronounced yellowish-brown tint. In fact, after what we have already seen we can identify the cells without hesitation as porocytes of a most typical kind. Pl. 41, fig. 41, shows the two types of epithelium in a decalcified section. On the lower (outer) side we see the typical dermal epithelium, and on the upper (inner) side we see the layer of granular porocytic epithelium, for so we may term it without further preamble.

Had the drawing (fig. 41) been continued as far again to the right, collar-cells would have appeared in the section.

If now we follow up the layer of granular epithelium lining the oscular rim in the direction of the margin of the osculum, it is found to lose its distinctive characters by degrees, becoming more and more similar to the epithelium of the dermal surface. Pl. 41, fig. 42, shows an epithelial cell from about halfway up the oscular rim, obviously intermediate in its characters between the cells figured in figs. 38 and 40 respectively. Finally, near the actual margin of the osculum the epithelium is quite of the ordinary type, and is continuous where it turns the edge with the general flat epithelium covering the exterior. There is, in fact, a gradual transformation in the oscular rim of ordinary cells of the flat epithelium into porocytes, the changes consisting of (1) increase in size of the cells; (2) increase in the number and size of the granules; (3) slight increase in the size of the nucleus, and corresponding decrease in its staining powers.

If, on the other hand, we follow the porocytic epithelium downwards instead of upwards, we find as we approach the limits of the collar-cell layer (Pl. 41, fig. 38) the porocytes becoming more compact and definite in their outlines, and hence less like epithelial cells. Lower still (figs. 38 and 39) we find them lying between the collar-cells, and there becoming gradually transformed into the familiar pores. In other words, the collar-cell layer continually extends its upper limit by proliferation of its cells, and as it does so the porocytes become surrounded by and enclosed amongst the collar-cells. As soon as this occurs they spread out greatly and form a large cavity, open towards the gastral space, and by perforation of the outer wall of this cavity they acquire a dermal aperture. In fact, they go through just the changes which a contracted pore cell goes through when it becomes expanded.

In all the Ascons of the genus Clathrina studied by me, and apparently also in the species of Leucosolenia, certainly in Ascandra falcata, the same state of things is to be found. I found the oscular rim in the species blanca,

coriacea, clathrus, primordialis, cerebrum, reticulum, lacunosa, spinosa, and contorta, besides "Clathrina, sp. dub." Yet Lendenfeld, after publishing a monograph of the Calcarea, not only had not seen this very obvious structure, which Lieberkühn described with great clearness in 1865 (see below), but he even characterised my temerity in venturing to point out its existence as showing a total lack of "Disciplin." I am not quite sure that I know what "Disciplin" means, but if it implies failure to see very obvious things on account of preconceived notions, it is a quality which I am very happy not to possess. Bidder also (1891) described the oscula of cerebrum as "lined with collared cells continuously up to the granular lip." I am unable to agree with this statement, though it is true that in cerebrum the oscular rim is shorter than in any other species known to me. I have sections through an osculum of cerebrum where the upper limit of the collar-cell layer is only separated from the extreme edge of the osculum by about five epithelial cells in the section. But in general, even in this species, the oscular rim is deeper than that. The shortness of the oscular rim in cerebrum leads, however, to one interesting deviation from the more usual condition, such as has just been described in contorta. The transformation of the epithelial cells into porocytes in the former does not take place wholly within the oscular rim, but commences before the epithelium has turned the edge, some way down on the exterior of the oscular tube. In contrast to cerebrum, the specimens of reticulum which I have examined were remarkable for the extreme length of the oscular rim, the region devoid of collar-cells often extending down to the commencement of the tubes which converge to open into the cavity of the oscular tube.

Before leaving this subject there is one point to notice. Both the sieve membrane,[1] formerly described by me in

[1] In some specimens of coriacea there is a ring-like sphincter, such as I described in clathrus. I have seen what is obviously a transition between the two structures, namely, a sieve membrane with a very large aperture in the centre surrounded by smaller apertures peripherally. In some specimens

coriacea, and the ring-like sphincter which I described in clathrus, as well as similar structures in other species, are made up of the porocytic epithelium of the interior of the oscular rim—a strong proof of the contractile nature of these cells. I may repeat an earlier statement of mine, namely, that when contracted the epithelium lining the oscular rim never assumes the mushroom form characteristic of the epithelial cells of the exterior when in a similar condition.

Do the porocytes multiply amongst themselves? I have stated above that they do not, which means I have never seen them do so, at least not when fully differentiated; while on the other hand I have, I think I may say, abundant proof of their origin individually from ordinary epithelial cells. But Pl. 41, fig. 38, shows a cell of the porocytic epithelium of the oscular rim containing two nuclei, proving that they may multiply in this region. I have never found functional pore-cells multiplying to form more pores, though it would be rash to affirm too positively that they could never do so, but at least it is a rare occurrence. To sum up briefly the results of this investigation upon the pore-cells, we have found—

(1) That the porocytes form a definite and well-characterised layer of cells. (2) That their characteristic features are the same as those of the ordinary dermal epithelial cells, but greatly exaggerated. (3) That they arise by modification and transformation of ordinary dermal epithelial cells, which come to lie amongst the collar-cells in two ways: at the oscular rim by inclusion amongst the collar-cell layer as it grows upwards; elsewhere by actual immigration from the surface into the

there is no trace of either sieve membrane or sphincter. In others again, incrusting forms of opaque, leathery appearance, with the tubes forming a network in one plane, the whole gastral cavity is traversed by a network running in all directions, not only at the oscular rim, but everywhere. This network has the same structure as the sieve membrane, and its threads are made up entirely of granular porocytes surrounding an axis apparently of jelly. The specimens which show this network are very resistent and firm even when expanded, and no doubt derive support from it. Compare the similar network figured by Dendy in "Leucosolenia" proxima (1891 [2], pl. viii, figs. 1, 2).

interior. (4) That the pore-cells are eminently contractile, and when contracted have received very different interpretations from different authors.

Connective-tissue Layer.—This consists of the spicules and their secreting cells embedded in the structureless jelly which constitutes the greater part of the body-wall.

The triradiate spicules have each a single cell applied to the extremity of each ray. These spicule-cells, as we may term them shortly, have the protoplasm very clear and almost free from granules. The nuclei are slightly smaller, and stain more deeply than those of the flat epithelial cells, appearing oval in profile view, circular in surface view (fig. 14).

I desire to correct the statement made by me in a former paper, to the effect that each spicule in coriacea had a nucleus at the extremity of each ray, and a fourth at the confluence of the rays (1892 [1], p. 265). When a spicule ray crosses the centre of another spicule an appearance may be produced of a cell at the centre of the spicule, which is not, however, the case. There are no other cells on the fully formed spicule than the three at the extremities of the rays. What applies to the triradiates applies also to the basal rays of the quadriradiates. The apical rays of the quadriradiates have, as we shall see, a variable number of nuclei upon them. The large monaxons of many species of Clathrina are covered by a number of cells, but exactly how many is a point very difficult to determine.

As we shall describe fully below the origin of the spicule cells, it is not necessary to enter into a further discussion upon them here.

Amœbocytes.—The wandering cells are very important and conspicuous elements of the sponge body. They give rise, as is well known, to the sexual elements, and their complete history will, I believe, furnish some points of considerable interest when worked out. At present, however, I am still far from being able to give a complete account of them, and will therefore content myself with describing them as they occur ordinarily in the adult sponge.

In the first place, there occur always large cells of lobose and irregular form, densely packed with refringent granules. These cells seem to possess a nutrient and distributive function, and are abundant in all parts of the sponge. A striking point about them is that their appearance is very different in different species, though fairly constant in individuals of the same species. To such an extent is this the case, at least for the species in which I have studied them, that it would be easy to identify and distinguish preparations of blanca, coriacea, clathrus, cerebrum, reticulum, contorta, and my undetermined species by their wandering cells alone. With these differences they exhibit certain constant points of structure common to all, which makes it easy to distinguish them in preparations. In size they are normally a good deal smaller than the porocytes. Their outlines are rounded, and their form either compact or irregular, with short lobed processes, never fine and pointed. Their nucleus when visible through the granules is seen to be large and rather pale, with a vesicular structure and a very distinct and large nucleus. Their granules are large, very refringent, and of a very glassy appearance after osmic and picrocarmine, quite different from the opaque dull granules of the porocytes and the flat epithelium. As a rule the nucleus can only be discerned with difficulty as an indistinct patch of colour, if stained, through the mass of granules.

Of all the species mentioned, the wandering cells of this type are most remarkable in clathrus, where they have a peculiar greenish or greenish-yellow colour in osmic-picrocarmine-glycerine preparations, so that the eye can easily pick them out in the preparation, even with a low power of the microscope. The granules are of moderate size, rounded or oval in form, and of refringent, glittering appearance.

In coriacea (Pl. 38, fig. 10, *am.c.*[1]) the wandering cells are yellowish, but very distinct in colour as well as in general appearance from the yellowish-brown porocytes. They contain large pale granules which have almost the appearance of vacuoles, and also small dark granules with a steely glitter. The nucleus is very hard to see.

In "Clathrina, sp. dub.," the densely packed granules of the amœbocytes are of moderate size and glassy appearance, without any particular tint (Pl. 39, fig. 19, *am. c.*[1]), and the nucleus is quite obscured by them.

In blanca, cerebrum, and reticulum also the wandering cells are colourless, but clearer than in the other species, and of very compact form. The nuclei are distinct. Of the three, blanca has the least granular cells, reticulum the most granular.

In contorta the granules are small and very numerous, of rounded or oval shape, and the cells are often of very irregular form (Pl. 41, figs. 43, 44).

Besides the granular and refringent amœbocytes just described, there appear to be others constantly present, which have clear, finely granulated protoplasm. In coriacea (Pl. 38, fig. 10, *am.c.*[2]) the cells are found to vary very much in size, but are distinguished by their nucleus, which has a very distinct nucleolus, and is large in comparison to the cell body. The cell itself is of irregular form, often with sharp processes. In Clathrina, sp. dub., the clear wandering cells, so far as I have studied them, are very similar to those of coriacea. In contorta, on the other hand, these cells are similar in their characters to the granular wandering cells, and only differ in the minute size of their granules (Pl. 41, figs. 46, 47).

It is by no means certain that these cells are really different from the granular cells. I am inclined to think that in the case of contorta, at any rate, the clear cells are simply those in which the supply of nutriment is exhausted. Perhaps they correspond to the two classes of cells which Fiedler (1888) has distinguished as "Fresszellen" and "Nahrzellen."

Finally, I have to mention very peculiar elements which I have found in all the species. These are very minute cells with a small faintly staining nucleus. Sometimes they are of globular shape, but more often elongated, with the nucleus at one extremity; the former may be the condition of rest, the latter that of active locomotion (Pl. 39, figs. 17, 19, and Pl. 41, fig. 41,

am.c.[3]). In some preparations of coriacea these minute elements appear to be connected by a series of transitions with the clear amœbocytes already described; that is to say, the latter by repeated division seem to break up into these excessively minute cells. It is possible that this process represents simply the method by which the wandering cells multiply. I make this suggestion with all reserve, but am guided to it by appearances, which I hope to describe in a future paper on the embryonic development. For the present it is sufficient for me to have described these cells simply for purposes of recognition, in order to distinguish them from the cells of the dermal layer proper.

Apart from the sexual cells, of which it is not my intention to treat in the present memoir, we can recognise two constituent layers in the sponge body, besides a class of cell elements which seem, properly speaking, to belong to neither layer. These are—

(1) The gastral layer, consisting of the collar-cells lining the interior.

(2) The dermal layer, consisting of—

(i) The external neuro-muscular flat epithelium.

(ii) The internal connective-tissue layer, consisting of the spicules and their formative cells.

(iii) The porocytes scattered about more or less evenly in the wall.

(3) The amœbocytes or amœboid wandering cells met with in all parts of the sponge.

(*c*) System and Nomenclature.—In a former paper (1896 [2]) I have put forward the outlines of the classification which I intend to adopt. I recognised four genera of Ascons, one of which has been seen as yet only by Haeckel. For these genera I employed the names Clathrina, Gray; Leucosolenia, Bowerbank; Ascandra, Haeckel; and Ascyssa, Haeckel.

The genus Clathrina is characterised by its reticulate form, equiangular triradiate systems, collar-cells with basal nuclei, parenchymella larva, and "protascetta" stage in the

development. Ascandra is similar in all its characters, but has the gastral layer folded by reason of the great development of the gastral rays of the quadriradiates. The genus Leucosolenia, on the other hand, has an erect or arborescent form, sagittal triradiate systems, collar-cells with terminal nuclei, amphiblastula larva, and "protascyssa" stage in the development. Of the genus Ascyssa, all that can be said at present is that the skeleton consists entirely of monaxon spicules.

My classification has not yet been in the field long enough for it to have received the criticisms which I hope it will call forth. The only point which as yet has been raised is with reference to the name Ascandra. Lendenfeld has raised objections to my retention of this name, coined by Haeckel, in quite a different sense, for the species falcata, one out of the many species which Haeckel referred to this genus. I will not revive the discussion, since Lendenfeld has admitted ('Zool. Centralbl.,' iv, No. 7, p. 231) that the course taken by me was "vielleicht formal richtig, real aber jedenfalls unpraktisch." If the retention of the name Ascandra is formally correct I desire no more, for I am quite of opinion that in a question of nomenclature, which should be treated purely as a matter of names, finality can be attained only by rigid adherence to rule, even if inconvenient.[1]

I propose now to develop my scheme of classification further by dividing the Ascons into two families, corresponding to their

[1] The abstract of my classification given by Lendenfeld (l. c.) misrepresents one point completely. In my diagnosis of Clathrina I gave as one of the characters "principal spicules of the skeleton, equiangular triradiate systems," having previously defined the term "principal spicules" as those "of which the general skeleton is composed, and which are found in all parts of the sponge," as distinguished from spicules forming "a special dermal or other layer restricted to some region of the sponge colony." Lendenfeld cites this point in my diagnosis as "spicules principally equiangular" (Nadeln hauptsächlich gleichwinkelig), which gives quite a wrong impression. Of Leucosolenia I stated "triradiate systems always sagittal," exception of course being assumed of those abnormal and irregular spicules which are to be found in every specimen. This, again, is quoted as "spicules principally sagittal."

affinities as represented graphically in my former paper in the form of a genealogical tree. The first family would be the Clathrinidæ, and includes the genera Clathrina and Ascandra. The second family would be the Leucosoleniidæ, to include Leucosolenia and probably Ascyssa. Their systematic position is as follows:

Class CALCAREA.

Sub-class Homocœla.

Order Ascones.

I. Family Clathrinidæ.
Genera Clathrina and Ascandra.

II. Family Leucosoleniidæ.
Genera Leucosolenia and (?) Ascyssa.

II. Descriptive: Observations upon the Development of the Spicules.

Preliminary Remarks.—For the sake of clearness it may be permitted to anticipate in one point the results of the investigations of which the description follows, with reference, namely, to the relations between the triradiate and quadriradiate spicules. As is well known, each triradiate spicule is formed of a system of three rays, and each ray is placed tangentially in the body-wall, entirely enveloped in the dermal layer, and never projecting freely from it, neither towards the exterior nor the interior. Typically one ray—the posterior ray—points away from the osculum, and therefore downwards, in an erect olynthus; while the other two, the lateral rays, slant upwards. In the species we are about to consider the three rays meet at equal angles, and the posterior ray is only marked out from the lateral rays by its position in the sponge, and sometimes also by its greater length (Cl. blanca). The three rays do not, however, lie all exactly in the same plane; but, as might be expected from their position in the wall of a cylinder, a plane containing any two of the rays is met by the third at

an acute angle, so that if the spicule were placed on a plane surface with its gastral aspect downwards, the tips of the rays would rest on the plane surface, while the centre of the spicule would be raised up from it.

The quadriradiate spicules consist of a basal system of three rays, orientated in the sponge body exactly as are the rays of the triradiate spicule, and a fourth, the gastral or apical ray, which arises from the centre of the basal system, making in the genera Clathrina and Ascandra equal angles with the three basal rays, and projects free into the gastral cavity, passing between the collar-cells.

Not only is the basal system of the quadriradiate spicule similar in all its relations to the whole of a triradiate spicule, but it also develops in exactly the same manner. In the species investigated by me not the least difference was observed between the development of those triradiate spicules which remained as such and those which, by addition of a fourth ray, became quadriradiates. This accounts for the fact that in nearly all species of Ascons the triradiate spicules and the basal systems of the quadriradiates are so exactly similar, at least in the case of the spicules of the general skeleton, and differ, if at all, only in size. This fact is even more striking in the species of Leucosolenia, in which the spicular systems have a bilateral form, than in the Clathrinidæ. At its first appearance it is not possible to predict to which class the spicule is destined to belong, since neither the fourth ray nor its secreting cell appears until the basal system has attained a certain size. The fourth ray is, in fact, an adventitious element, superadded to the basal system from a totally different source. For this reason I have proposed in a former paper the term triradiate system, to denote in a general way both the triradiate spicules and the basal systems of the quadriradiates.

It will therefore conduce greatly both to brevity and to simplicity to describe first the development of the triradiate systems, both of those which remain triradiate spicules and those which become the basal systems of quadriradiates, and

then to proceed to describe the origin and formation of the gastral rays.

(*a*) The Development of the Triradiate Systems.—The origin of the triradiate systems can be traced back to certain cells of the dermal flat epithelium, which have wandered inwards and have come to lie between the flat epithelium and the collared epithelium of the gastral layer. This is true equally of the first spicules formed in the young sponge after the fixation and metamorphosis of the larva, and of all spicules formed during subsequent growth in the adult sponge. The immigration of the spicule-secreting cells in the young fixed embryos first causes the dermal layer to become differentiated into a more internal connective-tissue layer and an external contractile epithelium. In the adults, where the connective-tissue layer is well established, the skeletogenous cells migrate from the epithelium into it, so that the connective-tissue layer is continually being recruited, as it were, from the dermal epithelium.

In the present memoir I propose to deal more particularly with the formation of the spicules in the adult, a few stages from embryos being described for comparison. I have observed the formation of the triradiate systems in the adults of Clathrina coriacea, Cl. reticulum, Cl. cerebrum, Cl. contorta, and Cl., sp. dub., and in the embryos of Cl. cerebrum, Cl. reticulum, and Ascandra falcata. Since in all these cases the development proceeds in an essentially similar manner, and differs only in points of detail, I have taken coriacea as a type (figs. 1—16), in order to avoid unnecessary multiplication of similar figures. The description to follow refers, therefore, to coriacea, unless the contrary is stated. The differences shown by other types will be mentioned in their place, and where necessary illustrated by figures.

The mother-cells of the spicules can be found without difficulty in actively growing parts of the sponge (figs. 1—3 and 10, Pl. 38, *act. bl.*). They are compact, irregularly rounded cells, which in all the characters of their nucleus and cytoplasm show an agreement amounting to identity with the

cells of the epithelium. Like them they are packed with large opaque granules, some appearing quite dark, others of a much paler tint in preparations. The spicule mother-cells, in fact, only differ from the dermal epithelial cells in their position and shape. Instead of being spread out into thin plates they form compact lumps, and hence acquire a rather denser and darker appearance. From all other cell elements they are easily distinguished. They are much smaller and less opaque than the contracted pore-cells, and their nuclei are slightly smaller and stain more deeply. On the other hand, their granules and the structure of their nuclei distinguish them at once from the various kinds of wandering cells, from which they differ so markedly that it will be sufficient to refer to Pl. 38, fig. 10, *a.m. c.*^{1}, *am. c.*^{2}, and to the description of the wandering cells given above.

I have just spoken of these cells as "spicule mother-cells," but this term is to be avoided if it conveys the idea that one such cell produces a whole spicule, for, as we shall see, each of the cells in question is responsible for one ray only of the spicule. The commonly used term "scleroblast" is perhaps a sufficiently vague term, meaning only a cell which secretes a spicule, and not committing us to any theory or opinion as to whether the cell so termed secretes the whole or part of a spicule. It is, however, better on the whole to invent a term which connotes accurately the relations of these cells to the future spicule, and they may be conveniently termed "actinoblasts."

Sometimes these skeletogenous cells are found singly (Pl. 38, fig. 10, *act. bl.*), or at least too far from any others for their connection with them to be apparent. More usually they are met with in trios (Pl. 38, figs. 1—3), and, as a rule, in such close contact that their apposed surfaces become more or less flattened against one another by mutual pressure, giving a figure like a trefoil. Trios may, however, be found in which there are considerable intervals between the component cells (fig. 1); and this fact, taken in connection with the not infrequent occurrence of isolated 'actinoblasts,' show that in many

cases, at least, the cells approach one another after immigration to form the trios. The next stage in the development is the division of each cell of the trio into two cells, giving rise to a figure which may be termed the sextet (Pl. 38, fig. 4, coriacea; Pl. 39, fig. 17, Clathrina, sp. dub.; and fig. 22, embryo of falcata). The division of each actinoblast takes place in such a way that the plane of cleavage is parallel to the inner and outer surfaces of the body-wall, so that the two daughter-cells are placed one above the other when seen in a surface view of the body-wall. Hence the two cells arising from the division of a single actinoblast may be termed the inner and outer formative cells respectively. The whole sextet is made up now of two superposed cell-trefoils, if the phrase may be allowed; so that when examined in surface view, three cells with their nuclei can be made out at the higher focus, and three others, exactly similar to the first and immediately below them, at a lower focus.

In spite of much searching I have not been able to make out the manner in which the division of the nuclei takes place. Indeed, though I have studied very carefully the actively growing portions of the sponge, cell division in the tissue cells generally remains a mystery to me. I have never seen any karyokinesis, nor have I come across any stages of direct nuclear division. It is not uncommon, however, to find stages intermediate between the trio and sextet,—that is to say, two nuclei in a cell as yet undivided. In Pl. 39, fig. 22, is figured a stage from an embryo of Ascandra falcata in which one cell of the trio has completely divided, while in the other two the division has not gone beyond the nucleus. It, is, moreover, impossible in surface views of a sextet to say for certain whether the division is complete between the upper and lower cells; the sextet shown in Pl. 39, fig. 17, is an instance of this.

The sextet is now ready to secrete the spicule, and the first preparation for this event appears to consist in a fusion of the formative cells at the centre of the sextet (fig. 4); if not actually fused, the cells are at least in such close contact that

their limits are indistinguishable towards the interior. The young triradiate spicule appears in the central portion of the sextet, and is so placed that each of its rays corresponds to one of the three pairs of formative cells (Pl. 38, figs. 5, 6, coriacea; Pl. 40, figs. 31, 32, embryo of reticulum; and Pl. 41, fig. 48, contorta). The exact relation of the spicule ray to its two formative cells is difficult to determine in surface views. So far as can be made out by carefully focussing the different structures, the ray lies between the two nuclei. It is, however, impossible to discover by this method exactly which of the two formative cells is concerned with the first appearance of the spicule ray. In the absence of direct evidence on this point there are some grounds for believing it to be the inner formative cell which secretes the minute ray at its first appearance, both on account of the subsequent relations which we shall shortly describe between this cell and the ray, and for another reason which may be mentioned here. In many preparations made by the osmic acid and picrocarmine method the spicule-secreting cells are remarkable for the fact that their nuclei stain much more brightly than the nuclei of other cells of the dermal layer. Not only is this the case with the nuclei of separate cells, but it even occurs, as we shall see, when a cell contains two nuclei, one of which is destined to be the nucleus of a scleroblast (figs. 18, 18 *a*, 19, 19 *a*). This interesting reaction to staining fluids is not the rule in preparations made by this method, and I am unable to state upon what it depends, or to give any directions for obtaining this result; but in a series of preparations mounted from a specimen of the sponge which I denote for the present as Clathrina, sp. dub., the deeper colour of the nuclei in the spicule-secreting cells was very obvious (see Pl. 39, figs. 17—21).[1]

Fig. 17 shows a sextet in which the cells are still perfectly distinct from one another, and no sign of the spicule is to be

[1] This colour reaction suggests a connection between the nucleus and the secretion of the calcareous matter, for which we shall find further evidence when discussing the growth of the adventitious gastral ray.

found. It will be noticed that the three lower (inner) cells are more deeply stained than the three upper (outer) ones. In fact, the three lower nuclei are as deeply coloured as the nucleus of the neighbouring spicule-cell, while the three upper nuclei are scarcely at all deeper than the nuclei of the flat epithelium.

The above facts are strong evidence for believing that it is the inner formative cells which are concerned with the first

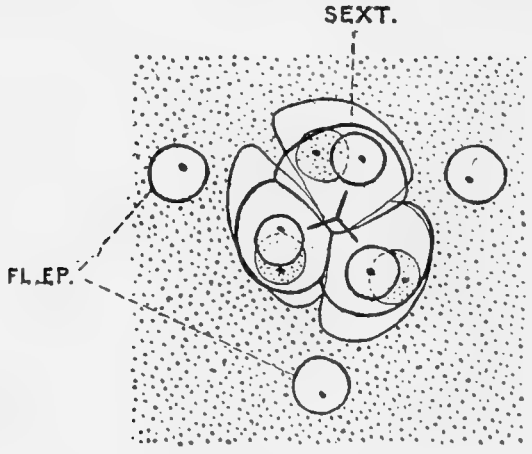

FIG. A.—Very young triradiate system in its sextet. × 1600. FL. EP. Flat epithelium. SEXT. Sextet.

appearance of the spicule rays, but the point cannot be considered as established with certainty.

A very important point with reference to the young spicule is the fact that its three rays appear to be at first quite separate from one another. In some cases the distinctness of the rays is extremely obvious, especially in the very young spicules of Cl. contorta (Pl. 41, fig. 48); in other cases it is not so obvious, and fusion of the rays seems to take place very early (Pl. 40, figs. 31, 32). The smallest spicule seen by me was in a preparation of Cl. coriacea, and the three rays were like three bacteria, about $1\frac{1}{2}$ μ in length; they appeared to be in contact at their inner ends, but the minuteness of the spicule and the thickness of the cells rendered it impossible to make out their exact relations (see woodcut, Fig. A). As the rays increase in size their separation from one another becomes more marked, and is generally quite distinct in spicules with

rays up to 5 μ in length (figs. 5 and 48), sometimes even in much larger spicules (fig. 23). But in spicules with rays more than about 5 μ in length complete fusion has taken place, as a rule, between the rays (fig. 6), and instead of three separate spicules, we have a single spicular system.

The spicule continues to grow, and at the same time its formative cells begin to shift their position. Each ray, as we have seen, is surrounded by two formative cells, one lying internally to it, the other externally. At first the ray is small in proportion to its formative cells, and completely embedded in them; but it soon grows to a length far exceeding the diameter of the cells. As it does so, the inner formative cell remains at the apex of the ray, impaled, as it were, upon its point, while the outer formative cell remains at the base of the ray (Pl. 38, fig. 7). In consequence of these relations, the six cells of the sextet become widely separated from one another, at least as far as their principal mass is concerned. The exact limits of the cells are very difficult to determine on account of the refraction of the spicule. It is evident from the manner in which the spicule grows that it must be covered everywhere by a layer of protoplasm. But except in the vicinity of the nucleus the investing layer of protoplasm cannot, as a rule, be made out, so that the bodies of the formative cells appear distinct from one another and adherent to the rapidly growing spicule. The spicule does not appear naked when exposed by the separation of its formative cells, but it is covered everywhere by a delicate layer or sheath, very hard to see in freshly made preparations, but very distinct in preparations kept some time in glycerine, in which the spicule is gradually dissolved. This is the first appearance of the well-known spicule sheath. It becomes more distinct as the spicule grows in size, but can scarcely be distinguished until the spicule ray has outgrown its formative cells, and it appears as a structureless membrane or layer intervening between the spicule and the secreting protoplasm.

The result of these changes is a stage very commonly found in the growing spicule, and very characteristic. Pl. 38, fig. 8,

shows it for Cl. coriacea; Pl. 39, figs. 18, 19, and 21, for Clathrina, sp. dub., figs. 23 and 24 for embryos of falcata, and fig. 27 for embryos of cerebrum; Pl. 40, fig. 33, for reticulum; Pl. 41, fig. 38, for contorta. In coriacea when the rays of the spicule have attained a length of about 20 μ, each ray has on it two cells, one at the base and one at the apex. The basal cell appears as a more or less fusiform mass, applied to the spicule ray, and containing the nucleus in its thickest part. The surface of the cell is usually smooth and rounded, without processes. The apical cell, on the other hand, has a very irregular outline, and runs out into processes in a way which gives the impression of its being amœboid and motile. It is placed quite at the tip of the spicule ray, its nucleus lying on a level with the point or even beyond it, and the portion of the cell body in contact with the ray is relatively very small. All the formative cells have now become much denser through gradual absorption of the granules with which at an earlier stage they were packed, and the diminution and disappearance of the granules goes on pari passu with the growth of the spicule. The apical formative cells appear rather clearer and less granular than the basal cells, which is perhaps partly due to their being more spread out and less compact.

The next event in the history of the spicule is the disappearance of the apical formative cells. The period at which this occurs is somewhat variable, not only in the case of different species, but in the spicules of the same individual, and even for the rays of the same spicule. In my preliminary account I stated for Cl. coriacea that the apical formative cells, which I then wrongly identified with the outer cells of the sextet, disappeared when the spicule rays had attained a length of 10 μ or 15 μ. More extended observations have shown me that this is far too low an estimate for the average length of ray at the time when this event takes place. Although the apical cells are often not to be found at this early stage, they more frequently persist until the spicule ray has attained a length of at least 20 μ, and sometimes even to a much later stage of growth.

In coriacea it is, in my experience, rare to find a spicule with rays exceeding about 20 μ in length which has apical cells present on all the rays, but here and there a spicule ray occurs which far exceeds this length, and still bears a formative cell at its apex, while the other rays of the spicule may show no trace of any such cell. In Pl. 38, fig. 9, is seen a spicule with rays averaging about 20 μ in length, and only one of the rays retains its apical formative cell. The spicule in fig. 10 has rays of about 45 μ in length, and still bears a formative cell at the apex of one of its rays. Fig. 14 shows a ray of about 60 μ in length, in which the apical cell appears to be in the act of leaving the ray. This is the longest ray I have ever seen bearing an apical cell in this species, and as a rule the apical cells disappear long before the ray reaches so great a length.

In other species, so far as my observations go, the apical cells may persist for a much longer time,—that is to say, until the spicule ray has attained a much greater size. This is especially the case in Clathrina, sp. dub. (see Pl. 39, fig. 19), in which species the apical cell disappears relatively late in the history of the spicule; in fact, it is usually found adherent to the tip of the ray after the basal cell has begun to migrate from the base outwards, and sometimes the basal cell may be found to have approached fairly close to the apical cell before the latter disappears.

In Clathrina contorta also the apical cells persist to a late stage (Pl. 41, fig. 38). It seems as if there was some relation between the size to which the spicule is destined to grow and the persistence of the apical cells. In the species characterised by larger spicules the apical cells persist to a relatively later period, as measured by the growth of the rays, than in the species with smaller spicules. Unfortunately it is in species of the former kind that the facts are most difficult to ascertain, on account of the greater thickness of the wall and the number of spicules crossing each other in all directions.

We see, then, that the period at which the apical formative cell disappears varies very much, and that in all cases it

persists long enough to show that it plays an important part in the growth of the spicule. Its disappearance is, indeed, a very remarkable fact, taking place as it usually does at a time when the sister formative cell is still at or near the base of the ray, and in all cases so far removed from the apex that a considerable stretch of the ray is left exposed and, to all appearance, covered only by the sheath.

So far as the further growth of the spicule is concerned, the apical cell no longer exists for us, but the question as to what becomes of it is of considerable interest. Unfortunately this question is one very difficult to answer positively. When the apical cell disappears, it is not to be supposed that it has any other fate than that of becoming merged in some class of cells, amongst which it is, or rapidly becomes, indistinguishable in its characters and appearance. It is scarcely possible that it should become a collar-cell or a wandering cell, both on general grounds and because it is sufficiently distinct in all its characters from cells of either class. It is also highly improbable for the latter reason that it should become a pore cell, for which office its inferior size alone would disqualify it, so to speak. If all these possibilities are excluded, there remain only two classes of cells into the ranks of which it could be received; namely, the flat epithelium and the connective-tissue layer. It is by no means impossible that after leaving the apex of the spicule ray it should become an actinoblast, and join with other cells like itself to form a spicule. All the actinoblasts, however, which I have seen were excessively granular, while the apical cells have few or no large granules, since those which they originally possessed became absorbed, as we have seen, during the growth of the ray. I have seen no appearances which suggest that the actinoblasts originate from the cast-off formative cells of spicules already formed, but many which indicate an origin for them from the flat epithelium by immigration. If, however, the actinoblasts come from the epithelium, there seems no great difficulty in supposing that their daughters, the formative cells, return there. The one feature of the latter which at all marks them

off, namely, their poorness in the coarse granules characteristic of the epithelium, is one that would not be very distinctive, since the epithelial cells vary a good deal in this respect, and it might be supposed that when once back in the epithelium they would rapidly re-acquire the granules which they lost when engaged in secreting the spicule.

While, however, it seems a reasonable supposition, indirectly supported in many ways, that the apical cell after leaving the spicule ray returns to the flat epithelium, direct proof of this is, from the nature of the case, very difficult, if not impossible, to bring forward. Appearances such as that shown in Pl. 38, fig. 13, seem to show that the apical cell wanders away from the spicule by amœboid movement. Its destination could only be determined with certainty by keeping it under observation when living, and this is scarcely possible. The matter has to be studied by observing and comparing different stages in preparations of preserved material, and by this method it is not possible to obtain more than circumstantial evidence. It can only be said, therefore, that the balance of evidence makes it a matter of extreme probability that the apical formative cell returns to the surface after leaving the spicule, and forms part of the flat epithelium from which it indirectly took its origin.

The history of the spicule subsequent to the disappearance of the apical cell is comparatively simple. The rays at this period have a sharply conical form, whatever may be the form of the complete spicule. Thus in coriacea the fully formed spicule rays have the form seen in Pl. 38, fig. 14,—cylindrical, or very nearly so, for their proximal half, and then tapering gradually to a blunt point. Contrasting fig. 14 with the figures preceding it, especially figs. 9, 10, and 12, we see that the spicule ray is built up to its full thickness at the base before it has attained its full length, and that the formative cell remains at or near the base until it has done its work there. It then migrates slowly towards the tip, building up the spicule as it goes, till finally in the fully formed spicule we find the definitive spicule cell persisting at the extreme tip of the

spicule. Comparing figs. 11 and 14 with figs. 9 and 10, one striking point brought out is the great activity of the originally basal formative cell. The apical cell usually disappears at so early a period that by far the greater bulk of the spicule ray must be secreted by the basal cell alone. Further, a comparison of different stages makes it evident that the spicule ray grows in length as well as in thickness after the apical cell has gone, and while the remaining formative cell is still at the base. This is conclusive evidence that the ray is enveloped completely by a layer of protoplasm even when the formative cell is quite at the base and the apex is apparently exposed, although the refraction of the spicule makes it impossible to distinguish clearly any such enveloping layer of protoplasm except near the nucleus.

During the growth of the ray the formative cell undergoes certain changes. First, the granules, which, as we have seen, have all along been diminishing in number and size, continue this process until they are at last completely absorbed. At the same time the protoplasm also appears to diminish slightly in quantity during the period of most active secretion, probably on account of its being spread over the whole spicule ray; as a consequence, the nucleus becomes slightly compressed between the spicule and the surface of the cell, and has an oval outline in side view and a circular outline in surface view. As the result of these changes the condition is attained which is characteristic of all fully formed spicules—a clear finely granular cell, with a slightly compressed nucleus, adherent to the extreme tip of each spicule ray.

The above account of the formation of the spicules is based, as has been said, upon their development in coriacea, but in the other species studied by me the process of spicule growth is so similar in all essential features that I have no hesitation in regarding it as the normal and typical mode of development for the triradiate systems of the genera Clathrina and Ascandra.

The differences in the development of the spicules in different species affect only matters of detail, and are largely such as are

due to the variations in the characters of the dermal epithelium, which have already been discussed above. In none of the species examined by me are the cells of the flat epithelium and the actinoblasts so granular as in coriacea (compare Pl. 39, figs. 17, 19, 20, and Pl. 41, fig. 40, with Pl. 38, figs. 4—7). In consequence the granules, being fewer, are absorbed more quickly, and the cells rapidly assume the clear, finely granulated appearance characteristic of the older spicule cells (Pl. 39, figs. 18—21; Pl. 40, fig. 33; and Pl. 41, fig. 38). We have noticed already the difference in the period at which the apical cells disappear. In coriacea the apical cells have not, as a rule, absorbed all their granules before their flight, but in contorta and the undetermined species they become perfectly clear. The spicule formation in cerebrum and reticulum does not call for any special remark, and beyond satisfying myself that it follows in these species the typical plan I have not studied it in great detail. It is indeed by no means an easy matter to follow the details closely in thick-walled species. The great mass of refractile spicules crossing each other in all directions makes it very difficult to follow minute details. For this reason I have not verified the scheme of development for the spicules of the adult Ascandra falcata, but have done so for the larvæ, as I shall proceed to describe.

The development of the spicules is not difficult to follow in larvæ which have fixed themselves on cover-slips. Clathrina blanca is rather an unsuitable species for this purpose, on account of the compactness and consequent opacity of the embryos, but cerebrum, reticulum, and falcata, on the other hand, are favorable objects. The fixed embryos should be preserved and stained with osmic acid and picrocarmine, and mounted either in glycerine or Canada balsam. The first spicules appear on the second day, i. e. about twenty-four hours after fixation; but new ones are continually being formed during subsequent development and growth. The embryos consist of a superficial layer of cubical or flattened dermal epithelium, enclosing the gastral layer, which is represented at first by a compact mass of cells, in which a cavity appears,

and grows until the gastral cells form a single layer round it. To see the spicules and their cells one must focus the microscope just below the dermal epithelium and external to the gastral layer. In this way I was able to prove to satisfaction that the triradiate spicules, from their first appearance onwards, are formed just as in the adult. This is shown in Pl. 39, figs. 22—26, for falcata; fig. 27, for cerebrum; and Pl. 40, figs. 31 and 32, for reticulum. On the other hand, my former statement with regard to cerebrum (1896 [1], p. 51), that the spicule-forming cells commence to secrete the spicule when still on a level with the dermal epithelium, was not confirmed by more extended investigations. This statement was based upon the examination of living specimens, at a time when material was scarce. Working a year later, with abundance of material carefully preserved, I was unable to find a single instance in which even the smallest spicules were not covered by the layer of dermal epithelium; and I must therefore retract my former statement, or at least restrict its application. The origin of the spicule-secreting cells from the dermal epithelium is very easily made out in the larva, both in surface views and sections. I reserve completer proof of this point for a work on the embryology. A point of difference between the young and the adults is found in the relatively large number of irregular spicules in the former. It is, if anything, rather the exception to find the rays meeting at regular angles; I have even observed instances in which one ray was lacking entirely, the two remaining rays meeting, perhaps, at an acute angle. Later, when the young sponge assumes the adult structure, the spicules formed are quite normal, and the irregularity becomes less marked. In spite of their tendency to variation, the first spicules can always be recognised plainly as belonging to the triradiate type, and that only. Monaxons and quadriradiates do not appear, as far as I have observed, till after the osculum is formed and the sponge has begun to grow.

To summarise the facts here brought forward with regard to the origin of the triradiate systems, we can recognise a scheme

of development common to all, with a number of constant and definite stages, as follows:

(1) Formation of "trios" by immigration of cells from the flattened epithelium.

(2) From the trios arise the "sextets" by division of each cell into two.

(3) The spicule appears with each of its rays corresponding to two sister-cells of the sextet, i. e. to two cells which have arisen from the division of one of the cells of the trio.

(4) As the rays increase in length the inner formative cells of the sextet remain at the apices, the outer formative cell at the bases, of the rays.

(5) Disappearance of the apical formative cells.

(6) The basal formative cells, after building up the rays to their full thickness at their bases, migrate slowly to the extreme tips of the rays, where they remain adherent as the definitive spicule-cell.

(*b*) The Origin of the Fourth or Gastral Ray of the Quadriradiate Spicules.—It has already been stated that the three basal rays of the quadriradiate spicule develop exactly as do the triradiate spicules, and that the fourth ray is an adventitious structure, derived from an entirely different source. In fact, while the mother-cells of the triradiate system are derived from the flat epithelium, that is to say, from the dermal surface of the body-wall, the mother-cell of the fourth ray is derived from the gastral surface of the body-wall, and from the layer of cells which we have termed above the porocytes.

Referring for a moment to what has already been stated with reference to the origin of the porocytes, we have seen that they originate in two ways in two different regions: (1) at the blind ends of diverticula, as well as throughout the sponge generally, from large granular cells of the flat epithelium which migrate inwards through the dermal layer, and come to lie between the collar-cells; (2) at the tip of the osculum, from the layer of large granular epithelial cells lining the interior of the oscular rim, which in their turn are

continuous at the oscular margin with the general flat epithelium covering the exterior.

The mother-cells of the adventitious rays—the gastral actinoblasts, as they may be termed—also originate in ways which, though essentially similar, yet differ in details in the two regions of the sponge body just mentioned. In the first case a porocyte, which is actually performing the functions of a pore, divides and gives off a cell which becomes a gastral actinoblast. In the second case one of the granular epithelial cells which line the oscular rim, and are destined to form pores, gives rise to a gastral ray without ever having been functional as a pore. Thus in all cases the gastral actinoblast arises from a porocyte; but while the commonest method is for the porocyte to become a functional pore-cell, which in its turn gives off an actinoblast, we have in a particular region a layer of porocytes giving origin to functional pore-cells on the one hand, and to actinoblasts on the other.

For demonstrating these facts Clathrina contorta is a particularly favorable object, on account of the yellowish-brown colour which characterises its pore-cells in preparations made with osmic acid and picrocarmine. The porocytes are in consequence very sharply marked off from other cells, and it is very easy to trace them. It is only necessary to lay out a piece of the sponge wall with the gastral surface uppermost, and if the piece selected contains gastral rays in an early stage, there is no difficulty in finding them. By the study of Cl. contorta I was able to confirm and to extend observations which I had made a year earlier upon Clathrina, sp. dub.

I will commence with the more usual mode of origin. Pl. 42, fig. 49, shows in Cl. contorta a young triradiate system, lying at a deeper level, below the collar-cell layer. Immediately over the young spicule is a coarsely granular cell, the gastral actinoblast, wedged in amongst the collar-cells and still in continuity with a pore-cell close at hand. The pore-cell and the actinoblast resemble each other exactly in the characters of the nucleus and cytoplasm. The nucleus of the actinoblast lies over the centre of the spicule, and as yet no

trace of the gastral ray is to be seen. Fig. 50 on the same plate shows almost exactly the same state of things, except that the rays of the triradiate system have grown slightly, and at their junction a minute gastral ray has appeared in the gastral actinoblast, the nucleus of which is now no longer placed over the centre of the spicule, but to one side.

Fig. 51 shows a condition considerably in advance of the foregoing; the actinoblast is quite separate from the pore, and its nucleus has divided in two, while the ray secreted by it has also grown considerably. In fig. 52 the condition is much the same, except that the actinoblast still retains its connection with the pore.

In Clathrina, sp. dub. (Pl. 39, figs. 18—21), I was able to observe some important stages in the formation of the fourth ray, which place the origin of the actinoblast beyond all doubt. The preparations were mounted in the usual way after removal of the collar-cells as described above, so that it was possible to make out clearly the actinoblasts of the basal system. Fig. 18 shows a triradiate system with its formative cells, and extending over it is a pore-cell, perfectly typical and normal except in possessing two nuclei. In fig. 18*a* the pore-cell is drawn separately. One of the two nuclei is much deeper in colour, and lies close to the minute gastral ray which has already appeared at the junction of the rays of the triradiate system. The pore-cell shows a commencing constriction, dividing off the portion with the more deeply stained nucleus as an actinoblast from the pore-cell proper with the paler nucleus.

Figs. 19 and 19*a* show a similar state of things, except that the actinoblast has nearly left the pore-cell, being connected with it by a drawn-out neck of protoplasm which, as can be seen from its indented outline, extended between the collar-cells. Finally, figs. 20 and 21 show the actinoblast completely cut off and secreting the growing gastral ray.

The stages figured in the two species enable us to construct with certainty the following history for the first appearance of the gastral ray. When a gastral ray is to be added to a tri-

radiate system the first sign of activity is the division of the nucleus in a neighbouring pore-cell. An outgrowth from the pore-cell containing one of the nuclei then extends over the triradiate system, and in this extension of the cell the gastral ray arises close to the nucleus. Finally the outgrowth becomes nipped off from the pore-cell to form the actinoblast. We see that the gastral ray may make its appearance when the actinoblast has become all but separate from the pore-cell (Pl. 42, figs. 49 and 50), or long before this event takes place (Pl. 39, figs. 18 and 18*a*). The amount of separation between the two cells probably depends upon how far distant the pore-cell originally was from the triradiate system.

The origin of the gastral rays in the oscular rim has not been followed by me in the same detail, but only sufficiently to make their origin clear, which I think is done by Pl. 41, fig. 38. Here we see a triradiate system with its six cells lying under the coarse granular epithelium of porocytes; one of the latter, which already contains two nuclei, is engaged in the formation of the fourth ray. The figure shows well the great difference between the basal formative cells and the gastral actinoblast. Pl. 41, fig. 41, shows an almost identical state of things in a section of a decalcified specimen of the sponge. I have not seen the earliest stages of the gastral actinoblasts in this region, so cannot say whether they represent the whole of a porocyte, or whether, as is more probable, a porocyte gives off a cell to form a gastral ray, as in the cases already described.

Having, as I think, made sufficiently clear the origin of the gastral actinoblasts, it only remains to follow their subsequent history, which I have done in the case of Clathrina contorta, Cl. reticulum, and Cl. cerebrum. Their behaviour in the different species presents some points of interest, and is most conveniently studied in sections, which should not be too thin —10 μ at least.

To begin with contorta. We have seen that the nucleus of the actinoblast divides into two (Pl. 41, figs. 38 and 41; Pl. 42, figs. 51—53). The cell does not, however, divide Each nucleus soon divides again (Pl. 42, fig. 54). Finally

an exceedingly long and slender ray is formed, projecting far into the gastral cavity, enveloped in a continuous mass of protoplasm, a true plasmodium which contains four nuclei scattered along the spicule ray (Pl. 42, fig. 55).

In cerebrum (Pl. 40, figs. 28—30) there are two classes of quadriradiates, distinguished by the characters of their gastral rays. In one class the gastral ray is comparatively short, and beset with spines near the extremity (fig. 29) ; these spicules are very characteristic of the species, and have been noticed by all the authors. The other class of quadriradiates, on the other hand, seems to have been generally overlooked, though it is the more abundant in many individuals; it has the gastral ray long and tapering, often slightly curved at the tip, and without spines (fig. 30). Typical examples of these two classes are distinct enough, but nevertheless they are connected by numerous intermediate forms. Thus in some spicules the gastral rays are spiny like that represented in fig. 28, but have the ray prolonged considerably beyond the spiny region ; in others a long tapering ray like fig. 30 may have minute rudiments of spines upon it.

In the young spicule the gastral ray is slender, straight, and smooth (fig. 28). After the ray has attained its full thickness at the base the actinoblast migrates towards the tip, leaving the cylindrical basal portion apparently exposed. The nucleus of the actinoblast does not divide, but in the spiny spicules granules resembling chromatin make their appearance, scattered about in the region of the spines (fig. 29). In the smooth spicules similar granules are present, but instead of being scattered they are collected together at the extreme tip of the spicule ray, the nucleus being at the opposite extremity near the basal limit of the actinoblast (fig. 30). I am unable at present to explain these appearances, which were observed in all the spicules examined, and both figs. 29 and 30 could have been repeated, if necessary, to any extent. The impression given is that granules of chromatin are budded off from the nucleus to superintend, as it were, the centres of secretion which give rise to the spines, though it cannot be said that

each such granule corresponds to a spine. In the smooth spicules, on the other hand, the granules seem to be about to form a second nucleus, and this is perhaps the manner in which nuclear division takes place; but I have not succeeded in finding an actinoblast containing two definite nuclei, but only the state of things shown in fig. 30, one nucleus and a bunch of granules. If this really represents nuclear division it would be rather an unusual type, though essentially similar to the manner in which, according to Fiedler (1888), the nucleus of the ovum of Spongilla buds off little masses of chromatin which represent the polar bodies.

In reticulum (Pl. 40, figs. 33—37) some quadriradiates have the fourth ray comparatively short (fig. 35), while others have it excessively long (fig. 37). The former have one nucleus, the latter two, in the actinoblast. It is very easy to find instances in which the nucleus has only recently divided (figs. 33, 34, and 36), though I have not seen the actual process of nuclear division, but this would probably be a favorable object in which to study it. The interesting point to notice, from the point of view of the growth of the spicule, is the variation in the period at which nuclear division takes place. We may assume that if the two nuclei are close together the division must have taken place recently, since they afterwards become so widely separated, as shown in fig. 37. In this way we see that the division sometimes takes place at an extremely early period (figs. 33 and 34), and sometimes only after the ray has attained a considerable size, as in fig. 36, where the small size of the two nuclei is a further proof of their recent division. Thus it would seem as if a ray was sometimes destined from the first to attain a great length, while at other times an elongated ray arises by some change at a later period in the secreting cell of a ray originally destined to be short. From the large number of spicules that occur with gastral rays similar to that drawn in fig. 35, it can hardly be doubted that many never go beyond this stage, and are, so to speak, adult spicules. But a comparison of figs. 35, 36, and 37 shows how gastral rays of the short kind may be converted into rays of

the long kind by division of the nucleus after the actinoblast has retreated from the base of the ray. On the other hand, in fig. 33 the spicule ray is shown to be in its infancy from the way in which the actinoblast extends down to the base, and the presence of two nuclei gives every promise of a future great development in size of the spicule ray.

The facts both in cerebrum and reticulum point to an incipient differentiation of the quadriradiates into two classes, already quite distinct in typical examples, but as yet connected by transitions. In reticulum, at least, the spicules of one class can probably become actually converted into spicules of the other class. In view of the many instances amongst Ascons and Calcarea generally of the spicules of a particular kind—triradiates, quadriradiates, or monaxons—being differentiated into two classes as a specific character, these facts are not without interest.

There remains one fact to mention with regard to the gastral actinoblasts, and that is the frequent occurrence in them of rod-shaped or needle-like bodies of crystalline appearance (Pl. 39, fig. 18, Pl. 40, fig. 30, and Pl. 41, fig. 41, *x*). I am unable to state what may be their nature or significance. That shown in fig. 41 was most distinct, and is interesting as occurring in a decalcified specimen.

To sum up the facts observed with reference to the quadriradiate spicules, the fourth or gastral ray is an adventitious element superadded to the triradiate system, and secreted by a mother-cell which is derived from a porocyte. The nucleus of the secreting cell may remain single, or divide into two or into four nuclei; but in all cases the cell itself remains undivided, forming a plasmodium-like investment to the spicule, or at least to its terminal portion.

(*c*) Some Observations on the Formation of the Monaxon Spicules.—In a former paper (1896) I described the formation of the monaxon spicules in young stages of Leucosolenia variabilis, and showed that they originated each in a single cell of the flat epithelium. I studied at

Banyuls the growth of the large monaxons of the species termed by me Clathrina, sp. dub., but did not succeed in finding the earliest stages. In the youngest stage found the spicule bore three cells, two fusiform cells attached to the shaft, and a branched cell at the extreme apex of one end, apparently the proximal end. The two cells on the shaft each closely resembled a basal formative cell on the ray of a triradiate system, and the cell at the extreme tip resembled an apical formative cell. A later stage had five cells, four on the shaft and one at the apex. A still later stage had the apical cell and a number along the shaft, but it was difficult to determine exactly how many on account of the great size of the spicule (Pl. 39, fig. 20, *spic. monax.*).

The stage with five cells is obviously to be derived from the stage with three by division of each of the two cells applied to the shaft in the earlier stage. This suggests that the stage with three cells is derived in a similar manner from a stage with one cell on the shaft and one at the apex; this would be a state of things exactly comparable to what is found ordinarily on the ray of a triradiate, with its basal and apical formative cell. The huge monaxons would then be formed just as a single ray of a triradiate system, with the difference that the basal formative cell repeatedly divides to furnish a row of cells which build up the spicule. In this connection we may refer to the interesting lines of growth described by Ebner (1887, Pl. 41, figs. 51—53) in the large monaxon spicules of Leucandra alcicornis and aspera, each system of lines being probably referable to a separate secreting cell.

Many of the large monaxons in Clathrinidæ, on the other hand, are almost certainly not true monaxons, but derived by modification of a triradiate system (cf. Haeckel, 1872, p. 350). In this way one can distinguish true or primary monaxons from what may be termed secondary monaxons. A good instance of the latter is to be found, probably, in the large elbowed monaxons in the stalk of Clathrina lacunosa. I have long had a belief that the large monaxons found in Clathrinidæ would turn out to be in all cases secondary

monaxons. My observations, so far as they go, have by no means dispelled this suspicion.

Lendenfeld employed the presence or absence of monaxon spicules to classify Ascons. If the term "monaxon spicule" be employed without further qualification, it represents a character which, if used for systematic purposes, yields, to my mind, a very artificial classification. But I think it extremely probable that primary monaxons will be found restricted to the Leucosoleniidæ, where they are the first spicules formed, and that in Clathrinidæ, where the triradiate systems appear first, the monaxons are secondary. This is, however, at present a pure speculation. I hope at some future time to be in a position either to prove or to demolish these views.

Appendix.—A few points in connection with the formation of the spicules or with their cells seem to call for special notice before leaving the subject.

(1) The Triradiates of Clathrina clathrus.—In 1892 ([3] p. 183) I described the spicules of this form as having on their rays sometimes one cell, sometimes two, and sometimes a cell with two nuclei close together. As this is rather a different state of things from anything I have found in the species investigated in this paper, I examined the point again, and find my description perfectly correct. Looking now at a series of drawings made by me at that time with the aid of the camera lucida, I have come to the conclusion that the apparent anomaly is due to the frequent persistence of the apical formative cell on the fully formed spicule rays of this species. When, as is frequently the case, there are two nuclei in a single cell, adherent to the tip of the spicule, I believe this to be brought about by the basal formative cell having travelled to the apex of the spicule ray, and there fused with the persistent apical cell.

That the apical cell should persist in this way is rather unusual, but may perhaps be connected with the cylindrical form of the rays, which in this species terminate abruptly

or are even slightly clubbed—an uncommon form of spicule ray. Whether this form of ray with persistent apical cell is more primitive than the more pointed rays or not I am unable to say, but it seems probable. Even in Cl. clathrus, however, the apical cell by no means invariably persists. In the majority of cases the spicule ray simply has a single fusiform cell at or near its distal extremity, as in the forms described above.

(2) The Influence of the Nucleus on the Secretion of Lime.—It may be of interest to bring together the facts observed which indicate a relation between the nucleus and the secretion of the spicule. These are (*a*) the deeper stain, in many cases, taken by the nuclei of the secreting cells; (*b*) the fact that the nucleus places itself in the region of greatest activity; for instance, in the case of the rays of the triradiates the nucleus of the basal cell remains near the base of the ray till it is fully formed, and then moves along towards the tip, superintending, as it were, the work to be done; (*c*) the division of the nucleus where the secretion is carried on over a considerable length of ray, as in the case of the long gastral rays of reticulum and contorta, or the large monaxons of Clathrina, sp. dub.; and (*d*) finally we may refer to the chromatin-like bodies scattered over the spinous portion of the gastral rays of cerebrum. So far as can be judged from surface views, the minute sclerite always makes its appearance in the immediate neighbourhood of the nucleus.

(3) The Granules of the Dermal Layer.—We have seen that all the cells of the dermal layer contain very characteristic granules, to which in the first place the colour of the sponge is due. When the sponge is any other colour than white, the colouring matter is contained in the granules of the dermal layer, and is rapidly dissolved out of them by alcohol. The granules attain their greatest development in the porocytes, where they were clearly described by Metschnikoff; but they are also present in the flat epithelium, and usually in great abundance. They are always found in the young spicule-secreting cells; but while in the case of the triradiate systems

they are absorbed more or less rapidly as the spicule grows, in the case of the gastral actinoblasts they persist throughout in undiminished size and quantity. As I am not acquainted with any species with quadriradiates which shows colour varieties, I am unable to state whether the granules in the gastral actinoblasts would retain the vivid coloration which the granules often show in some species,—coriacea, for instance. In the latter I have seen an orange-red specimen which had a sieve membrane across the osculum, and, as might have been expected, the sieve membrane was also of an orange-red colour.

I am not able at present to make any positive statements about these granules, but will briefly notice some views that have been put forward about them.

Bidder (1892 and elsewhere) regards the granules as excretory, especially in the porocytes. They seem, however, too constant an element in the cells of the dermal layer for Bidder's theory to be a complete explanation of them. There is, moreover, a strong primâ facie argument against the cells of the flat epithelium or the porocytes being concerned in excretion, as then the excreted products would immediately be carried back into the sponge by the currents. On the other hand, it must be confessed that the porocytes which line the oscular rim would be in a particularly favorable situation for exercising the function of excretion.

Topsent, on the other hand, as we have seen, regarded the granules of the porocytes, or "cellules sphéruleuses," as representing reserve nutriment. It might be doubted if Topsent would have come to this conclusion had he known that the "cellules sphéruleuses" were simply contracted pore cells. He seems, further, to have overlooked the fact that similar granules occur in the cells of the flat epithelium.

It seems to me probable that the granules in question subserve more than one function, but as a peculiarity of all the cells in which they occur is their contractility, especially in the case of the porocytes in which they are most abundant, I have long thought they might have some connection with this function. Bütschli has shown how, on the alveolar theory of

protoplasm, the granules might exercise a great influence on internal movements of the protoplasmic framework, such as those which result in contraction in one or another direction (1892, pp. 207—209; English translation, pp. 323—327). The distribution of the granules in the dermal layer favours this view of their function, since they are most abundant in the very contractile porocytes, of which any specially contractile organs—sphincters or sieve membranes—are formed, while in the spicule-secreting cells they rapidly disappear. An exception to this is apparently to be found in the case of the gastral actinoblasts, which, however, according to Lieberkühn (1865, p. 737), are retractile, a fact that would explain the persistence of the granules in them. It is noteworthy also that while the dermal layer of the Clathrinidæ is remarkable both for its richness in granules and for its contractility, the corresponding layer in the non-contractile Leucosolenias is very clear and free from large granules.

The theory that the granules aid in the contraction is, however, devoid of any actual basis of experiment or observation, and is to be regarded merely as a possible hypothesis, which is at least worth testing.

III. Historical: Earlier Observations upon Calcareous and other Spicules.

(*a*) Observations upon Spicule Formation.—Direct observations on the origin and growth of the spicules in calcareous sponges are few and far between in the literature of the group; confident assumptions, on the other hand, with regard to the way in which it is supposed a priori that the spicules should and must arise, are to be found in abundance. In the almost total absence of facts, the foundation for the current beliefs concerning spicule formation in the Calcarea is to be sought partly in deductions from theory, partly in analogies from the well-established facts as to the origin of siliceous spicules. It is best, therefore, to begin by a brief survey of what has been ascertained in non-calcareous sponges.

1. Observations upon Siliceous Sponges.—For the spicules of Monaxonida the classic researches of Lieberkühn and Carter established the fact so far back as the fifties that the spicules of Spongilla originated each within a single cell. At a still earlier date Carter had been of opinion that spicules and horny skeleton were "formed in the intercellular substance, and not within the cells;"[1] but direct observations caused him to abandon this opinion. Lieberkühn gave a full and clear description of the formation of the spicules of Spongilla in 1856 (pp. 407—409, figs. 17—30); and a year later, but independently, Carter published an account confirming that given by Lieberkühn (1857, p. 23, fig. 8). Carter afterwards extended his observations to marine sponges, and described a similar origin for the spicules in Esperia ægagropila, Jnstn. (1874 [1], pp. 101—105, and [2] p. 456, pl. xxi, fig. 26), and in Microciona armata, Bwk. (1874 [2], p. 457, pl. xxi, fig. 27). Schmidt had already (1864, p. 5, Taf. i, fig. 13) described the formation of the spicules of Reniera, sp., and he is quoted by Sollas as describing in detail their origin and growth in Esperia, in a work which I have not been able to see.[2] Kölliker also figured and described the monaxon spicule in its formative cell (1864, p. 61, woodcut 15), and Keller described the spicule formation in the embryo of Chalinula fertilis (1879, p. 334, pl. xix, fig. 20). Sollas gave further instances of spicule development in a series of papers dealing with sponges of Norway; the bundles of trichites originating, each bundle, in one cell (1880, pp. 141, 142, pl. vii, fig. 21); the formation of the sterrasters of Geodia Barretti, Bwk., each in a single cell (1880, pp. 256 and 401, pl. xi, fig. 18, and pl. xvii, figs. 18—22); and the monaxon spicules arising each in a cell (1882, p. 159, pl. vii, figs. 12, 17, 18). It is not necessary to multiply these instances further; all observers are of one accord in describing the monaxon spicules and microscleres of Demospongiæ gene-

[1] 'Ann. and Mag. Nat. Hist.' (2), iv, 1849, p. 95.

[2] 'Zoologische Ergebnisse der Nordenfahrt,' p. 120; see Sollas (1888), p. xlv.

rally, as arising each within one single mother-cell. Sometimes even, as in the case of the bundles of hair-like spicules known as trichites, more than one spicule may arise in the scleroblast. In view of this pleasing unanimity with regard to the first origin of the siliceous monaxon spicule, it is to be regretted that so little seems to be known of the fate of the primitive scleroblast. Does the mother-cell persist on the fully formed spicule? Does the nucleus of the secreting cell remain single, or does it ever divide? These are questions yet to be answered. Carter discusses the point, and is of the opinion that "ornamental or subsidiary parts, such as the spines, &c., are subsequently added, probably after the spicule has left the mother-cell, and has got into the intercellular sarcode, as shown by the central canal never extending into them" (1875, p. 12). Here, then, we see the belief very clearly expressed that the mother-cell is not responsible for the whole spicule, even in simple forms; and as no one would now ascribe any skeletogenous function to the jelly or "sarcode" of Carter, it must be supposed that other cells besides the original mother-cell take part in aiding the growth of the spicule. Ridley and Dendy are also of opinion that "it is pretty certain that the larger forms at any rate become free from the parent cell (silicoblast) before attaining their full size" (1887, p. xiv). On the other hand, Maas found in Spongilla larvæ spicules of $\frac{1}{2}$ mm. in length, with only one silicoblast nucleus, and thinks "that the whole duration of the growth only claims a single cell, the more so as he has never seen spicules with cells applied to them like an epithelium" (1890, p. 539). Delage also (1892) figures many instances of spicules and their cells, but never more than one cell to a spicule. But in the "shovels" (anisochelæ) of the Esperia larva Maas found four nuclei to each shovel, one on each side of the blade and one on each side of the shaft (1892, p. 420, Pl. xxviii, fig. 19). These shovels are united into rosettes, and this fact suggests, according to Ridley and Dendy, that each such rosette originates within a single cell (1887, p. xx). From all this conflict of evidence and opinions the only thing clear is that the

subject is ripe for further investigations, and that at present no certain conclusion can be drawn as to the fate of the mother-cell.

Of the origin of the triaxon spicule of Hexactinellids we know at present nothing whatever. But with regard to the tetraxon siliceous spicule, the evidence is all in favour of an origin within a single mother-cell. Schulze's observations on Plakina (1880) are, so far as I know, the earliest in this field. The author figures in the plainest manner the tetractines in the young sponge as enclosed each in a single cell (pl. xxii, fig. 29), and the theory is advocated that the primitive siliceous spicules were irregular thorny bodies, in which the rays first became concentrated in a point, giving rise to irregular multiradiate spicules; that then the number and direction of the rays became fixed, so that there were formed hexactines and tetractines; and that from these forms arose triactines, diactines, and even monactines, by reduction of the rays (1880, p. 445). A year later Schulze figured and described in Corticium "a distinctly formed regular tetractine" "in a rounded mesoderm cell containing a few pigment granules," situated close to the outer surface of the sponge; the spicule was "still so completely embedded in the protoplasm of the cell that its tips did not even project into the surrounding ground substance" (1881, pp. 426, 427, pl. xxii, fig. 10).

The next author to deal with the origin of the tetractines is Sollas (1888). He says, "In the Choristida all the spicules, both large and small, originate each in a single scleroblast, which persists throughout the life of the spicule. The scleroblast in the case of the large spicules is a large granular cell, extending all round the spicule, which it has formed as a siliceous secretion" (p. xlvi). Reference is then made to two figures (pl. ii, fig. 20; pl. xiii, fig. 10), both of which, however, only show one arm of the spicule, the rhabdome, with a cell upon it; but in Tribrachion Schmidtii the author figures very clearly entire orthodiænes of various sizes, each with a single scleroblast upon the rhabdome (pl. xvii, figs. 12 and 20). On the other hand, Sollas also figures three cases in

which the shaft of a large spicule has a series of pyriform cells along it, considered by the author to be derived from the "collenchyma" (pl. iv, fig. 30; pl. v, fig. 6*a*; pl. viii, fig. 37; compare p. 12). Sollas further states that in the Lithistida the crepis of the desma is formed in a single scleroblast, but that other cells are found on the arms of the desma. "Each of the four depressions which occur about the centrum in the angle between the arms of a tetracladine desma appears to be occupied by a scleroblast, and others may possibly be distributed along the sides of the arms" (p. xlvi). Reference is given to figures (pl. xxx, figs. 20 and 21) showing the minute tetractines, each enclosed in a cell. Finally, Lendenfeld (1894 [1], p. 166) expresses an opinion with regard to the tissues and asters of Geodia and Ancorina, which is the same as that which Sollas enunciated in more general terms. He believes the spicules to arise within single cells as a refractile body near the nucleus, afterwards embracing it. Some of the cells have a process and are believed to form triœnes; others show a radial striation and form asters. No figures are given in support of these statements.

From a study of the literature of siliceous spicules, and without going beyond the statements of the authors, one would be justified, I think, in making the following generalisations:—(1) Every spicule originates within a single cell, which perhaps in many cases receives assistance afterwards from other cells, and certainly does so in the case of the complicated desmas of the Lithistida. No evidence for division taking place in the mother-cell or in its nucleus. (2) The forms with few rays are derived from those with many, by reduction and loss of some of the rays. This applies in the first instance to the spicules of the Hexactinellids and Tetractinellids, but it perhaps holds good also for the phylogeny of the simple spicules of the Monaxonida.

(2) Observations on Calcareous Sponges.—The earliest recorded observations upon the relations of calcareous spicules to the tissues of the sponge are those of Kölliker (1864) upon

a species from Villafranca which he termed Nardoa spongiosa.[1] Kölliker discovered the spicule sheath left behind after the spicule is dissolved by acetic acid. On the rays of the quadriradiates which project into the gastral cavity the author observed "another problematic structure, namely, a dark, granulated, conical mass, which envelops the calcareous ray, and also, as it seemed to me, the sheath. Seen in surface view these structures appear as round cells, and only profile views make clear their true relations (pl. vii, fig. 10). In some cases this dark granular mass is continued in reduced quantity (verschmalert) over the portion of the calcareous ray lodged in the epithelium; but I was unfortunately unsuccessful in discovering the true significance of this curious structure" (p. 65). It is easy to recognise in this account a description, perfectly accurate as far as it goes, of the cells I have termed the gastral actinoblasts, the secreting cells of the gastral rays. The cells which the author mentions further on in his description as occurring on the walls of the canals near the ciliated epithelium, "rather larger rounded cells, singly or in groups, the significance of which remained unknown to me" (p. 65), were very probably porocytes.

[1] Haeckel identifies Kölliker's "Nardoa spongiosa" with either "Ascaltis Gegenbauri" or cerebrum. I am inclined to dispute both these identifications for the following reasons:—(1) Haeckel's "Ascaltis Gegenbauri" is figured by him as having the gastral layer folded like Ascandra falcata; this is a point which certainly would not have escaped Kölliker, who neither mentions nor figures any such peculiarity; (2) the excellent figures of the sponge given by Kölliker (pl. ix, figs. 6, 7) are not from a specimen of Clathrina cerebrum; (3) there is a distinct reference on p. 54 of Kölliker to monaxons in his sponge, though he does not mention them in the special description. From Kölliker's figures of the sponge and of its long and slender gastral rays (pl. vii, fig. 10; and pl. ix, fig. 8) I am inclined to identify it as contorta, which is one of the commonest Ascons in the Gulf of Lyons; or possibly as spinosa, Lendenfeld, which, as I have stated above, I believe to be identical with contorta, differing only in the total absence of the monaxon spicules so variable in size and number in the true contorta. The gastral ray figured at pl. vii, fig. 10, is not full-grown, as shown by the position of the actinoblast near the base.

As Kölliker had ten specimens of his sponge there may possibly have been more than one species amongst them.

In the following year (1865) were published Lieberkühn's observations upon "Grantia botryoides" = Leucosolenia Lieberkühnii, O. S., of which it is not too much to say that his descriptions attain a far higher degree of truth and correctness in many points than some which have appeared more than a quarter of a century later. He recognises at the outset the fact of the body-wall being composed of two distinct layers, "a layer of contractile parenchyma and a layer of ciliated cells which clothe the inner surface" (p. 734). Here we have the two layers for which I have revived Haeckel's terms, dermal and gastral layer; in the former "spherical, oval, and stellate corpuscles, at varying distances from one another, can be distinguished in the transparent homogeneous parenchyma" (p. 735). On the same page we are told that just below the oscular margin the ciliated lining ceases, and the aperture is surrounded by a simple layer of jelly substance; what better description could be given of the oscular rim, of which it has so strangely fallen to my lot to maintain the existence and to point out the significance? Further on (p. 738) the author repeats the observation, and gives it a more general application; and on p. 742 he shows that a similar region occurs in Sycons. With regard to the spicules Lieberkühn observes (p. 736) that on many of the projecting gastral rays "a fine layer of the contractile substance [i. e. of the dermal layer] can be seen pushing its way out between the ciliated cells, and either covering the spicule completely or partially in a fine layer, or enclosing only the root of it in a thicker mass (Anhaufung)." Treatment with acetic acid dissolves the spicule, "and the contractile substance remains behind as a more or less thin-walled sheath." Hereby the author thinks that Kölliker's observations, which he quotes, receive an explanation; and he further observes, "In favorable cases the conical mass can even be traced through the epithelium, and recognised in continuity with the contractile substance, in many places very granular. Besides this, the same forms of thickened sheaths of the contractile substance can be found singly on the free outer surface [doubtless the

scleroblasts on the projecting monaxons]. These, as well as those of the inner surface, are retractile"—an observation which, if true, would be of great interest.[1]

Not less remarkable than these early observations of Kölliker and Lieberkühn, which so nearly solved the question of the origin of the gastral ray, is the fact that no further observations upon the gastral rays are to be found in all the voluminous literature of calcareous sponges until we come to those of Dendy, to be mentioned in due course, published in the present decade.

The next author to formulate opinions upon the formation of calcareous spicules was Haeckel, but his views were purely theoretical and devoid of any basis of fact or observation. Haeckel regarded the dermal layer of the sponge as a syncytium, formed by the fusion of cells originally distinct in the embryo. He supposed the ground substance or sarcodine to be made up of the fused protoplasm of the cells; and that in this sarcodine, "at once the outer covering of the body and its 'skeletogenous layer,' its contractile and its sensitive tissue" (p. 164), the spicules arise by a sort of crystallisation, the spicule sheath being formed by "a thickening and separation from the sarcodine" (p. 167). Altogether a very clear and logical theory, did it but harmonise with the facts.[2]

Schulze, in his classical memoir on Sycon raphanus (1875), did not make any observations on the origin of the spicules, but appears to have been largely of Haeckel's opinion.

[1] Haeckel, having never seen a scleroblast—incredible though this may seem,—understands Lieberkühn to mean that the sheath is retractile, and denies that this is the case. I do not attach this significance to Lieberkühn's statements. The scleroblast may quite well be retractile.

[2] It is remarkable that Haeckel should never have seen and figured the gastral actinoblasts, especially after the clear descriptions of Kölliker and Lieberkühn. In such a figure as that of Ascaltis Gegenbauri (1872, pl. ix, fig. 7) we are astonished to see nothing of these cells, usually so conspicuous. A closer inspection, however, of the figure, to which reference has been made, reveals a number of sperm masses in close proximity to the spicule ray, in such a way as to provoke the suspicion that Haeckel confused two totally distinct kinds of cell elements.

He believed the external protoplasm of the cells of the dermal layer to be "completely fused and modified into a homogeneous and possibly contractile ground substance" (p. 252), and he agreed with Haeckel in regarding the spicule sheath as a condensed layer of the ground substance closely surrounding the spicule (p. 254). That Schulze at this period agreed with Haeckel as to the mode of formation of the spicules may be further inferred from the fact that in his work on the development of Sycandra raphanus (1878), while figuring clearly the monaxon spicules each in its secreting cell (pl. xix, figs. 10, 11), he yet stated in the text that the spicules arise in the hyaline substance between the two layers.

With the year 1879 we come to the only important observations, as apart from theories, on the formation of calcareous spicules which have been made since the sixties, namely, those of Metschnikoff. This author first demonstrated that calcareous, like siliceous spicules, arise within cells, and not free in the ground substance of the sponge body. Valuable as were Metschnikoff's results, they are nevertheless not free from important errors of misinterpretation as regards the details of the development, though based on accurate and careful observation.

The specimens of Ascons which Metschnikoff investigated seem to have been for the most part more or less contracted, since in sections he found a dermal epithelium composed of flask-shaped cells. We have already noticed his observations on the porocytes, which he believed to be granular mesoderm cells. In Clathrina primordialis he found these cells giving rise to the skeleton. "The smallest calcareous spicules are formed in the interior of such cells" (p. 361, and pl. xxii, figs. 4, 5, 8, and 12 *s*.). This is a most important statement in view of what has been said above, namely, that both the porocytes and the spicule-forming cells originate from the dermal epithelium. The cell masses surrounding the smallest spicules do in fact resemble closely in appearance the contracted porocytes, a resemblance due to the possession of similar cytological characters derived from a common origin. Hence it is not

difficult to understand how Metschnikoff should have confused them in contracted specimens. The youngest spicules are figured by Metschnikoff in the middle of rounded granular protoplasmic masses, which become trefoil-shaped as the spicule grows. There can be no doubt from his description that he regards each of these protoplasmic masses as a single cell. He appears, however, to have seen them only in the fresh state, and in his figures no nuclei are shown; had he applied suitable reagents, he would of course have found each of these masses to be six cells, and not one only. It is a little strange he should not have seen any cell outlines, but they are not very obvious until the spicule has begun to grow and to cause the cells to separate. In any case Metschnikoff was the first to point out that the spicules "always arise in the cell protoplasm, and not in the jelly-like ground substance." He does not figure cells on the rays of the spicules, but the cell *a* on pl. xxii, fig. 5, is obviously a spicule-cell detached from the spicule (compare his description, p. 361). On the whole, I think I may fairly claim that Metschnikoff's observations confirm, or even forestall, my own, though his interpretation of them requires modification.

Poléjaeff, in his work on the '"Challenger" Calcarea,' observed cells which he interpreted as scleroblasts on some of the spicules, monaxon as well as triradiate (1883, p. 32). The example which he figures (pl. vi, fig. 3, *c*.) is certainly very remarkable in appearance, the result, perhaps, as the author suggests, of preservation in alcohol.

In 1885 Lendenfeld published a series of papers on Australian calcareous sponges, which contain some observations on the spicules and their origin. Of the spicules he states (1885 [2], pp. 979, 980) that by prolonged action of gold-potassium chloride the spicule splits into prisms "parallel to one another, radiating from the axis." "The radial structure first makes its appearance in the interior, close to the inner axis, which is a cylindrical chord of organic matter without lime." "The inner part, the part produced first, of the spicule is softer, and contains more organic matter, whilst the outer

layers, the youngest part, is [sic] harder, and resists the action of reagents; the whole spicule is composed of prisms formed as cuticular productions by the cells clothing the spicule from without" (p. 980). "The spicules first make their appearance within cells, and the axial rod (not canal!) is part thereof. The succeeding layers are cuticular productions of endothel cells." The author gives no pictorial illustrations of his researches on spicule structures, but he has a figure showing "mesoderm cells" forming an "endothel" "in the the shape of a hollow tube" covering the rays of a triactine of "Ascetta procumbens" (1885 [1], pl. lxiii, fig. 3; and description, p. 1146; also [2], p. 980). He is more successful in his delineations of hard structures than of soft, since he draws quite correctly the sharply conical form of the rays of the young triradiate as compared with their more cylindrical contour when fully formed.

Lendenfeld's later statements with regard to calcareous spicules are not much happier than his early efforts in this direction, and a few quotations may serve to make clear his standpoint. On p. 198 of his work on Adriatic sponges (1891) we read, "On the surface of the spicules [of Clathrina primordialis] one observes not infrequently flattened cells, sometimes provided with processes, which singly or united in small groups partly envelop the spicule." In opposition to Metschnikoff the author finds that "the irregularly shaped lumps of protoplasm" which envelop young spicules, or are applied to them, are fairly transparent and free from refractile granules (p. 199). "The spicules probably arise in cells, but it must be pointed out that even the youngest of the large spicules which are to be found in the sponge body are much longer than any known sponge cell with exception of the ripe ova. The further growth of the spicule takes place by means of numerous cells, which settle on the surface of the young spicule and precipitate spicular substance upon it. . . . . The skeleton-forming elements are always cells of the intermediate layer (Zwischenschicht)" (p. 383). These three sentences furnish a good instance of

this author's habitual method of stating dogmatically, and as if from his own observation, propositions which are the result merely of imagination, and deduced a priori from, in this instance at least, unsound premises.

In the present decade Dendy has put forward views on the origin of calcareous spicules, which can best be made clear by quotations from his works. "It is generally admitted," he says, "that both calcareous and siliceous spicules originate within special mother-cells, but probably in both cases they subsequently receive additional layers of the calcareous or siliceous material from other cells. . . . There are probably two kinds of calcoblasts, primary and secondary. The primary calcoblasts are the mother-cells in which the spicules originate, and the secondary calcoblasts are the cells which secrete additional layers of calcareous matter around the spicule after it has been formed" (1891 [1], p. 25 ; compare 1891 [2], p. 15). He states his belief in the same passage that the calcoblast is "an ordinary stellate mesodermal cell." "The spicule sheaths . . . . are formed by a slight concentration of the structureless mesodermal jelly around the spicule" (1893, p. 223). Supposed primary calcoblasts are figured on the uniaxial spicules of Leucandra phillipensis, and it is stated that "their characters certainly justify the assumption that they are but slight modifications of ordinary stellate cells" (1893, p. 225, figs. 44—47). Dendy quotes my former statements (1892 [1], p. 265, fig. 15, *a*, *b*), which I have stated above to be erroneous, as to the presence of a fourth cell at the centre, in addition to the three on the rays, of the triradiates of Cl. coriacea; and considers that the cells on the rays represent the secondary calcoblasts, "for we can hardly suppose that the spicule is originally formed by more than one cell" (1893, p. 225). In so far as this remark applies to the triradiate systems, I think the observations described above sufficiently refute it, but in so far as Dendy wishes to express his conception of what constitutes a true spicule in general, as distinguished from compound spicular systems, I quite agree with him.

In considering Dendy's views of spicule formation, it must

be borne in mind that his "stellate mesodermal cells" are the same as my "spicule cells." Dendy believes the mesoderm of Calcarea to be packed with stellate connective-tissue cells, for the most part independent of the spicules, and similar in nature to the stellate cells in the jelly of a Medusa; I, on the other hand, have been unable so far to find stellate connective cells of this kind at all in Ascons, and am of opinion that what has been taken for them is the spicule-cells, which in sections are generally found separated from their spicules, as the mechanical result of section-cutting. Hence Dendy's "stellate cells," being identical with my "spicule-cells," we are agreed in stating that they are "calcoblasts." Where I must differ from Dendy is with regard to the existence of a special mother-cell for the triradiate systems. In the assumption of "primary calcoblasts," of which the existence has certainly not been demonstrated, we can trace the influence partly of an analogy with siliceous spicules, partly of Metschnikoff's descriptions and figures.

Dendy considers that "the calcoblasts, at any rate in the case of large spicules, must be amœboid, for unless they be so I cannot understand how the spicules can increase uniformly in thickness" (1893, p. 225). I have shown, however, that they do not increase uniformly in thickness, but are built up to their full thickness, first at the base and afterwards at the tip.

The apical ray of the quadriradiate system, according to Dendy, "is not naked, but clothed by an investing sheath of plate-like nucleated cells," which "resemble the cells of the ectoderm" (1891 [2], p. 14, pl. vi, fig. 2). Dendy considers these sheaths as mesodermal structures, though "there is nothing to prove that they are not endodermal;"[1] probably, however, they are "calcoblasts," derived from the stellate connective-tissue cells. The last thing probably with which Dendy would have thought of connecting them is the "yellow granules," as

[1] Bidder, in his review of Dendy's work, regards these cells as endodermal (1891, p. 630), and has some curious remarks on the subject of the mesoderm. This discussion seems to me a proof of how meaningless are the terms "ectoderm," "endoderm," and "mesoderm," when applied to these forms of life.

he termed the porocytes. Even in zoology truth is sometimes stranger, or seems so, than fiction.

In 1895 I published a preliminary account of the origin of the triradiate systems of Clathrina coriacea, and in 1896 I showed that the monaxon spicules in the young Leucosolenia variabilis are formed each by a cell of the flat epithelium. The last author who has written upon calcareous spicules is Döderlein in the present year. In Petrostoma Schulzei the author finds young quadriradiates in the cortex. "From them . . . by reduction (Verkummerung) of one of the four arms arises the triradiate. This derivation of the triradiate calcareous spicule seems to me much more natural than the opposite view" (p. 21). The author does not clearly state what he means by "the opposite view," and his remarks might at first sight be supposed to refer either to Haeckel or to my theory, published two years before (1895); but as he makes no mention of my preliminary note on the origin of the triradiate systems, the author's words must be supposed to have a more abstract reference, and to be intended simply as an exposition in general terms of his views on the phylogeny of calcareous sponge spicules.

Since Döderlein was only able to study dried specimens of Petrostoma, we learn nothing from his description as to the relation of the young spicules to the soft parts.

To sum up the results of our researches in the literature of calcareous spicules: we might divide the authorities in this field conveniently into two classes or groups, of which the first would comprise Kölliker, Lieberkühn, and Metschnikoff; the second all the remaining authors. From the former we obtain valuable observations, but no theories; the latter give us, on the one hand, plenty of theories, which would be valuable if they had any foundation of fact; but of observations, on the other hand, next to nothing. Until the publication of Metschnikoff's work (1879) the predominant authorities were in favour of an origin for the calcareous spicules in the gelatinous ground substance of the body-wall. Metschnikoff having destroyed this notion by demonstrating a cellular origin for

the spicules, a mode of formation analogous to that supposed to occur in siliceous sponges, has been universally assumed. Two notions have dominated all the writings on this subject: (1) that spicules must arise each in a special mother-cell; (2) that their subsequent growth must be aided by other cells of the body-wall. To these principles some authors add a third, as obviously based on the analogy of siliceous sponges, namely, that the tetraxon spicule must be the primary form, from which the others arise by reduction of the number of rays. I shall discuss the last of these three propositions more fully in the theoretical portion of this paper, and hope to show with regard to it what is sufficiently obvious in the case of the first two, namely, that a priori reasoning, founded on pure analogy, has led to conclusions hopelessly at variance with the facts.

(*b*) Observations upon the Structure and Composition of the Calcareous Spicules.—The chemical and physical nature of the calcareous spicules has been so thoroughly worked out by Haeckel (1872), Sollas (1885), and Ebner (1887), that as I have nothing to add to the subject, I shall content myself with the references to these authors, and a brief summary of the results reached by the careful investigations of Ebner as a preliminary to a theoretical discussion.

The most striking, and in many ways unexpected peculiarity of these bodies, of which the triradiate and quadriradiate forms are so clearly shown by their development to be compound structures, built up from different sources, is the fact that each spicule behaves optically as a single crystal. This was first shown by Sollas and confirmed by Ebner, who further denied the presence of organic matter in them, postulated by Haeckel under the name of spiculin. "Each spicule behaves as a single crystal individual, and an organic substance cannot be demonstrated in it. . . . The spicule by no means consists of pure calcium carbonate in the form of calcite, though very similar to it from the point of view of crystallography (in krystallographischer Beziehung), but the spicule substance

contains in addition considerable quantities of other inorganic constituents, amongst which sodium, magnesium, and sulphuric acid are demonstrated, together with, probably, water" (1887, p. 132). "The spicules of calcareous sponges are mixed crystal individuals, consisting principally of calcite, and containing no organic substance;[1] their outer form, not limited by the surfaces of a true crystal, is conditioned by the specific activity of a living organism, and their inner structure, though completely crystalline, is in relation with the outer form by a peculiar distribution of the constituent parts" (p. 134). "The calcite first secreted contains the greatest amount of impurities (the central thread), and as the tip of the spicule continues to grow a substance corresponding to the central thread is first formed" (p. 133).

IV. Theoretical: on the Origin and Evolution of Calcareous Sponge Spicules.

The spicules of sponges, and especially of calcareous sponges, are structures which seem in a certain sense to connect the two worlds of living and dead matter. Composed, as a rule, of simple inorganic materials, they show the geometrical regularity of form which we associate with the mineral kingdom. Formed, on the other hand, within a living body as a product of its vital activity, they exhibit a diversity of character and a progressive evolution of form such as we find only in living organisms. As in the case of crystals, we can reduce them to a few fundamental types, constructed according to simple geometrical patterns. As in the case of living bodies, on the other hand, we can distinguish in them the characters of classes, orders, or families, until we come down to specific characters, and finally to racial and individual variations. Bodies which lie so close in many ways to the border-line between the organic and the inorganic must excite an interest, which is a sufficient justification for embarking upon a discussion as to their nature, and for considering the causes which

[1] For further remarks on this point see Addendum A (infra, p. 569).

have been instrumental in producing the fundamental types under which they occur.

Four theories have been put forward to explain the forms of the spicules of Calcarea or of sponges generally, which we may term respectively the Biocrystallisation Theory of Haeckel, the Adaptation Theory of Schulze, the Mechanical Theory of Sollas, and the Alveolar Theory of Dreyer.

Haeckel, whose views on the secretion of spicules we have already noticed, regarded calcareous spicules as the products of a process of "biocrystallisation," "i. e. a combination of the crystallising activity of calcium carbonate and the organising activity of the sarcodine." Calcareous spicules are "biocrystals, i. e. form individuals, which represent a mean between an inorganic crystal and an organic secretion." "Their first origin depends on a compromise between the crystallising efforts of the calcium carbonate and the formative activity of the fused cells of the syncytium." The primitive secretion of calcium carbonate assumed a semicrystalline nature, and gave rise to bodies "which were utilised by natural selection as spicules for building up a skeleton, and which afterwards, by the interaction of adaptation and heredity, became modified in form and differentiated in very various ways in the struggle for existence" (1872, p. 377).[1]

Haeckel considered the primary forms of spicule in the Calcarea to be the triradiates, and the monaxon or acerate forms. He regarded the quadriradiate form as undoubtedly secondary, and derived from the triradiate by addition of the gastral ray. He discussed the relationship between the two primary forms, and considered (p. 350) the three possible theories—(1) that the monaxon and triradiate spicules are independent formations; (2) that the monaxon spicule has arisen from the triradiate; (3) that the triradiate spicule has arisen from the monaxons.

[1] Schmidt at an earlier date (1870, p. 4) had attempted to explain the regular tetraxon siliceous spicule by the action of crystallisation, but considered any such explanation inapplicable to calcareous spicules. Schmidt evidently thought that in siliceous sponge spicules the silica was in the crystalline form of quartz, whereas it is now known to be in the colloid form of opal.

The last of these possibilities he sets aside at once, as entirely improbable and unsupported by facts. The second is regarded as undoubtedly true in some cases; but the first is the true relationship between monaxons and triradiates in general. The vast majority of monaxon spicules in Calcarea are regarded as primary forms, fundamentally distinct from the other primary form, the regular triradiate spicule. There are thus two stems or lines of descent marked out in Calcarea, the first starting from the genus Ascetta, the second from the genus Ascyssa. The two primary forms of spicule are traced back by Haeckel to two distinct types of biocrystal. "The fundamental form of all monaxon spicules is the absolutely regular spindle, or a cylinder, on the basal surfaces of which are seated two similar cones with curved mantle surfaces. The original and fundamental form of all triradiates and quadriradiates is . . . the absolutely regular triradiate, which can be considered as a hemiaxon form of the hexagonal crystalline system, in which calcium carbonate crystallises as calcite" (p. 377).

Schulze, in his great work on Hexactinellids (1887, pp. 497—504), put forward a general theory of the forms of spicules, not only in Calcarea, but in sponges generally. He first of all attempts to trace the main lines of descent in the phylum of sponges, and recognises three main stems, each leading back to a distinct ancestral form, characterised by a peculiar type of skeleton. The three lines of descent are (1) the Calcarea, with a calcareous skeleton; (2) the Tetraxonia, with siliceous spicules typically of the tetraxon type, retained in the modern Tetractinellids[1] and Lithistids; the Monaxonida are considered as having arisen from the Tetraxonia by reduction of the rays of the spicule, and the Keratosa from the Monaxonida by loss of the spicules and their replacement by spongin; (3) the Triaxonia, with siliceous spicules of the Triaxon type, represented by the modern Hexactinellids. Each of these three types must have acquired its skeleton indepen-

[1] Under the Tetractinellids, Schulze includes such forms as Plakina, Corticium, &c., now usually separated in the division Carnosa.

dently of the other two, since there are no transitions between them so far as the skeleton is concerned, and their common ancestor can only have been a simple primitive form without a skeleton.

Schulze next discusses what is the most primitive form of spicule in each of the three great groups into which sponges are thus separated, and tries to show that the form of the primitive spicule is in each case a direct adaptation to the simplest type of anatomical structure, as seen in the more primitive or least specialised examples of the groups, or in young specimens. It would be out of place here to discuss at length his explanation of the origin of the triaxon and tetraxon siliceous skeleton, and we will only consider in detail his theory as applied to Calcarea.

Schulze does not consider any crystallisation theory as a sufficient explanation of the form of a spicule as a whole: (1) because symmetrical forms occur not only in the case of spicules composed of a crystalline material such as calcite, but also in the case of siliceous spicules composed of a colloid substance, namely, opal; (2) because the rays frequently deviate from the typical angles, and are often markedly curved. He considers even the fundamental types of the spicules to be determined solely by the matrix in which they lie. In Calcarea the most primitive group is that of the Ascons, represented by the olynthus stage in the development of the higher forms, and the most primitive type of spicule is the regular triradiate, with three equal rays meeting at equal angles of 120° in a plane. An Ascon or an olynthus has the form of a thin-walled tube, open at the free end, with the wall perforated by uniformly distributed pores. The triradiate spicules occur embedded in the wall, their rays lying tangentially to the surface, each spicule being typically so orientated that one ray is directed away from the osculum, while the other two run obliquely forwards, or rather upwards. In the angle between any two rays a pore is situated,[1] and the regularity of the

[1] Schulze suggests also an alternative arrangement, which to the best of my belief, however, does not occur in nature, and need not be discussed.

angles is to be explained in much the same manner as in the well-known instance of the honeycomb. The triradiate systems are, in fact, the form of spicule best fitted to support the porous wall of the Ascons, and the anatomical structure and arrangement of the soft parts show the utility of the specific structure of the skeletal elements. A similar theory is worked out by Schulze with great ingenuity and thoroughness for the primitive tetraxon spicule of the Tetraxonia and the triaxon spicule of Hexactinellids. In the case of the latter his view certainly receives great support from the structure of the Cambrian Protospongia, the oldest known fossil sponge.

Sollas, in his monograph of the Tetractinellida (1888), made an attempt to explain the forms of spicules as the mechanical result of forces acting upon them during their growth. After claiming that the forms of bones or other skeletons in higher animals are due to the effects of pressure and tension exerted upon them during their growth by the surrounding tissues, he thinks (p. lxxv) that "were it possible to connect the special forms of spicule with these forces an explanation would be reached which would fulfil" the necessary conditions; namely, that a theory to explain the forms of spicules should be "independent of the nature of the material, and capable of being applied to all organisms in which spicular forms are developed." The principle of this explanation is "that all spicular structures tend to grow along lines of least resistance." "The simplest form of spicule is a minute granule, generally more or less spherical," and from this as a starting-point Sollas derives the various forms by the aid of his theory. We need not follow here in detail the ingenious manner in which the principle of "least resistance" is applied to explain the various spicular forms of Tetractinellids and other sponges. For the calcareous sponges he says (p. lxxxi), "Let a scleroblast be situated near the surface of a sponge, as it must be in the Ascones; the surface tension will here also lead to the growth of three actines inclined at angles of 120° to each other, and thus the triradiate spicule so common in the calcareous sponges may have arisen."

Dreyer, in his studies on skeletal formations (1892), is more ambitious than either Haeckel or Schulze, since he tries to find a theory to explain the forms of the spicules, not only in sponges, but Radiolaria and Echinoderms as well. Like Sollas, he invokes mechanical principles, but only to explain a supposed universal type of primitive sclerite. He will have nothing of either crystallisation or adaptation in explanation of the forms of spicules. Crystallisation he rejects, with Schulze and Sollas, as an agency which determines the forms, on the ground of the occurrence of spicules similar in form but composed of very different materials, such as lime or silica, or even substances of organic nature. Hence he concludes that the morphology of the sponge spicule is quite independent of the nature of the materials composing it (p. 299). Adaptation he considers inadequate, on the ground that natural selection is "an externally regulative," not an "internally formative" principle (p. 349).

Dreyer regards the tetraxon type of spicule as the primitive form of skeletal element, not only for sponges, but also for Rhizopods and Echinoderms. To explain this universally recurring type of spicule it is necessary to seek for some constant cause; and this he finds in the vesicular structure of living matter. All living bodies are built up of three orders of vesicular elements, namely, (1) cells, (2) vacuoles, and (3) the alveoli of the protoplasmic framework, according to Bütschli's theory of the ultimate structure of protoplasm, with which he agrees (p. 350). The spicules being formed by living bodies, and being therefore deposited in the midst of these vesicular structures, have primitively the tetraxon form as a direct mechanical result of vesicular tension (Blasenspannung). Each such primitive tetraxon is, in fact, regarded by the author as laid down in the nodes of the alveolar framework in such a way that each arm lies in the interspace between three of the four contiguous alveoli, which naturally touch each other at a node.

The most unfortunate gap which at once presents itself in this theory is the fact of its being totally inapplicable to the

triaxon spicules of the Hexactinellids; and Dreyer is obliged to leave them entirely out of consideration.

In considering these different views, certain objections to each one of them readily suggest themselves.

Haeckel's theory is entirely borne out as regards the molecular structure of the spicules by Ebner's investigations (1887), and this author proposes to retain for them Haeckel's term "biocrystals." But crystallisation cannot be taken as an adequate explanation of the external form of the spicules, at least as they now occur. For in the case of the monaxons, Haeckel's "absolutely regular spindle" is a form which never occurs, and though it might possibly have been the form under which the spicules first appeared at a very remote period of the earth's history, yet Haeckel himself is of opinion that the monaxon spicules as we know them are the results of natural selection acting upon the primitive biocrystal; in other words, it is natural selection, and not crystallisation, which is the factor which models the various forms assumed by the monaxon spicules at the present day. A similar train of reasoning, strengthened by additional arguments, applies to the case of the triradiate spicules, and insuperable objections to Haeckel's theory in this instance are furnished, it seems to me, by the observations upon the origin of these bodies set forth above by me. The case against Haeckel may be summed up as follows:

1. Ebner has already pointed out (1887, p. 134) that the morphological axis of a triradiate spicule cannot possibly be compared to a crystalline axis.

2. The occurrence in other sponges of forms of spicule even more symmetrical and regular than those of Calcarea, composed of non-crystalline materials, such as colloid silica or spongin, has supplied such a weighty argument to all Haeckel's critics, that to attack his theory on these grounds may seem to many like flogging a dead horse. Since, however, it is clear that in other cases the form of the spicule is independent of the nature of the material, it is reasonable to suppose that the same may be true of calcareous spicules.

3. And, finally, my own investigations prove, to my mind,

that the triradiate spicule is not a simple and primitive skeletal element, but is built up from three monaxon spicules; in fact, that it arises in just the manner which Haeckel dismissed without further discussion as improbable and unfounded. It is, therefore, clearly impossible that the triradiate system should owe its form, as a whole, to the action of crystallisation.

While unable to accept biocrystallisation as an explanation of the primitive forms of the calcareous sponge spicule, I fully agree with the views Haeckel has put forward with regard to their evolution. With Haeckel I consider that there are two primitive types of spicule in the Calcarea, the monaxon and the triradiate, which are independent of one another in the form in which we now meet with them, though I shall try to refer both to modifications of a yet simpler monaxon type. With Haeckel I believe that these two forms of spicule are associated with two lines of descent in the Calcarea, the one starting from forms such as those constituting Haeckel's genus Ascetta, the other from his genus Ascyssa; and I may refer to my own later attempts (1896) to give this theory further support, and to introduce a classification of the Homocœla modelled upon it. With Haeckel, finally, I am fully of the opinion, of which I think the researches here brought forward are a convincing proof, that the quadriradiate spicule is a secondary form, derived, by the addition of an adventitious ray, from the triradiate system.

The view advocated by Schulze—namely, that the fundamental forms of the spicules are to be explained by adaptation to the primitive types of structure in sponges—is the one which seems to me to contain the true solution of the problem, at least in the case of calcareous sponges; and I shall presently try to show that my investigations at once confirm and extend Schulze's conclusions. The one defect, to my mind, in Schulze's presentation of the case is that he left out of consideration the monaxon spicules, which are certainly of as primitive a type as the triradiates. No scheme of evolution seems to me complete which fails to indicate the relations of the monaxon

spicules to the other forms. This gap I shall endeavour to fill, but will first consider in more detail the views advocated by Sollas and Dreyer.

Sollas's theory was put forward to explain the forms of spicules originating within a single cell, and his explanation of the triradiate system was based upon the assumption that it developed in this manner. Since, however, the triradiate system owes its origin to three mother-cells, it is clear that Sollas's theory would not apply to it, still less to the quadriradiate. No explanation based upon the principle of growth along lines of least resistance would explain why the gastral actinoblast should attach the monaxon spicule which it secretes to a triradiate system. Sollas's theory is therefore reduced at the outset to an explanation of, if anything, the simple monaxon sclerite from which the more complex forms are put together. In the case of monaxon sclerites secreted by a single cell, it seems to me highly probable that the mechanical principle involved by Sollas does operate largely in producing the form of the sclerite. But the principle is, after all, only an explanation of how the scleroblast lays down the sclerite, and not of why it has that form and no other. It is as if one should try to "explain" laughter by describing accurately the facial and other muscles concerned, and the manner of their contraction. If the lines of least resistance are such as to cause the secretion of calcite to take an elongate, needle-like form while still completely embedded in the scleroblast, this presupposes a certain polarity in the cell; but to what is the polarity itself due? I am unable to understand how the analogy of a bone, supposed to owe its form to the tension of the muscles and other tissues, can give us any help in the present case; for the problem is to explain why the molecules of calcium carbonate should be deposited more at the two opposite ends of the cell than in the middle, and this can hardly be a matter simply of pressure and tension on the sclerite itself.

I cannot, therefore, consider Sollas's theory as more than explanation, at most, of the mechanics of growth in the case of the simplest skeletal elements, and not as a solution of the

problem why the sclerite should have the monaxon form, still less of the triradiate and quadriradiate type.

Dreyer's theory assumes the well-known theory of Bütschli (1892) as to the ultimate structure of protoplasm. Let me begin, therefore, by declaring myself also a firm believer in Bütschli's views; the translation published by me of his great work may perhaps be taken as a sufficient guarantee of my acquaintance with his theories. Bütschli regards protoplasm as having the structure of a very fine emulsion or foam; droplets of a watery fluid or enchylema are suspended in a denser and more viscid fluid which constitutes the alveolar framework. The relations of these two parts are similar as regards structure to those of an ordinary froth or lather, the enchylema corresponding to the air, the alveolar framework to the liquid, whatever it may be, of which the froth is composed. In cross-section the alveolar framework would appear as a fine network containing meshes of various sizes which were filled originally with the enchylema. In addition to these two parts, all known protoplasm shows a great number of granules or microsomes, varying greatly in size, consistency, appearance, and chemical reactions, but always contained in the alveolar framework and lodged at the nodes of the reticulum. Protoplasm, therefore, consists of three constituent parts,—alveolar framework, granules, and enchylema. The vacuoles, on the other hand, which are so often a conspicuous feature of protoplasm, and which may contain various bodies, are not to be regarded as another primary element, but as produced by the fusion or running together of alveoli, just as in a froth a large bubble may arise by the breaking down of smaller ones.

Dreyer assumes that the sclerites are first deposited at the nodes of the alveolar framework, and that the supposed primitive tetraxon type of all spicules is the mechanical result of this fact. In the first place it may be pointed out that this assumption remains to be proved, for Calcarea at any rate. It is by no means impossible that the spicule at its first appearance is deposited in something corresponding to a vacuole, and if so, Dreyer's explanation would no longer apply. We may

pass over this point for the present, however, and assume with Dreyer that the first appearance of the sclerite takes place in a node of the framework; in other words, that the minute sclerite has the value of a granule, and is to be classed as such. Now the granules commonly found in protoplasm are very variable, as has been said, in nature and consistency; but they always present themselves, whatever their size, as rounded bodies of a more or less spherical form. In spite of their position at the nodes of a framework, where, as Dreyer assumes, they would be subject to "vesicular tension," no granules have been recorded, to my knowledge, which show any approach to the supposed primitive tetraxon form. If the sclerites have, as a result of their position at the nodes of the framework, a peculiar form and shape which all the other granules occupying the same position conspicuously lack, it follows that there must be some other cause to produce this anomalous result than their position simply. If vesicular tension cannot in any other instance cause the granules at the nodes to assume a tetraxon form, why should it do so in the case of the sclerites? If now we try to find a distinction between the sclerites and the other granules which would account for their difference of form, we can find none but the fact that the sclerites have, ex hypothesi, a supporting or skeletal function. If, however, their form be correlated not with their position, but with their function, we are thrown back at once, as it seems to me, to seek an explanation for the alleged primitive tetraxon, not in the mechanical results of its position and origin, but in adaptation to its surroundings,—in fact, to just the theory which Dreyer rejects.

In any case there seems no reason why the sclerite should have had a tetraxon form, unless it had at the same time a supporting function. It is extremely probable, however, that the material of which skeletons of any kind are built up appeared at first as mere concretions, or perhaps excretions,—in fact, as products, and perhaps as waste products, of the metabolism, before they were utilised for any function. If such a view be admitted, there is then no reason at all why the

sclerites should not at their first appearance have been similar in form to any other cell granules; that is to say, simply little lumps of rounded or irregular, or perhaps of crystalline form.

Whatever may have been the course of evolution, the sclerites must at first have been extremely minute, and no sooner did they appear than there was at once an opening, so to speak, for the action of adaptive and selective forces, related to the life of the organism as a whole, as apart from the molecular or mechanical forces acting upon the sclerite itself. Dreyer's hypothetical tetraxon sclerite, if it were really the fundamental spicular form, could only be explained, it seems to me, in this way. Natural selection, we will admit and proclaim, is not an "internally formative" principle; it could not have caused the first appearance of sclerites, which must be attributed primarily to variations in the cell metabolism. But natural selection is, we are told, an "externally regulative" force; and given a simple sclerite of whatever form, there is immediately something upon which external regulation can be exercised; but there is no reason to postulate that the primitive sclerite should be of the tetraxon rather than of any other type.

In the foregoing discussion of Dreyer's theory I have endeavoured to follow up his conclusions entirely from his own premises; and in order not to confuse the argument, I have purposely refrained from mentioning one constant accompaniment of the spicules of calcareous sponges which seems to me to make Dreyer's theory altogether inapplicable to that group at least. I refer to the spicule sheath, which is nothing less than an envelope of structureless organic material, inserted between the spicule and its secreting cell.

All the authors who have expressed any opinion as to the spicule sheath, namely, Haeckel, Schulze, and Dendy (see above), agree in regarding it as a layer derived from the general gelatinous ground substance condensed round the spicule. But since the sheath appears between the spicule and its secreting cell, it obviously cannot be derived from the ground substance outside the cell. It may, however, be an

intra-cellular secretion of the cell of the same nature as the intercellular secretion which produces the gelatinous matrix of the dermal layer. In the absence of precise observations on this point, my statements with regard to the spicule sheath are to be taken as provisional; but I think it highly probable that the spicule sheath represents a matrix secreted by the cell in which the spicule is subsequently deposited. I imagine to myself the spicule mother-cell secreting a minute drop or vacuole of the substance, whatever it may be, of which the sheath is composed, and in this vacuole the sclerite appearing as a minute concretion. The sclerite may have had originally a perfectly crystalline form, as Haeckel supposes, or it may have been a simple globular concretion like the crystalline substances deposited in an organic matrix in Rainey's [1] interesting experiments. In either case it early assumed a definite form in correlation with the functions imposed upon it, and as it grew into a spicule, such as we are familiar with, the matrix in which it was first deposited grew with it to form the sheath.

If this interpretation of the spicule sheath be correct, it is obvious that the calcareous sclerite cannot be regarded, in sponges at least, as having the value of a granule deposited at a node of the protoplasmic framework, but rather as a simple concretion within a vacuole, not subject to any force of the nature of vesicular tension. This representation of the mode in which the spicule is deposited is of course purely speculative, and lacks as yet any support from actual observation in the case of sponges. There are, however, instances of sclerites deposited in this manner in both animal and vegetable cells.[2]

[1] 'British and Foreign Medico-Chirurg. Review,' xx, 1857, pp. 451—476; see also Harting, 'Quart. Journ. Micr. Sci.' (n. s.), xii, 1872, pp. 118—123 and Ord, ibid., pp. 219—239, pls. xv, xvi.

[2] Compare, for instance, Leger's observations on the clinorhombic crystals of calcium oxalate in the cysts of Lithocystis Schneideri ('Ann. Mag. Nat. Hist.' [6], xviii, 1895, p. 479). Sclerites are figured in cells by Semon ('Mitth. Zool. Stat. Neapel,' vii, pl. ix, fig. 3) and Blochmann ('Die Epithel-Frage bei Cestoden u. Trematoden,' Hamburg, 1896), but neither

For all these reasons I am most decidedly of opinion that Dreyer's theory of a primitive tetraxon spicule, produced by the influence of vesicular tension upon a sclerite deposited at a nodal point of the protoplasmic framework, must be rejected for calcareous sponges at least. My own observations upon the formation of the spicules seem to me to indicate clearly that the primitive form of skeletal element in the Calcarea was a rod-shaped or fusiform sclerite; that the triradiate system has arisen from a junction of three such simple elements; and that the quadriradiate system, the last stage in the evolution, and not the first, was further built up by the addition of yet another monaxon sclerite to the triradiate system. The primitive monaxon sclerite probably differed in one point from the ordinary monaxon spicules that we are familiar with in existing Calcarea. The latter always project free from the surface of the sponge, only the proximal portion of the spicule being embedded in the sponge wall. In the triradiate systems, on the other hand, the rays are placed longitudinally, and completely embedded in the body-wall, at least in all the more primitive Ascons. Hence it is probable that the ancestral monaxon spicules lay tangentially in the body-wall, and did not project from it. The spicules of this character underwent two divergent courses of evolution. Some remained single, but acquired a portion projecting free from the surface of the dermal layer, becoming the existing monaxon spicules. Others again acquired no such projecting portion, but, remaining completely embedded in the body-wall, became united with one another in trios to form the triradiate spicules as we know

author notices their relation to the protoplasmic structure. The formation of sclerites in plants—the so-called raphides—offers the best instance to the point. Vacuoles of mucilage arise in the cell protoplasm, and run together to form a large vacuole in which the raphides are deposited as crystals. See Haberlandt, 'Physiologische Pflanzenanatomie (Leipzig, 1896), p. 449, figs. 190, 191; de Bary, 'Comparative Anatomy of the Phanerogams and Ferns' (Oxford, 1884), pp. 137—139; Gardner and Ito, "On the Structure of the Mucilage-secreting Cells, &c.," 'Annals of Botany,' i (1887), pp. 27—54, pls. iii, iv.

them. In this way arose from a common source the two primitive types of spicule which we find in the Calcarea.

Before proceeding to discuss the factors which may be supposed to have brought about this course of evolution, I will first examine all the objections which occur to me against my theory of the origin of the triradiate and quadriradiate system by fusion of skeletal elements primitively distinct.

1. A great objection, at first sight, to regarding the many rayed calcareous spicules as compound bodies, is the well-established fact that they behave optically as single crystals (Sollas and Ebner, see above). This fact certainly seems strong evidence for regarding them as simple and uniform bodies. But it may be pointed out that, whatever their ancestral history, each ray does actually arise from a separate mother-cell in the course of the development. It is just as difficult to comprehend why the united products of a number of independent cells should behave as a single crystal, as to believe that a body of this kind arose ancestrally from a fusion of separate elements. This is especially so in the case of the quadriradiate systems, where the fourth ray has an origin quite distinct from the basal system, and is even formed at a later period. I am justified therefore, I think, in claiming that my theory of a fusion of skeletal elements to form a structure which behaves as a single crystal individual, does not present greater difficulties than do the ascertained facts of development.[1]

2. It might be urged against the views here advocated, that no other sponge spicules are known to arise by fusion of separate elements. This is an argument which at most would only excite a bias against the theory, but could not afford a positive disproof of it. It is certainly true that, so far as is known, the polyaxon spicules of other sponges arise always each in a single cell. On the other hand, instances of secondary deposit of skeletal material uniting primitively distinct spicules are not wanting. We may cite the case of the compound desmas of the Lithistida; the secondary siliceous

[1] For further remarks on this point see Addendum B (infra, p. 572).

deposits uniting the spicules in the Dictyonina; and finally the secondary deposits of calcite uniting the calcareous spicules in the Pharetrones, of which Döderlein has so recently (1897) described a living example in his Petrostoma Schulzei. These examples are sufficient to show, were evidence required, that spicules may become united together secondarily to form compound structures.

3. A quite trivial difference, as it seems to me, can be pointed out between the formation of a ray of a triradiate, and of a primary monaxon spicule. While in the former the actinoblast divides, in the latter, so far as is known, the mother-cell remains single. But the history of the gastral ray shows plainly that it depends on the size of the spicule to be formed, whether or no the mother-cell remains undivided. And for my part I much doubt whether, in the case of primary monaxons which attain a large size, the secreting cell always remains single.

None of these objections are in any way a serious obstacle, it seems to me, to regarding the triradiate and quadriradiate systems not as true or primary spicules, but as compound spicular systems. To my mind it is essential to the definition of a true spicule or primary spicular element that it should be formed by a single mother-cell, or by the descendants of one such cell. In this way each ray of a triradiate system represents a single spicule, homologous with a primary monaxon, and the whole structure is a compound or secondary spicular system. Examples of primary siliceous spicules are the ordinary monaxons and tetraxons which arise each in a single cell, while the desmas of Lithistida are instances of secondary systems.

This view of the origin of the triradiate systems completes, as it seems to me, and extends Schulze's theory that they arose as an adaptation to the structure of the body-wall of the simplest Calcarea. The primitive monaxon sclerites lying in the body-wall would soon take on an arrangement suited to its structure, and came to lie probably one between every pair of adjacent pores. Then, by union of the monaxons in trios, the

characteristic arrangement of the triradiate systems was brought about which we may still observe in the olynthus of any calcareous sponge, or in the oscular tube of the Ascons (Fig. B).

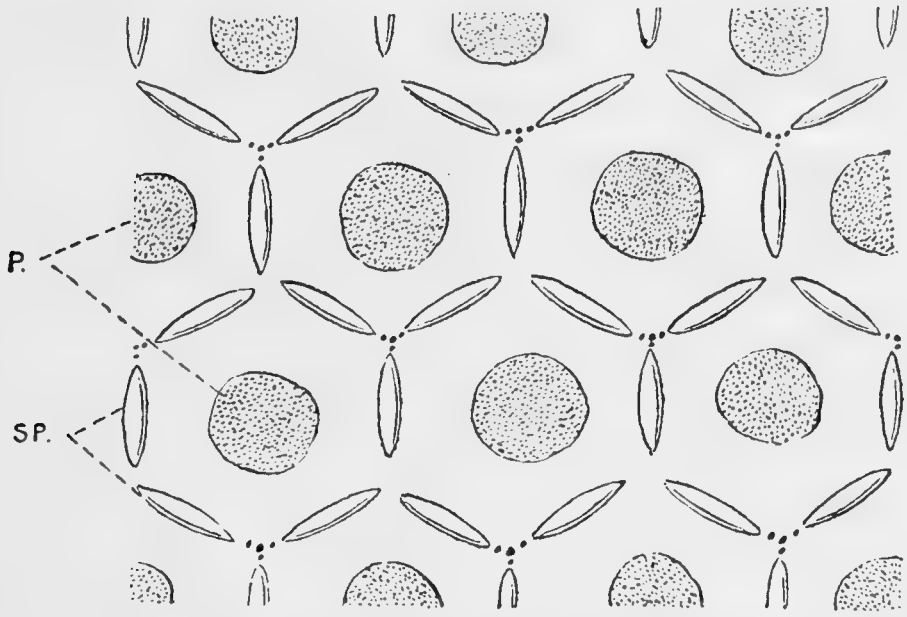

Fig. B.—Diagram showing the hypothetical origin of triradiate systems, each from three monaxon spicules (sp.) lying between the pores (p.)

If it be asked why only three sclerites should have fused together, and not two or four or any other number, it is impossible to say more than that the triradiate arrangement represents a compromise between too great flexibility on the one hand, and too great brittleness on the other. Such a question would, in fact, be as difficult to answer off-hand as to say why insects have only six legs, or mammals seven cervical vertebræ. We can see that a skeleton of monaxon sclerites would afford little support to an erect oscular tube, while the union of many such sclerites into a lattice-work would result, in the case of objects of the size of a calcareous sponge, in an excessively fragile structure.

The theory that the triradiate systems arose in this way, as an adaptation to the structure of the body-wall, seems to me to explain not only why they should be formed, as we see they are formed, of three simple monaxons, but also their extraordinary and striking regularity. It is the old problem of the

honeycomb over again. I can understand, however, that an attempt might be made to explain this characteristic regularity in a different manner. It might be urged, since each spicule is formed by three cells of more or less equal size, closely pressed together, that therefore the rays would naturally meet at angles of 120° as the result of their being formed in the axis of the cell; but after carefully weighing this theory I am obliged to reject it as an adequate explanation, for the following reasons. First, because the actinoblasts are never exactly equal or perfectly regularly placed, nor are the rays formed exactly in the axis of the cell, but almost always a little to one side or the other; hence if that were the only factor at work, we should rather expect irregularity to be the rule, and equality between the angles to be a rare exception. Secondly, any such explanation, even if accepted for the spicules of the Clathrinidæ, which have the three rays meeting at equal angles, would fail completely in Leucosoleniæ, where we find indeed great regularity, but of a different type. In Leucosolenia the triradiate systems are of a pronounced bilateral type, with two curved lateral rays, each meeting the straight posterior ray at an angle much less than 120°, and enclosing anteriorly an angle much greater. We may express it by saying that while each lateral angle is, in a projection $(120-n)°$, the anterior angle is $(120+2n)°$. Now if the orientation of the rays is to be referred to the arrangement of the actinoblasts, the bilateral form of the triradiate systems of Leucosolenia could only be explained by a corresponding arrangement or difference in size of the actinoblasts, which would bring us to an explanation which would itself require to be explained.[1]

I cannot, therefore, regard the regularity of the triradiate systems as due to the arrangement of the secreting cells. On the other hand, the view that this, their most striking characteristic, arises from adaptation to the structural requirements

[1] So also Ludwig, discussing the calcareous bodies in the Holothurians, refers the branching at angles of 120° to the arrangement of the cells ("Echinodermen," I, in 'Bronn's Thierreich,' ii, 3, p. 60). But what explains the arrangement of the cells?

of the sponge as a whole, receives further support from the fact mentioned above, namely, that in the case of the triradiate systems first secreted after the fixation of the larva, extreme irregularity prevails. The sponge has not at this stage assumed its cylindrical form, but is a compact mass of cells; the spicules are formed in the superficial layer just as in the adult, and were their regular form due to the arrangement of the secreting cells, there is no reason why the rays should not at this early stage meet at the same constant angles as in the adult. It is not, however, until the young sponge has assumed its characteristic tubular form that regularity becomes the rule, and not the exception, amongst its spicules. I can only interpret these facts by supposing that the forms of the spicules are influenced by the requirements of the sponge as a whole, and not by the arrangement of its cells, so that the spicules do not attain their characteristic form until the sponge itself has the structure to which that form is an adaptation. And this conclusion seems to me still further supported by the above-mentioned differences between Clathrina and Leucosolenia. In the former the reticulate form of the sponge colony is associated with the possession of equiangular triradiates, which in the latter become bilateral in correlation with the upright form of the sponge as a whole.

If the triradiate systems furnish a strong argument, as I believe they do, for the influence of adaptation on the spicules, the course of events in the growth of a quadriradiate system greatly strengthens this impression. We have seen that after a triradiate system has been laid down in the usual way, the gastral ray is tacked on to it from quite a different source, namely, by a porocyte. We have seen, further, that the porocytes come from the dermal epithelium, and we know that in Leucosolenia each cell of this epithelium can secrete a monaxon spicule. Hence there is no difficulty in supposing that the porocyte in secreting a monaxon spicular element is simply exercising a function which was primitively possessed by every cell of the dermal layer. We can invoke the aid of such well-known principles as heredity, atavism, and even

metabolism, to explain why the porocyte secretes the gastral ray. But none of all these factors of evolution can explain why the presence of a young triradiate system in the vicinity should stimulate the porocyte to resume its long-forgotten functions, nor, above all, why it should not only secrete a monaxon spicule, but should go so far as to stick it on to the triradiate system. The formation of the gastral ray is, indeed, a most crucial test of the theories of spicular origin, and all simply mechanical theories are at once, as it were, impaled upon its relentless point.[1]

We have in the foregoing pages considered some very different hypotheses in our search for the principle which determined and modelled the fundamental types of the calcareous sponge spicule. We have examined the rival, and often conflicting claims of crystallisation on the one hand, and of the mechanical effects that might be supposed to follow from the ultimate structure of protoplasm, from the dynamics of the cell, or from the number and arrangement of the secreting cells on the other hand. None of these theories, however, stand the test of a thorough analysis, or can supply more than a part of the explanation. All the evidence which we have at our disposal drives us to seek the principle which guided and directed the evolution of the spicular forms in a process of

[1] While the general method in which the spicules arise seems to me explicable only by adaptation, there are characters in all of them which are certainly difficult to explain in this way. There are the peculiarities which separate and distinguish the species, and which consist in small details of relative size, length, curvature, or sharpness of the rays. A peculiarity of this kind in the quadriradiates of Leucosolenia complicata, Mont. (= Ascandra complicata, H., + A. pinus, H.), is worth mentioning in this connection, since it seems to have escaped notice hitherto. In this species the gastral ray does not arise from the junction of the three basal rays, but from the straight posterior (unpaired) ray near its base—a fact which would be difficult to reconcile with the theory of a primitive tetraxon spicule. Specific characters of this kind are difficult to explain by any principle which requires that they should be of utility to the organism, and they seem rather to have arisen by the perpetuation in some unexplained way of a sport or variation not in itself "useful" to the whole sponge. This is, however, no place to raise the much-disputed question of the utility of specific characters.

adaptation to the needs of the whole organism, resulting in the form best suited for the function which the sclerite has to perform—the function, namely, of supporting the tissues in the manner required by their structure and arrangement in the primitive forms in which the spicules first arose.

In explaining the main types of calcareous spicules by adaptation, we are referring them to a very wide-spread and universal category of structures characteristic of living beings. There is probably no living organism which does not possess some special features, owing their characteristics or even their existence to the fact that they are of utility to the organism in its efforts to maintain and perpetuate its existence in the way in which its environment necessitates. If now it be asked how this process of adaptation came about, we find ourselves confronted with one of the most important and fundamental questions of evolution. The most commonly received explanation of adaptation is that put forward by Darwin—namely, that it is due to the natural selection of favorable varieties in the struggle for existence, resulting in the survival of the fittest types and the gradual elimination of the less perfectly adapted.

Very recently, however, Delage (1895) has put forward a very different view. Natural selection, according to him, is not a factor of progress in evolution, but one which tends to maintain the fixity of the species by elimination of harmful variations. Adaptation, we are told, is the rock upon which both Darwinian and Lamarckian hypotheses founder, since natural selection is an inadequate explanation, and acquired characters are not inherited (p. 839). There is no phylogenetic adaptation; that is to say, species are not adapted to their mode of life, but it is the individual which becomes adapted by its reaction to its environment—"auto-regulation" (p. 828). "Phylogeny creates organs without regard to function; ontogeny . . . adapts them to the necessary functions:" in other words, "in phylogeny it is the organ which makes the function; in ontogeny it is the function which makes the organ" (p. 831). The cause of this ontogenetic adapta-

tion is to be sought in "functional excitation," under the influence of which "the individuals adapt themselves regularly, without interruption, and in all their organs" (p. 828).

Space would not permit of a thorough discussion of all the questions raised by these propositions, but it is a legitimate task to discuss here how far the ontogeny and phylogeny of the spicules support or refute Delage's views, and to test them by the facts of spicule development. At the outset some consequences may be pointed out which follow from his theories. If the adaptation be always ontogenetic, and due to a reaction on the part of the individual organism to a functional excitation, then an adaptive structure might be expected to arise only when the conditions to which it is an adaptation have come into operation. If, on the other hand, the adaptation is phylogenetic, acquired in the past history of the evolution of the species, and fixed and handed on by heredity, there would be nothing astonishing in finding that an adaptation shows itself before it is functional or useful. A prophetic adaptation of this kind would, however, be fatal, it seems to me, to the view that adaptation is always ontogenetic.

To return now to our spicules. We believe that in phylogeny they arose by the union of three separate monaxons, which first came to lie one between each pair of pores, and then fused together in trios. A consideration of the structure of the wall shows at once how, when union took place, they met at quite regular angles of 120°. So far all is simple and natural, and there is nothing to contradict the view that in phylogeny the regular forms of the triradiate spicules were produced by "auto-regulation" in response to functional excitation. The matter is, however, quite otherwise when we come to the ontogeny. Whatever may have been the past history, the three spicule rays are not laid down at the present day first as three full-sized monaxon spicules, and then secondarily united into one system. The supposition that they ever were formed in this way is purely theoretical. They are, on the contrary, united to form a triradiate system when still excessively minute and enclosed within their secreting cells. This

being so, it is difficult to see how the functional excitation, so feasible in phylogeny, can influence the ontogeny, since the triradiate systems have their characteristic and definite form long before they are able to exercise their function of support. The adaptation of their form to the structure of the sponge is therefore entirely prophetic. The only way in which functional excitation could modify their form would be to produce curvature of the rays at a late period of their growth. Such a curvature is found normally in the spicule rays of Leucosolenia, and exceptionally in Clathrina, and is perhaps the result of a functional excitation exercised as follows. If we look at a young triradiate system surrounded by its six cells (compare especially Pl. 38, fig. 8, Pl. 39, fig. 21, and Pl. 41, fig. 38), it is impossible to avoid the idea that the apical formative cell exerts in some way a directive function in laying down the ray. Going ahead at the extreme tip of the ray, it seems to mark out its future direction. Of the two formative cells, the basal cell might be termed the secretive cell, the apical cell the directive element. If now an apical formative cell, acting in this way, were deflected from its path and turned to one side by a pore or other tissue element, a curved ray would result. I hope at some future time to be able to bring forward observations on this point, and especially to trace the history of abnormal spicules, and investigate the causes of their abnormality. In any case the functional adaptation could only produce, as has been said, a late curvature of the rays, and could not influence the fundamental angles at which they meet, for the latter are determined at a very early period within the secreting cells. The apical formative cell might represent a medium for auto-regulation and ontogenetic adaptation, but in the case of spicules which lose their apical cells at an early period, variations in the curvature of the rays due to a functional stimulus would be impossible. The spicule must, it would seem, in such cases grow straight on, like the incisor of a rodent, which grows into its brain if not worn down, and if a pore is in the way of the spicule it is the pore which must shift its position.

The ontogeny of the spicules, therefore, points clearly to their regular form being a phylogenetic adaptation, which has become fixed and handed on by heredity, appearing in the ontogeny as a prophetic adaptation. Nowhere is this fact more evident than in the rim of a growing oscular tube of an Ascon, since in this region we find triradiate systems being laid down and even attaining to their full size in a perfectly regular manner before any pores are formed. At a later period, as the gastral layer grows up, pores are formed amongst and between them, but the spicules appear first, long before the wall has the structure to which we find that their form is an adaptation. This is true equally of the triradiate systems of Clathrina with straight, and those of Leucosolenia with curved rays, showing that neither form can be explained as produced by functional excitation during the ontogeny.

The facts already mentioned with regard to the development of the embryo show that the hereditary impulse or tendency does not come into full play until the sponge as a whole has the structure to which the triradiate system is an adaptation, and that the spicules first formed are very irregular in consequence. But in spite of this influence exerted by the structure of the whole organism, it is not possible to connect the regularity of the spicules with any functional excitation in the ontogeny, though this is a possible explanation of the phylogeny. But at this stage of the inquiry we find ourselves confronted by a dilemma. If functional excitation produced the adaptation in phylogeny, then it is a case of an acquired character being inherited. If, on the other hand, acquired characters be not inherited, then the adaptation must have arisen from innate or constitutional variations, which became gradually fixed, doubtless by selection, as a constant hereditary tendency of the organism.

If, therefore, we wish to avoid the hypothesis, not as yet established by a single clear instance, of the transmission of acquired characters, we are brought back, it seems to me, to the old familiar theory of natural selection, in order to explain the constant recurrence and regular form of the triradiate

system, and for the operation of this principle the spicules offer a splendid field. Few structures are more variable than spicules. In every specimen a certain percentage of irregular, or as we say abnormal spicules are to be found, and sometimes quite a high percentage of them.[1] Ascons, again, are for the most part shore forms, and nothing gives a more vivid idea of what the struggle for existence means than, after becoming familiar with the fragile and delicate structure of these organisms, to watch a stormy sea breaking over the rocks upon which one knows that Ascons are actually living and growing. The wonder is rather that any specimen ever survives. That natural causes produce a high death-rate among these creatures can be further deduced from the fact that a single specimen produces an enormous number of larvæ, and as the latter exercise but little choice as to the localities in which they fix themselves, a very large proportion must be wiped out of existence by the first storm that breaks over them. All these arguments are perhaps somewhat trite, but they may serve to emphasise the fact that the field is in every way a favorable one for the operation of natural selection. There is variation to supply the material for selection, and there is an environment calculated in every way to favour the survival of individuals distinguished by even slight improvements in the supporting framework. Into the question of the origin of the variations on the one hand, and of how the characters of the fittest, after they have survived, are fixed and become perpetuated on the other hand, this is no place to enter. If, however, the application of the principle of natural selection raises some difficulties in the present state of our knowledge, especially as regards matters of detail, the same is true to a much greater degree of any other theory. Many of the objections so often brought forward against the theory of natural selection seem to me to amount simply to the following argument: there are many facts which natural selection cannot explain, therefore natural selection explains nothing. For my

[1] Compare also the facts brought forward above (pp. 521—523) with regard to variation in the gastral rays.

part I find a great difficulty in explaining some facts by natural selection, but I am none the less of opinion that, in other cases, it is the only possible explanation.

Summary.

1. The first appearance of a calcareous spicule or spicular element, both ancestrally and in the actual development, was probably a minute vacuole in a cell of the dermal layer, filled with an organic substance perhaps identical with the intercellular ground substance, within which the minute sclerite appeared as a crystal or concretion.

2. The ancestral sclerite, though crystalline in structure, soon assumed a non-crystalline form as a whole, as an adaptation to its secondarily acquired function of support, and as it grew in size the contents of the vacuole formed the spicule sheath.

3. The ancestral form of spicule in the Calcarea was a simple monaxon, placed tangentially and completely embedded in the body-wall, lying between two adjacent pores.

4. From this ancestral spicule the forms of spicule now occurring in the Calcarea arose as follows: (*a*) the primitive monaxon acquired a distal portion projecting from the surface, as in the existing primary monaxons; (*b*) groups consisting each of three primitive monaxons became united by their contiguous ends to form a single triradiate system; (*c*) to some of the triradiate systems thus formed a fourth ray was added, secreted by the pore-cell, giving rise to the quadriradiate system; (*d*) some of the triradiate systems, by loss of one ray and placing of the other two in a straight line, or by loss of two rays, perhaps became modified into secondary monaxon spicules.

5. The power of secreting a monaxon sclerite was primitively possessed by every cell of the dermal layer, and this condition appears to be retained in Leucosolenia. In Clathrina, on the other hand, all the skeletogenous cells migrate inwards from the dermal epithelium, and form a con-

nective-tissue layer distinct in function from the contractile, undifferentiated dermal epithelium. In Leucosolenia also the actinoblasts of the triradiate systems form a deeper layer, but the dermal epithelium secretes primary monaxons—at least in the young form—and is non-contractile.

6. The forms of the spicules are the result of adaptation to the requirements of the sponge as a whole, produced by the action of natural selection upon variation in every direction.

ADDENDA.

A. On the Presence of an Axial Organic Filament in the Spicule Rays.

(Note to p. 543.)

Since the above paper went to press I have succeeded in demonstrating very clearly the existence of an axial organic thread in the spicule rays by means of the following staining mixture, recommended to me by my friend Dr. Ritchie, Lecturer on Pathology in the University of Oxford:

Nigrosin, 1 per cent. in H_2O . . .	1 volume.
Picric acid, sat. sol. in H_2O . . .	9 volumes.

The action of this mixture upon the sponge tissue is that, in the first place, the calcite of the spicules is rapidly dissolved by the picric acid, which also colours the cytoplasm of the cells yellowish, while the nigrosin stains the ground substance a faint blue and the spicule sheaths deep blue, and also colours a very delicate filament in the axis of each spicule ray.

For showing the filaments, sections of material which has been decalcified before embedding are almost useless; on the other hand, the filaments are seen very well in sections not previously decalcified after treatment with the nigrosin mixture, especially in fairly thick sections, in which it is possible to find large pieces or whole rays of the spicules. I attribute this difference to the fact that after decalcification the filaments are quite unsupported within the spicule sheaths, and are readily

displaced from their position; even in the most successful preparations the filaments have a more or less undulating appearance (Fig. C), and show signs of displacement. The processes which a piece of tissue goes through in its passage from alcohol to paraffin are such as to set up a great many diffusion currents in the tissues, and in consequence, if the spicules are decalcified, the axial filaments are in nearly every case displaced and stuck against the spicule sheaths, and undistinguishable from them after staining.

The simplest method of demonstrating the axial filaments is the following. Take any spirit specimen of an Ascon, and put a small piece—a tube or part of one—first in water and then in the staining mixture for about half an hour. Then wash thoroughly in water, and pass through alcohol to clove oil. In the latter medium the tube can be split open with needles, and the collar-cells removed with a paint-brush, which generally brings away the pore-cells as well. Then the piece of the wall is laid out flat on a slide, and mounted in Canada balsam. I have never failed to demonstrate the axial filaments in the clearest manner in such preparations.

I also made the attempt to unmount and stain some of my preparations mounted in glycerine, but without great success. The stain in these cases acted feebly, and the delicate filaments could only be made out with great difficulty. On the other hand, the sheaths were well shown, and it could be seen that they appear very early in the development of the spicule when it is still quite enveloped in its cells. At first the sheath forms an excessively thin layer, which gets thicker as the development proceeds. In such a stage as that shown in Pl. 38, fig. 7, the sheath is perfectly distinct when stained, a fact that leaves no doubt as to its being, like the spicule, an intra-cellular secretion.

The axial filament runs to the extreme tip of the spicule ray, and joins the spicule sheath (woodcut, Fig. C), showing that the spicules must be perforated at the tips of the rays. The filaments are more distinct towards the tips of the rays than at the bases, and the behaviour of the filaments at the centre of

the spicule is often very difficult to make out; but in coriacea, which I studied carefully, I was able to see clearly the state of things in a large number of instances. As the filaments approach the centre they swell out slightly and become less

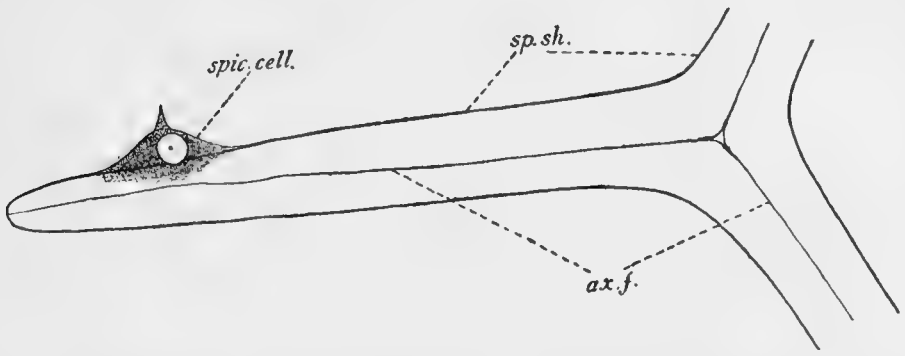

FIG. C.—Tracing with the camera lucida of the spicule sheath (*sp. sh.*) and axial filament (*ax. f.*) of Clathrina coriacea, after treatment with nigrosin and picric acid. × 800.

definite; finally they end at a short distance apart, and when seen with insufficient magnification appear independent of each other, but with a high power they are seen to be connected by three delicate curved filaments, forming a minute triangle enclosed by curved lines, concave towards the exterior.

This structure was seen by me in all the spicules in which I could make out clearly the central portions of the filaments; but considerable variations were observable in the dimensions of the central triangle, and in some cases it was so small that the filaments appeared to touch each other. I believe that the central triangle is easily explained by the development of the spicule, and the fact that the rays are at first separate from one another. The abrupt termination of the filaments before they meet represents the distance between the rays at their first origin, and the delicate connecting threads represent the secondary union of the three sclerites. I have not had time yet to make extended observations upon the union of the filaments in different species, as I hope to do, but I have made and examined a preparation of reticulum, a species which

seemed likely to yield interesting results, since it is a form in which the rays unite at a very early period (see Addendum B, infra). The preparation showed clearly filaments in the monaxons and in the gastral rays, but with regard to the latter I was not able to make out clearly in surface view their relations to the filaments in the basal triradiate system. In the triradiates which I examined I could only find one instance showing a distinct, but small, central triangle. In all the other cases the filaments appeared to meet at the centre, but closer observation showed that they were united by, or were in contact with, a distinct central granule which stained a little more deeply than the contiguous ends of the filaments. I think this observation, so far as it goes, confirms my view of the significance of the central triangle.

In any case I think these observations afford the most conclusive proof of the existence of an organic axial filament in the calcareous sponge spicule. Haeckel (1872) and Sollas (1885) affirmed the existence of this structure, while Ebner (1887) denied it. Lendenfeld (1885) gave a most detailed and circumstantial description of the axial filaments (see above, p. 538), but I find it difficult to believe that he saw anything of what he described; for when Ebner cast doubts upon the existence of an organic axis to the spicule, Lendenfeld in his very next paper on the subject (1891, p. 382) acquiesced in all Ebner's statements without a protest, not even so much as mentioning that he or any one else had ever described such a structure.

B. On Compound Spicular Systems behaving as Single Crystals.

(Note to p. 557.)

With regard to the apparent paradox which is presented by the triradiate and quadriradiate spicules, namely, the fact that while on the one hand they are bodies of multiple origin, they behave on the other hand as single crystals, I am indebted

for some valuable suggestions to my friend Mr. H. A. Miers, Fellow of Magdalen College and Professor of Mineralogy in the University of Oxford. Professor Miers pointed out to me that there were two possibilities as regards the early development of the spicules; either that the rays when first secreted are of a non-crystalline nature, and become crystalline as the result of contact and fusion; or that the rays are crystalline from the first. In the latter case there would be two further alternatives to be considered, namely, that the rays might be at first three separate crystals independently orientated, or that the rays, even when distinct from each other, might possess a molecular structure similarly orientated throughout the whole system.

Since these possibilities can be tested without difficulty by examining very young spicules between the nicols of a polarising microscope, I have searched through my preparations for the early stages of the development of the spicules. The preparations which are figured in this paper, being mounted in glycerine, and now for the most part more than two years old, are so much corroded by the glycerine that the youngest stages are almost all dissolved entirely; but I have found a number of young spicules in preparations mounted in Canada balsam, which are scarcely or not at all corroded. Besides three minute spicules in preparations of the adult coriacea, I have examined an embryo of contorta, two embryos of cerebrum, one of falcata, and one of reticulum. Of these only the embryos of cerebrum showed slight traces of corrosion; in the other embryos the spicules did not seem in the least damaged, though the preparations are eighteen months old. Slight corrosion, it may be pointed out, would not affect the crystallographic results, so long as the spicule be not completely dissolved; but in the case of the embryos of cerebrum it affects the question of the separation of the rays. Since the parts last deposited seem to be the first to be corroded, it is possible that in my preparations of cerebrum spicule rays which appear distinct had really become united and then separated again by corrosion. If so, my results with

regard to the time at which the rays become united, as measured by the length of the rays, would require correction.

The results of my investigations, in which I have to acknowledge much valuable assistance from Professor Miers, will be found below in tabulatory form; but I will first briefly indicate the conclusions to be drawn from them. The chief point of interest which I have discovered is that the spicule rays are not crystalline when first laid down; so long as the rays are quite separate they will not light up when rotated between crossed nicols.[1] After union of the rays they gradually become crystalline, the change appearing to start from the centre, and in fact from the secondary deposit of calcite by which the rays are united together. I have seen many appearances which indicate that the rays are united by a disc or globule of calcite deposited at the centre over the apposed extremities of the rays. In some preparations of cerebrum, viewed from the dermal surface, one sees at a lower focus the interspaces between the rays; at a higher focus a circular piece of calcite comes into view, sometimes so distinct that it looks like the rudiment of another ray. This central portion seems to be in many cases the part that first lights up between crossed prisms. On the other hand, spicules may be found which have the rays completely joined, but which fail to show any crystalline properties; others, again, light up so feebly between the prisms that it is very difficult to detect the feeble illumination, except by observing the slight degree to which it waxes and wanes as the stage is rotated. On the other hand, amongst and between spicules which remain dark there occur others scarcely differing in size, or even smaller, which light up with the greatest brilliancy, thus supplying a valuable contrast.

The next result which merits special notice is the fact that the period in the development at which the rays assume a crystalline character varies greatly in different species, in

[1] As this result would also be produced if the optic axis of the crystal coincided with that of the microscope, I have, where it was possible, tilted the preparation, and found that the spicules still remained dark.

different individuals of the same species, and in the different classes of spicules in the same individual. The embryo of contorta shows the latter point very well. On the third day the numerous spicules fall into two distinct classes: (1) those which have the rays more or less equal in length, and meeting at more or less equal angles; (2) those which have two larger rays placed in the same straight line, or nearly so, while the third ray may be only slightly smaller than the other two, or it may be very much smaller, or finally it may be absent altogether, in which case the whole spicule is simply spindle-shaped, and is a true monaxon spicule. It is evident that the spicules of the second class are the young forms of the large monaxons which characterise this species, and that these spicules are secondary monaxons derived from two rays of the primitive triradiate.

In the embryo of contorta the rays seem in all cases to unite when about 4 or 5 μ in length, or even earlier. The spicules of the first class, the true triradiates, remain non-crystalline for a much longer period, and only light up between prisms when the rays have reached a length of 16 or 17 μ. The spicules of the second class, on the other hand, the future monaxons, become crystalline much earlier. I have not found any which remained perfectly dark, while those with rays over 8 μ in length (i. e. with the monaxon shaft over 16 μ in length) light up with the greatest brilliancy.

In cerebrum also two classes of spicules are early distinct, plainly corresponding to the two classes just described in contorta. Those of the first class, the regular triradiates, are relatively more abundant. In one embryo examined (A) they become crystalline when the rays measure on the average about 13—15 μ; in another (B) this event takes place earlier, when the rays are about 10 or 12 μ in length. The spicules of the second class can be recognised as the young forms of the horn-like spicules peculiar to this species. In the adult, though variable, they have typically two thick rays, placed nearly in a straight line at their bases, but curving like horns towards the tips, while the third ray, which lies in a different plane, is

usually much smaller, and may be reduced to a mere nodule, or may even be quite absent. In the latter case, which is of very frequent occurrence, the spicule becomes simply a curved monaxon. Lendenfeld has figured what I take to be some spicules of this class (1891, pl. viii, figs. 3, *c*, *d*, *e*), but I cannot say that his figures represent the forms of these spicules as I am familiar with them. Bidder terms them tripods (1891, p. 627). I regard these spicules, which show every variation between two extreme forms, the one triradiate, the other secondarily monaxon, as the homologues of the monaxons of contorta, and like them they become crystalline much earlier than the regular triradiates, and can be picked out in the embryo by the brilliance with which they light up between crossed prisms. While the spicules become crystalline at a relatively late period in contorta and cerebrum, in falcata they do so much earlier, so far as my observations go; the rays are separate and non-crystalline till they have a length of about 3 μ, then they unite and show crystalline properties. In reticulum, finally, I have not been able to find any spicules, however small (2 μ) which have the rays either separate or non-crystalline. The smallest visible spicules glitter like stars between the prisms.

The comparison of different species seems to show that the triradiate spicules are the product of a long course of evolution in a certain direction, the furthest point in which has been attained by reticulum. If the view which I have taken of the phylogeny of the spicules be the true one,—if, that is to say, they arose primitively from fusion of three or four separate monaxon spicules,—it seems to me highly probable that the ancestral triradiate or quadriradiate spicules, when they first appeared, would not have behaved optically as single crystals; and that the fact that they do so now is a secondary character, the result of their ontogeny. Instead of now being formed as three (or four) distinct spicules which fuse together when full grown, the relations of the rays are in a certain sense predestined before they are secreted. They arise in close contact, and fuse when still excessively minute; hence

the fact, at first puzzling, that a compound spicular system behaves as a single crystal.

These speculations are impossible to test, unfortunately, unless by some happy chance an Ascon or calcareous sponge should be found in which monaxon spicules, laid down and built up separately, are joined together after attaining their full growth, to form triradiate systems. But it would be interesting to examine between nicols the compound masses formed by fusion of spicules in such a form as Petrostoma (Döderlein, 1897), in order to see whether the masses in question behave as single crystals or show a number of crystalline centres corresponding to the number of spicules which have been united.

As regards the significance, from the point of view of crystallography, of the fact that the spicules at their first appearance are not crystalline, I think I had better leave the explanation to those who are more conversant with the facts and theories of crystallisation than myself. I may, however, point out that since an organic axis to the spicule ray has now been demonstrated, it is evident that the greater part of the spicule ray at its first appearance would be organic in its composition; a fact which may perhaps furnish a clue to the mystery—if there be a mystery.

Summary of Addendum B.

(1) The rays are non-crystalline so long as they are distinct from one another.

(2) They may remain non-crystalline for some time after union has taken place.

(3) The crystallisation appears to start from the secondary deposit which unites the rays at the centre.

(4) With regard to the period at which the rays become crystalline, the species contorta, cerebrum, falcata, and reticulum form a diminishing series, the last-named being the species in which crystallisation sets in earliest.

(5) Those triradiate systems which, by hypertrophy of two

rays and diminution of the third, become modified to form the secondary monaxons, become crystalline much earlier than the more regular triradiates, especially as regards the two rays which are placed in the same straight line to form the shaft of the monaxon spicule.

Synopsis of Observations.

The numbers in brackets denote the length in μ of the rays of the spicules observed. Sometimes the rays vary in length, and the two extremes are given. In the case of spicules of the second class in contorta and cerebrum the length given is half the length of the shaft formed by the two rays placed in a straight line.

s. denotes that the rays were separate, *j.* that they joined; while those spicules too much corroded to judge of this point are marked *c.*[1]

(1) Coriacea, adult.—3 spicules examined. 2 remained dark (4 *s.*, 5 *s.*); 1 lit up feebly (6 *j.*).

(2) Contorta embryo.—26 spicules examined.

Class 1.

Perfectly dark.—8 (4, 6½, 7, 12, 13, 13, 16, 18).

Faintly illuminated.—1 (17).

Fairly bright.—4 (16, 17, 17, 17).

Class 2.—13 examined; all lit up.

Faintly.—3 (3, 6½, 6½).

Distinctly.—2 (4½, 7½).

The rest brilliantly.

(3) Cerebrum.—2 embryos.

Embryo A.

Class 1.

Perfectly dark.—13 (9 *c.*, 11 *s.*, 11 *c.*, 11 *c.*, 12 *c.*, 13 *s.*, 14 *s.*, 14 *c.*, 14 *c.*, 15 *s.*, 15 *c.*, 15—17 *s.*, 16 *s.*).

Faint illumination in centre.—8 (13 *j.*, 13 *j.*, 14 *j.*, 14 *j.*, 14 *j.*, 14 *j.*, 14—16 *j.*, 15 *j.*).

Rays faintly illuminated.—12 (13 *j.*, 13—15 *j.*, 14 *s.*, 14 *j.*, 14 *j.*, 14—16 *j.*, 14—17 *j.*, 15 *j.*, 15 *j.*, 15 *j.*, 16 *j.*, 16 *j.*).

Distinctly illuminated.—12 (13 *j.*, 13—14 *j.*, 14—16 *j.*, 15 *j.*, 15 *j.*, 15 *j.*, 15 *j.*, 16 *j.*, 16 *j.*, 17 *j.*, 17 *j.*, 17 *j.*)

Brightly illuminated.—2 (15 *j.*, 17 *j.*).

[1] Even when the spicule is perfect, it is often very difficult to be certain whether the three clearer lines usually seen at the junction of the rays indicate real interspaces, or lines of recent union.

In addition 1 was observed (13 *s.*) of which 2 rays lit up; 1 remained dark.

Class 2.—6 observed, all of which lit up; 3 distinctly (14 *j.*, 15 *j.*, 15 *j.*) and 3 brilliantly (13 *j.*, 14 *j.*, 15 *j.*).

Embryo B.

Class 1.

Perfectly dark.—3 (10 *s.*, 10—12 *s.*, 12 *j.*).

Faint illumination in centre.—9 (9 *s.*, 9—11 *j.*, 10 *s.*, 11 *j.*, 11 *j.*, 12 *j.*, 11—13 *j.* [?] 13 *j.*, 14 *j.*).

Rays faintly illuminated.—2 (10 *j.*, 11 *j.*).

In addition a triradiate was observed with unequal rays of 11, 9, and 6 μ; the first ray lit up distinctly, the second faintly, the third not at all.

Class 2.

Centre bright.—2 (12 *j.*, 15 *j.*).

Illumination fairly bright.—1 (11 *j.*).

Illumination brilliant.—6 (8 *j.*, 9—10 *j.*, 10 *j.*, 10 *j.*, 11 *j.*, 11 *j.*).

(4) Falcata, embryo.—8 spicules examined.

Perfectly dark.—3 ($2\frac{1}{2}$ *s.*, 3 *s.*, 3 *s.*).

The remaining five were slightly larger, and appeared to have the rays united; they all lit up distinctly.

(5) Reticulum, embryo.—All the spicules, even the minutest (rays less than 2 μ), seemed to have their rays united, and lit up brightly.

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EXPLANATION OF PLATES 38—42,

Illustrating Mr. E. A. Minchin's paper on "Materials for a Monograph of the Ascons."

All the figures are drawn with the camera lucida, using oc. 4 (Zeiss) and obj. 2 mm. immersion (Leitz), and are magnified about 1400.

Significance of the Lettering.

act. bl. Actinoblast. *am.c.*[1] Nutritive wandering cell (amœbocyte). *am.c.*[2] Clear wandering cell. *am.c.*[3] Minute wandering cell. *apic. form. cell.* Apical formative cell of the spicule ray. *bas. form. cell.* Basal formative cell of the spicule ray. *col.* Collar. *col. cell.* Collar-cell. *derm. ap.* Dermal (external) aperture of pore. *fl.* Flagellum. *fl. ep.* Flat epithelium. *gastr. act. bl.* Gastral

actinoblast. *gastr. ap.* Gastral (internal) aperture of pore. *gastr. ray.* Gastral ray of quadriradiate. *n. fl. ep.* Nuclei of flat epithelium. *por. c.* Porocyte. *sext.* Sextet of spicule-forming cells. *spic.* Spicule. *spic. cell.* Definitive spicule-cell. *spic. monax.* Monaxon spicule. *sp. sh.* Spicule sheath. *trirad. syst.* Triradiate system. *x.* Rod-like body in spicule-forming cells.

PLATE 38.

All the figures relate to Clathrina coriacea.

FIG. 1.—Surface view from the gastral side of the dermal layer to show three cells of the flat epithelium that have migrated inwards (actinoblasts). The drawing further shows five cells of the flat epithelium, two spicule rays, one of them bearing a spicule-cell, and a pore-cell.

FIG. 2.—Surface view from the gastral side of dermal layer showing a "trio" of actinoblasts near a spicule ray. Three nuclei of the underlying flat epithelium are figured to show the relations of the cells.

FIG. 3.—Surface view from dermal side of dermal layer showing a trio of actinoblasts underneath a spicule ray. At a higher level are three cells of the flat epithelium.

FIG. 4.—A "sextet" showing three formative cells lying just above three others. From the dermal aspect.

FIG. 5.—A sextet in which the minute triradiate system has made its appearance.

FIG. 6.—A slightly older triradiate system in its sextet of formative cells. From the gastral aspect.

FIG. 7.—A further stage in the growth of the triradiate system seen from the gastral side; the three inner formative cells have travelled to the apices of the rays, while the three outer ones remain at the base.

FIG. 8.—Typical six-celled stage in the formation of the triradiate system, seen from the dermal aspect; the three irregularly shaped inner formative cells at the apices of the rays, the three spindle-shaped outer formative cells at the bases of the rays.

FIG. 9.—Triradiate system in which two apical formative cells have disappeared, one still remaining. Dermal aspect.

FIG. 10.—View of dermal layer after removal of collar-cells, showing various cell elements from the dermal aspect. The drawing shows ten cells of the flat epithelium (*fl. ep.*), six pore-cells (*por. c.*), one actinoblast (*act. bl.*), one young triradiate system bearing three basal formative cells and a single apical formative cell (*apic. form. cell*) persistent on one ray; overlying this the ray of a fully formed triradiate system, bearing a spicule-cell (*spic. cell*), and finally one granular (nutritive) wandering cell (*am. c.*[1]) and five non-granular wandering cells (*am. c.*[2]).

Fig. 11.—Triradiate system showing one ray fully formed and the other not yet so. In the former ray the basal formative cell is at the apex of the spicule as the definitive spicule-cell; in the latter it is still not far from the base of the ray.

Fig. 12.—Triradiate system not quite full-grown; the rays are conical in form, and the formative cells still contain numerous granules. In the case of one ray the formative cell is quite at the base; in the case of the other it has begun to migrate towards the apex.

Fig. 13.—Triradiate system showing what is apparently an apical formative cell just detached from one of the rays at an unusually late period of its growth. The basal formative cell in this ray has travelled halfway to the apex; that on one of the other rays is still at the base.

Fig. 14.—A fully formed spicule ray, showing the spicule cell at the extreme edge.

PLATE 39.

Figs. 15 and 16.—Clathrina coriacea.

Fig. 15.—A porocyte in process of immigration from the flat epithelium, near a triradiate system. Dermal aspect. One portion of the cell has definite limits and an indented outline, and lies at a deeper level, in contact with the collar-cells. The other portion is spread out without clearly defined limits, and is quite superficial. The cell shows a large vacuole containing apparently calcareous matter, spicule remnants. Near the cell lies what appears to be a fragment of a broken spicule.

Fig. 16.—Surface view of the dermal epithelium, with a porocyte in process of immigration. For the latter compare description of last figure.

Figs. 17—21.—Clathrina, sp. dub.

Fig. 17.—Surface view of dermal layer from dermal aspect, showing three cells of flat epithelium, two spicule rays, one bearing a spicule-cell, two minute wandering cells, and a sextet of spicule-forming cells. The spicule has not as yet appeared.

Fig. 18.—A young quadriradiate surrounded by formative cells. Gastral aspect. The rays of the basal system are surrounded by a sextet of formative cells. The minute gastral ray is contained in a porocyte containing two nuclei, one close to the ray in question.

Fig. 18a.—The porocyte and spicule of the last figure drawn separately from the other cells.

Fig. 19.—View of dermal layer, gastral aspect; collar-cells removed. The figure shows twelve cells of the flat epithelium; several spicules and spicule rays, one of which shows a single spicule-cell at its

extremity, while another still bears both its formative cells; a very young quadriradiate, the basal rays enveloped in their sextet of formative cells; the gastral ray contained in a gastral actinoblast which is still continuous with a pore-cell; and finally, a granular wandering cell, partly covered by a spicule, and two minute wandering cells.

Fig. 19A.—The porocyte and gastral actinoblast of the last figure drawn separately.

Fig. 20.—View of dermal layer, dermal aspect. The figure shows seven cells of the flat epithelium, and a portion of another; one pore-cell; portions of three triradiate systems, one bearing a spicule-cell at the extremity of a ray, and the proximal extremity of a large monaxon spicule, bearing two spicule-cells; and finally a young quadriradiate surrounded by the six formative cells of the basal system, and the gastral actinoblast.

Fig. 21.—A young quadriradiate surrounded by the six cells of the basal system, and the gastral actinoblast.

FIGS. 22—26.—From embryos of Ascandra falcata.

Fig. 22.—A sextet from an embryo of the third day of fixation.

Fig. 23.—A young triradiate system from an embryo of the same date.

Fig. 24.—An older triradiate system from an embryo of the sixth day.

Fig. 25.—A ray of a triradiate, with its two formative cells, from an embryo of the same date.

Fig. 26.—A fully formed ray, with its single cell, embryo of the same date.

FIG. 27.—Young triradiate systems, with their formative cells, from Clathrina cerebrum, embryo of third day of fixation. The overlying nuclei of the flat epithelium are drawn in outline only.

PLATE 40.

FIGS. 28—30.—From sections of Clathrina cerebrum, adult.

Fig. 28.—Young quadriradiate with gastral ray enveloped by its actinoblast.

Fig. 29.—Fully formed spiny gastral ray with its actinoblast, containing a nucleus and scattered granules of chromatin.

Fig. 30.—Fully formed smooth gastral ray with its actinoblast, containing a nucleus at the lower extremity, and an aggregation of chromatin granules near the apex.

FIGS. 31 AND 32.—From embryos of Clathrina reticulum.

Fig. 31.—Young triradiate system surrounded by sextet of formative cells; the overlying nuclei of dermal epithelium drawn in outline only.

Fig. 32.—An older triradiate system, with cells as in last figure.

Figs. 33—37.—From sections of adult Clathrina reticulum.

Fig. 33.—A young quadriradiate spicule with formative cells; the basal rays, two of which appear in the section, have each two formative cells. The gastral ray is completely enveloped in its actinoblast.

Fig. 34.—A slightly older gastral ray with its actinoblast.

Fig. 35.—Gastral ray apparently fully formed, the actinoblast with its single nucleus having retreated to the extremity of the ray.

Fig. 36.—Gastral ray with actinoblast in which the nucleus has only recently divided, at a late stage in the growth of the spicule.

Fig. 37.—Very long and slender gastral ray, with two nuclei in the actinoblast.

PLATE 41.

All the figures refer to Clathrina contorta.

Fig. 38.—Surface view of the oscular rim in the region just above the limit of the collar-cells. Gastral aspect. The preparation contains eight porocytes, one with two nuclei; a quadriradiate spicule, each basal ray bearing the usual two formative cells, and the gastral ray enveloped in an actinoblast with two nuclei; and seven collar-cells, more or less completely shown. To show the identity of porocytes and gastral actinoblasts.

Fig. 39.—View of the gastral surface of the body-wall in a spot slightly below that figured in the last figure, showing collar-cells and four porocytes, three of them opened and functioning as pores. The collars of the collar-cells are seen in optical section at higher focus as irregular circles, in the centre of each of which the flagellum appears as a dot.

Fig. 40.—Surface view of dermal epithelium of oscular rim on the outer side.

Fig. 41.—Section of oscular rim, showing epithelium as in Fig. 40 on the lower side, and granular porocytic epithelium, as in Fig. 38, on the upper side. The section being fairly thick, and being decalcified, shows the ground substance full of spaces formerly occupied by spicule rays, to two of which formative cells are adhering, and a gastral ray projects on the upper side bearing an actinoblast with two nuclei. Note also four minute wandering cells.

Fig. 42.—An epithelial cell from the inside of the oscular rim, near the margin of the osculum, intermediate in its characters between the ordinary dermal epithelial cells and the porocytes.

Fig. 43.—A granular wandering cell.

Fig. 44.—A granular wandering cell near two sexual cells (spermatogonia?).

Fig. 45.—Two sexual cells similar to those in the last figure.

Fig. 46.—A finely granular wandering cell (the fine granules only drawn in part).

Fig. 47.—A finely granular wandering cell.

Fig. 48.—A minute triradiate system in its sextet of formative cells.

PLATE 42.

All the figures refer to Clathrina contorta.

Fig. 49.—View of the gastral surface of the body-wall, showing, amongst the collar-cells, a porocyte in continuity with a gastral actinoblast, which is overlying a young triradiate system. The gastral ray has not made its appearance. Only the gastral aperture of the pore has been figured, and the formative cells of the triradiate system have also been omitted.

Fig. 50.—A similar view of a slightly more advanced stage. The porocyte has the dermal aperture closed; the minute gastral ray has now appeared.

Fig. 51.—A similar view of still older stage. The gastral actinoblast has completely separated from the porocyte, and the nucleus of the former has divided into two.

Fig. 52.—Slightly older but rather anomalous stage. The gastral actinoblast has its nucleus divided into two, but is still in continuity with the porocyte. Collar-cells not figured.

Fig. 53.—Young quadriradiate showing the gastral ray a little from the side, enveloped in an actinoblast with two nuclei.

Fig. 54.—More advanced stage. The actinoblast now has four nuclei.

Fig. 55.—Fully formed gastral ray, from a section. The large actinoblast is spread over the gastral ray like a plasmodium, and contains four nuclei.

The Early Development of Amphioxus.

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With Plates 43—45.

The work which forms the subject of the present essay was carried out in the Cambridge Zoological Laboratory during the years 1895–7. The material on which my results are founded consisted of a collection of embryos and larvæ which had been obtained by Mr. Sedgwick and Dr. Willey during their visits to Faro in 1890–91, and I have to express my warmest thanks to Mr. Sedgwick for placing this valuable material at my disposal.

It will, no doubt, be the opinion of many zoologists that a fresh paper on the early development of Amphioxus needs considerable justification, since it may appear superfluous to attempt to improve on the admirably clear account given by Hatschek (3 and 5) of this subject, an account which has become incorporated in all text-books, and forms part of the classical literature of zoology. That such an opinion, however, would not be well founded, and that on the contrary a large number of doubtful points of great theoretical importance remained to be cleared up, will be evident when we briefly examine the present state of our knowledge on this subject.

The foundation of our knowledge of the development of Amphioxus was laid by Kowalevsky, whose two papers (6 and

7) may be considered together. In these the segmentation of the egg and the formation of the blastula and the process of invagination are described. Kowalevsky states that the blastopore is at first posterior in position, but becomes later shifted on to the dorsal surface; he describes the formation of the central nervous system and its appendix, the neurenteric canal. He also describes the formation of the cœlomic pouches (myotomes) as folds of the gut wall, and notes the fact that the first pair have thinner walls and a wider cavity than the rest, and communicate by a broader slit with the gut cavity. He likewise gives an account of the larval life of Amphioxus, and it is interesting to note that in his second paper (7) he anticipated the results which Lankester and Willey obtained later (9 and 13) as to the development of the gill-slits and the formation of the atrial cavity. The two longitudinal ridges, which by their union form the atrial cavity, were seen and figured by him, and the cavities they contained also described. His account of the gill-slits appeared, however, so extraordinary to his contemporaries, that it was supposed he was misled by pathological specimens, and it needed Dr. Willey's researches to convince zoologists of its accuracy.

A year or two after Kowalevsky's second paper was published Professor Hatschek undertook a re-investigation of the subject, and the account given of the development of Amphioxus in Korschelt and Heider's 'Lehrbuch der Vergleichenden Entwickelungsgeschichte' is taken directly from Hatschek's paper. Hatschek confirmed in his first paper (3) Kowalevsky's account of the segmentation of the egg; but with regard to the invagination he asserts that the blastopore is from the first in its definitive dorsal position, or, what comes to the same thing, that the closure of the blastopore is mainly effected by the backward growth of the dorsal (anterior) lip.

This statement has been seized on with avidity by certain workers in Vertebrate embryology as affording a possibility of twisting the developmental history of Amphioxus into accordance with theories which regarded the main developmental process in Vertebrates as a concrescence of two distinct halves

along the mid-dorsal line, or, as it was sometimes expressed, the meeting of the lips of a long slit-like blastopore.

Hatschek's account of the origin of the mesodermal structures is curious and interesting. Like Kowalevsky, he finds two longitudinal folds of the gut wall, but these he believes to terminate posteriorly in two large "pole" cells situated in the lip of the blastopore. By cross-folds the successive pouches are cut off from these longitudinal folds. Later two independent outgrowths from the alimentary canal form the "head cavities," of which the right retains longest its communication with the alimentary canal. The formation of a continuous ventral body-cavity by the fusion of the lower parts of the pouches (somites) is deduced from the fact that the divisions between the somites can no longer be traced in this part of the body. In a later paper (**4**) he describes in the larva what he terms "a genuine kidney," a tube lying in front of the mouth. This structure will be referred to throughout this paper as "Hatschek's nephridium." In his last paper (**5**) he gives an account of the derivation of the muscular and skeletal tissue from the cœlomic pouches, pointing out that everything in Amphioxus is essentially epithelial in nature, the connective tissue having no nuclei in it.

Lankester and Willey's paper on the development of the atrial chamber (**9**) confirms in most points Kowalevsky's statements, but the authors deny that the cavities in the metapleural folds are cœlomic in nature as Kowalevsky had imagined. The right atrial fold is described as extending much further forward than the left. They found also that Hatschek's nephridium opens into the gut; a result which Van Wijhe (**11**) announced as an independent discovery three years later.

Willey (**13**) in 1891 published an account of the development of the gill-slits and other organs appearing in the larval stage. He describes the internal and external openings of the club-shaped gland (an organ noticed already by Kowalevsky), which he regards as the fellow of the first gill-slit. He states that the oral hood which conceals the true mouth of Amphi-

oxus, and which was regarded by Lankester as a forward prolongation of the atrial folds, is a downgrowth of the upper margin of the præoral pit as far as its upper part is concerned, its lower part being formed beneath the mouth independently.

Lwoff's paper (**10**) deserves special attention not only because it is the first systematic study of the embryology of Amphioxus with modern methods, but also on account of the disagreement of the results he arrived at with those of Hatschek. Lwoff maintains that before invagination commences the cells constituting the blastula become sharply divided into two sorts, endoderm and ectoderm cells; that invagination first involves the endoderm cells, but that later the ectoderm bends round the dorsal lip of the blastopore, and displacing the endoderm forms the dorsal wall of the gut, and that the whole of this ectodermal stage is employed in the formation of the notochord, and in the production of mesoderm. Lwoff believes that the mesodermal "folds" are the mechanical result of the pressure of the nerve-tube and notochord on the upper wall of the gut; he asserts that the cavity of this fold disappears, and that the cavities of the somites appearing later have no connection with or relation to the enteric cavity; hence that Amphioxus is not an "enterocœlous animal." He finds that the pole-cells of Hatschek have no existence, therein confirming Wilson (**14**); and that the blastopore is closed not by the special growth of the dorsal lip, but from all sides.

It will thus be evident that the most interesting part of the development of Amphioxus, viz. the formation of the primitive germinal layers, was involved in great uncertainty, and it was with a view of clearing up the questions thus raised, and also from a feeling of dissatisfaction with the accounts given of the nature and origin of such structures as Hatschek's nephridium, and the cavities in the atrial (metapleural) folds, &c., that I was led to undertake a re-investigation of the whole subject. That the early development of Amphioxus is of great theoretical importance can, I think, be hardly denied; when we consider that in it we have the only instance of

Vertebrate development where, the yolk being evenly distributed, its disturbing influence is negligible; and when one recollects the weary controversies which have been waged round the meaning to be attached to the gastrulation and formation of the layers in the heavily yolked eggs of the higher Vertebrates, one must feel that a knowledge of the development of Amphioxus alone could bring these questions to a definite issue.

The results I have arrived at differ considerably from any obtained hitherto, and in claiming to have penetrated more deeply into the developmental processes than Hatschek or Kowalevsky I rely entirely on the higher grade of perfection which methods of dealing with small organisms have reached in the meantime.

All the embryos were embedded in the ordinary way in celloidin; but after hardening the celloidin with chloroform I adopted a plan of clearing on which really the whole success of the work depended. This was as follows:—The hardened celloidin was immersed for a minute or so in absolute alcohol, in order to remove any traces of moisture which might be present in the chloroform; it was then placed in cedar oil and left for a night in a warm place (the dish containing the cedar oil being placed on the top of the thermostat). In the morning the celloidin had become so transparent as to be almost invisible when looked at in the cedar oil. Little pieces of the block containing the embryos could then be cut out and examined with ease under a low power, and their exact orientation determined. They were then embedded in paraffin, and cut into series of sections from 4 to 5 μ thick, and stained on the slide.

The material at my disposal was preserved in a variety of ways, but except for the earliest stages my results are based only on specimens preserved in osmic acid. I wish to lay particular emphasis on this, as should any zoologist feel inclined to work over the same ground with a view of testing my results, and use such fixing reagents as corrosive sublimate or picro-sulphuric acid, he is foredoomed to failure. After such fluids the gut becomes swollen and the body-walls

collapsed, so that it is impossible to make out anything of the limits of the cœlomic cavities. The greatest difficulty I have found in dealing with Amphioxus larvæ is to stain the connective tissue; and I have, in fact, only found this possible with specimens preserved in osmic acid. Of course insufficient or careless preservation in this fluid is valueless, and leads as usual to maceration. For the stages up to the end of the gastrulation, however, before any real differentiation of tissues has taken place, almost any reagent gives fair results.

I do not intend in this paper to refer, except incidentally, to those features in the development of Amphioxus which have been satisfactorily worked out, and about which there is a general consensus of opinion. I may, however, remind the reader that the eggs are spawned in the evening about 7 o'clock; segmentation takes place rapidly, so that by 11 p.m. the blastula is complete and invagination has commenced; two hours later the gastrula stage is attained, and by 5 a.m. the mesoderm has appeared, and the embryo which has been for some time actually rotating within the egg-shell is hatched. On the morning of the next day about 8 a.m. the embryo has acquired the definite form of the larva, with a pointed snout, a swollen pharyngeal region, and a very attenuated body, and the mouth and first gill-slit are formed. No further development has been attained with embryos reared in aquaria, and in their natural habitat the rate of development is after this very slow, extending over several months. I shall divide my account of the subject into the following parts:—*a*. The gastrulation. *b*. The formation of the mesoderm. *c*. The fate of the cœlomic cavities. *d*. The origin of the atrial or "epipleural" folds.

1. The Gastrulation.

A specimen of the youngest embryos examined is represented in Pl. 43, fig. 1. It has the form of a regular sphere bounded by one layer of cells of approximately equal size, and is in fact a perfectly typical blastula. Although Hatschek figures a difference in size of the cells in the two poles of the

egg as being observable in the earlier stages of segmentation, I have been quite unable to find any trace of it in sections, and in this respect confirm Lwoff (**10**). In agreement with the latter author, I find when the first change ushering in the process of invagination takes place, namely, a flattening of one side of the blastula, that then for the first time a differentiation of the cells composing the embryo into two sorts become observable. Some of them, in fact, become taller and slightly narrower than the rest. Lwoff lays great emphasis on this phenomenon. He regards the more cylindrical cells as alone representing the endoderm of Invertebrates, any further portion of the blastula which may be involved in the process of invagination being denominated as ectoderm.

It is difficult to find words to adequately characterise the artificiality and arbitrariness of such a view. The only circumstances under which it could be maintained would be if the supposed endoderm were sharply marked off from the ectoderm, and if further there were a pause in the process of gastrulation after the so-called endoderm had been invaginated, but before the invagination of the ectoderm had commenced. A glance at Pl. 43, figs. 2 and 3, will show that the taller cells on one side shade imperceptibly into the shorter and rounder, so that it is impossible to say where the one begins and the other ends. On the other side, it is true, there is an abrupt transition at the point marked *x*. This point I have found by careful comparison with one another of successive stages to correspond to the dorsal (anterior) lip of the blastopore, the very place where Lwoff supposes ectoderm to be invaginated. This spot is easily recognisable in the earlier stages of invagination (Pl. 43, figs. 4, 5, and 6), but becomes less recognisable in the later stages. As a tentative explanation of it I may suggest that here an active multiplication of cells takes place, and that those which are added to the invaginated portion of the blastula become laterally compressed and columnar, whereas those added to the ectoderm remain stretched by the internal turgidity of the fluid in the blastocœle or segmenta-

tion cavity. As invagination proceeds, the future dorsal surface of the embryo becomes recognisable by the close apposition of the layers of ectoderm and endoderm which subsists here, whilst on the other side the outer and inner layers of the gastrula diverge from one another (at the point marked *o* in fig. 7, for example). A little later we are able to perceive that the future dorsal surface has become definitely flattened, this being the first preparation for the formation of the neural plate, the rudiment of the central nervous system; and as the appearance of this structure enables us to determine the long axis of the future animal, we are able to say that the blastopore is at first posterior. At the same time the peculiar character of the dorsal lip at this stage, with the abrupt transition from ectoderm to endoderm, and the close parallelism of the two layers enables us with certainty to identify it with the corresponding part in earlier stages.

Thus I regard the gastrulation as a fairly uniform pushing in of the under or flattened surface of the blastula, accompanied by division and multiplication of the cells, such multiplication being at first most active in the dorsal (future anterior) lip of the blastopore. The blastopore, which is still wide, becomes rapidly narrowed by the upgrowth of the ventral lip (Pl. 43, figs. 8, 9, and 10): in contra-distinction to what Hatschek (3) asserts, the dorsal lip remains relatively stationary.

Coincidently with this increased activity of growth in the ventral lip, a sharp, abrupt transition becomes now observable in it from ectoderm to endoderm, a fact which supports the explanation given above of a similar phenomenon observed in the dorsal lip. In support of his view that there is an invagination of ectoderm round the dorsal lip of the blastopore, Lwoff speaks of a frequent accumulation of cells just inside the blastopore on the dorsal side, and figures two longitudinal sections of gastrulæ in illustration of this point. I have to definitely state that no such appearances are ever seen in properly orientated sections, and that Lwoff has been misled by his inability to distinguish between oblique and sagittal

sections, though I do not admit that such an accumulation, did it exist, would prove his point.

If we examine a transverse section of a completed gastrula—such a one, for instance, as Pl. 43, fig. 11—we find no difference in character between the cells forming the dorsal wall of the alimentary canal and those forming the ventral wall, such as we should have the right to expect did Lwoff's hypothesis in any way correspond with the facts. Before leaving this subject, however, it is but just to notice a statement of Lwoff's, that had he been dealing with the development of Amphioxus alone, he should not have ventured to put forward the hypothesis of an ectodermal origin of the dorsal wall of the archenteron; but that as he found in other Vertebrates that this dorsal wall was entirely used up in the formation of the notochord and mesoderm, and did not take part in the definitive wall of the alimentary canal, and was in some cases apparently derived from ectoderm, he felt justified in reading this interpretation into the developmental processes of Amphioxus. Such an attitude of mind seems to me the entire converse of the proper one to be adopted under these circumstances. Quite apart from the superior value to be attached to the significance of the processes in Amphioxus owing to the primitive nature of the adult, it is one of the best known facts of embryology that the presence of large quantities of yolk clogs and utterly distorts the developmental processes, and that we have to interpret the cases where much yolk is present in the light of those where little yolk is present, and not vice versâ. Moreover, a very simple and natural explanation can be suggested why in the Vertebrate embryo the yolk should be confined to the ventral wall of the archenteron. We know that many, if not most, developmental processes are ultimately reducible to processes of folding, such as would be rendered entirely impossible were the tissue in which they have to take place clogged with yolk. Hence in the higher Vertebrates the processes of invagination itself are profoundly modified; and, as explained in detail in the careful work of Will (12) (who in

this confirms the ideas of Balfour[1]), the bulky ventral wall of the archenteron can no longer be folded in, and the persistent invagination of the yolkless dorsal wall has the appearance of an independent ingrowth of the ectoderm.

A good illustration of the influence of yolk is seen in the development of Molluscs. There it has been customary to regard the four macromeres as representing the endoderm. These cells are, however, much too heavily loaded with yolk to give rise to any definitive tissue; from them the smaller micromeres which are to form the ectoderm are budded off, and from them later, in continuity with these, the cells which give rise to the epithelium of the intestine. (See Balfour's 'Text-book of Comparative Embryology,' vol. i, Dev. of Mollusca.) It is significant that assertions of the share of the ectoderm in the formation of the alimentary canal should have been made principally in such cases (eggs of insects, cephalopods, &c.), where the accumulation of yolk is so great as to preclude any possibility of the yolk-bearing cells being directly converted into permanent tissue.

2. The Formation of the Mesoderm.

Shortly after the gastrulation is complete the outline of the alimentary canal, as seen in transverse section, ceases to be round. Its dorsal wall becomes flattened, and is then drawn out into two lateral angles, and almost immediately afterwards a median hollow ridge is formed, the first suggestion of the future notochord. The lateral angles mentioned are the expression of two longitudinal hollow ridges or folds of the gut wall. Lwoff regarded them as the mechanical effect of the downward pressure of the nerve-tube as it became folded into the interior of the body, and likewise of the notochord when it was formed. The absurdity of this view is well shown by Pl. 43, fig. 12; there these two lateral ridges are plainly seen, whilst the nerve-cord is still a flat plate, and the notochord is barely indicated. That these hollow outgrowths (which we

[1] "A Comparison of the Early Stages in the Development of Vertebrates," by F. M. Balfour, 'Quart. Journ. Mic. Sci.,' 1875.

may term the cœlomic grooves) are due to an independent process of folding, originating in the endoderm, is also shown by the fact that the endoderm is no longer in close contact with the ectoderm, a distinct slit-like blastocœle being observable in several places, which certainly would not be the case if the endoderm merely passively followed the foldings of the ectoderm.

Shortly after the appearance of the cœlomic grooves a fresh pair of outgrowths from the alimentary canal make their appearance anteriorly. These, which will be denominated collar cavities, are shown in fig. 13. As will be seen, they are situated slightly nearer the middle line than the cœlomic grooves (which in the figure are seen external to them, separated by a very narrow fold of the gut wall), and their lumina are at first excessively narrow (at least in preserved specimens), but they soon enlarge, and their openings into the cavity of the alimentary canal become clear (fig. 14, *c*). About the same time the front part of the cœlomic grooves becomes constricted off from the alimentary canal, and thus a definite "somite" or myomere is formed (fig. 14, *b*). Behind, however, the cœlomic grooves still open into the alimentary canal, as shown in fig. 14, *a*. If we compare with such a series of sections through older embryos, such as those figured in fig. 17, *d* and *e*, we shall arrive at a clear comprehension as to how the cœlomic grooves are converted into a series of somites. We can always find at the hinder end of the embryo appearances (like those represented in figs. 14, *a*, and 14, *c*) of the gut wall being folded so as to produce a pair of cœlomic grooves, and we can follow the walls of the fold constituting the cœlomic groove into the walls of the last somite (compare fig. 17, *d*). The cavity of no other somite communicates with the gut; it is only the last somite, for the time being, whose cavity is in communication with the gut cavity through the cœlomic groove; and hence we see that as the embryo grows in length, and the cœlomic groove with it, this latter becomes progressively constricted from the gut and divided into somites at the same time, each new piece which is con-

stricted off becoming formed into a somite, and nipped off from the open part of the cœlomic groove, which then again grows in length, and the process is repeated. The formation of a somite, then, is essentially a process of obliterating the cavity of the cœlomic groove for a certain space, and the so-called last somite is really the undifferentiated hinder end of the cœlomic groove.

The entire independence of the collar cavities from the cœlomic grooves is emphasised by the fact that for some time after the latter are shut off from the gut the collar cavities still retain their openings into it. This is shown in the transverse section (fig. 14, *c*) and in the longitudinal section (fig. 15, *a*). These collar cavities are the "first protovertebræ" of Kowalevsky, and in his paper (7) he notes the fact that they communicate by a broader slit with the alimentary canal, and retain this communication longer than the rest. Later, it is true, the right collar cavity becomes completely shut off from the gut, but the left retains its communication, as is shown in Pl. 43, fig. 16.

Shortly after this period the embryo begins to diminish rapidly in diameter, owing to the consumption of the yolk in the endoderm cells, whilst at the same time it increases in length, and the cavities in its interior diminish in size, owing to the gradual shrinkage. Hence one requires now to have specimens preserved in such a way as to give firmness and resistance to the outer tissue, if one is to make out anything of the internal anatomy at all. The yolk, which is present not only in the ventral but also in the dorsal ectoderm and in the walls of the cœlomic folds, acts whilst it endures, somewhat like paraffin, in preventing too great shrinkage; after it has gone nothing but osmic acid will give any help.

Just as the disappearance of the yolk is commencing, the third division of the mesoderm, the head cavities, make their appearance. The two head cavities really constitute the extreme anterior end of the alimentary canal, which grows out into two lateral horns. In Pl. 44, fig. 17, *a*, we see them still opening widely into the gut; but in the next section (17, *b*), taken

further back, we see them quite free from the gut, and so we can conclude that they are recurved. Hatschek (3) spoke of the two head cavities as distinct outgrowths from the gut, and further stated that the right one still communicated with the gut after the left had been cut off. This is not a correct account of what happens: the whole anterior part of the alimentary canal becomes shut off from the hinder part, and its two horns, which later become converted into the head cavities, still communicate with one another after separation from the gut has taken place (fig. 18, *b*).

We have thus seen that the mesoderm originates as five hollow outgrowths from the gut—an anterior median, viz. the head cavity rudiment, and two pairs of lateral ones, viz. the collar cavities and the cœlomic grooves. Thus in the formation of the primary layers Amphioxus is in fundamental agreement with Balanoglossus, as described by Bateson (1). The main difference between the two types is that whereas in Balanoglossus the trunk cavity remains undivided, in Amphioxus it becomes broken up into a series of segments, a difference which we may plausibly correlate with the different modes of life pursued by the two animals. Pl. 45, figs. 25 and 26, are two diagrams intended to make these relations clearer.

3. The Fate of the Cœlomic Cavities.

The two horns of the common head cavity rudiments rapidly become separated from one another; the right now shows itself as an irregular-shaped and thin-walled sac; the left, on the other hand, is composed of cylindrical cells, and remains small and round (fig. 18, *a*). The right soon after gets shifted ventrally, and forms the greater part of the cavity of the præoral snout during the whole of the larval period (compare fig. 21, *a*). I have not been able to identify it in the adult, and can only suppose that it becomes obliterated, the space corresponding to it being apparently occupied by connective tissue. The left, as is well known, acquires an opening to the exterior, and constitutes the præoral ciliated pit (fig. 21, *a*), which Hatschek first discovered. This præoral pit persists into

the adult condition as a ciliated area on the inner side of the præoral hood. By the time that the head cavities have commenced to appear, notochord and nerve-cord have become well advanced in their development. The notochordal fold has become completely shut off from the gut, and is quite solid in front, though still a groove behind, whilst the neural plate has passed through the stage of being covered by a flap growing from the adjacent ectoderm (figs. 13, 14, and 16), and has become converted into a tube (fig. 17, *b*, *c*, and *d*), still retaining an anterior opening, the neuropore (figs. 17, *a*, and 18, *b*). The collar cavities have now become large thin-walled sacs; the right extending by this time nearly to the mid-ventral line; the left does not extend so far, but it still retains its communication with the gut. This communication has by this time become drawn into an exceedingly narrow tube (fig. 17, *c*, *neph.*), and is in fact the rudiment of Hatschek's nephridium.

At the close of what we may term the embryonic development —that is to say, at the end of the second day of development —both collar cavities have undergone further changes. They have extended forward at the sides of the notochord, above the head cavities, to just beneath the neuropore (fig. 18, *b*); behind they have nearly reached the mid-ventral line, the left being more obliquely directed, as it has to pass over the area where the future mouth will be formed (fig. 19, *b*). When they have reached the ventral line they extend backwards to a considerable distance behind the first gill-slit, forming the ventro-lateral angles of the body, and giving to the transverse section a squarish appearance ventrally, which contrasts strongly with the rounded appearance behind the point they extend to (compare Pl. 44, fig. 20, *a* and *c*). The inner walls of the dorsal portions of the collar cavities become like the corresponding parts of the somites converted into longitudinal muscles, and constitute in fact the first myotomes; but during early larval life, at any rate, the persistent cavity of this "first myotome" on the right side remains in open and obvious communication with the ventral part of the collar cavity

(fig. 21, *a*). On the left side the front section of the ventral collar cavity seems to become solid, and from it are apparently derived the true oral tentacles, so well described by Lankester, and the muscles moving them. "Hatschek's nephridium" has now become a horizontally placed tube, the openings of which into the first myotome (collar cavity) on the left and into the gut are easily seen (fig. 21, *b* and *c*). It is curious that this internal opening completely escaped the observation of both Van Wyhe and Hatschek; it is perfectly easy to see in any good series of sections through a specimen preserved in osmic acid. Hatschek (4) states that this nephridium persists into the adult. I have, however, been able to find no trace of it after the metamorphosis.

The ventral extensions of the collar cavities appear in the larva to reach some distance behind the last gill-slit formed. In any series of sections in this region we get quite similar appearances to those represented in figs. 20, *a*, *b*, and *c*. The natural conclusion to be drawn from this is that behind the first gill-slit, as the gut grows and produces new gill-slits, the collar cavities grow pari passu.

The cœlomic cavities in the somites we saw at their time of formation to be exceedingly minute; in some cases it would be more correct to say that there was no cavity at all, but only a radiate arrangement of the cells round a virtual cavity. Such a state of things might in the minds of some suggest that there was something to be said for Lwoff's position that Amphioxus is not an enterocœlous animal, since its cœlom could not be traced into continuity with the alimentary cavity. Such a position, however, appears to me to be quite untenable, even apart from the fact that the collar cavity is at one time in open and obvious communication with the enteric space. What determines whether an animal is to be regarded as enterocœlous or not is whether or not evidence is forthcoming to show that the walls of the body-cavity have been derived from those of the alimentary canal by a process of folding, for it is the walls only to which we can attribute an objective existence; the presence or absence of a cavity at any moment

of development is due to the relations of growth and pressure subsisting between them. No one who has seen well-preserved sections can doubt that it is by a folding process that the mesoderm is formed in Amphioxus.

Later the cavities of the somites enlarge, and the dorsal portions of their inner walls are like the corresponding parts of the collar cavities converted into longish muscles (*Musc.*, fig. 20, *a*, *b*, and *c*), and form all the myotomes except the first. The ventral portions wedge themselves in between the gut and the posterior ventral extension of the collar cavities (*V. Tr.*, figs. 19, *c*, 20, *a*, *b*, and *c*). Whether in the living condition these ventral portions are completely hollow, or whether, as is suggested by the examination of sections, they are partly represented by solid tongues of tissue, it is impossible to settle. Of course in the adult the ventral portions of the somites give rise to the dorsal cœlomic canals, and to the canals running in the primary and secondary gill bars (Lankester); the dorsal cœlomic canals are clearly represented in the larva, but I have found it impossible to be certain whether the rest of the ventral portions of the somites are open spaces in the larva or not. Just at the close of embryonic life the "myotome" becomes separated from the rest of the somite by a septum, and the ventral portions of the somites acquire communication with each other, about the region where the dorsal cœlomic canals afterwards appear. This ventral fusion of the somites was inferred by Hatschek from the fact that he could not trace the dividing lines between the somites to the mid-ventral line. The specimens of later larvæ which were well enough preserved to rely on showed the trunk cœlom (derived from the ventral fusion of the somites) clearly only behind the gill-slits, where the collar cavities were dying out. Elsewhere the extreme difficulty of staining the connective tissue and peritoneal epithelium made it impossible to be certain whether a narrow slit-like cavity or only a wedge of tissue intervened between the gut wall and the collar cavity.

4. The Origin of the Atrial Folds.

Kowalevsky (7) was the first to discover that the atrial cavity was formed by the meeting in the mid-ventral line of two long ridges or folds. These, which were more exactly investigated by Lankester and Willey (9), are situated at the ventro-lateral angles of the body, and the atrial cavity is at first a small space situated in the middle line beneath the pharynx (fig. 23, *b*). Later the atrial cavity extends up at the sides of the pharynx, and the origin of the folds becomes consequently shifted up the body. This is the account of the origin of the atrial cavity given by Lankester and Willey ; but it must be remembered that as the dorsal limits of the atrial cavity are from the beginning conterminous with those of the gill-slits, the process might be more correctly described as a great relative growth of the ventral region of the pharynx and surrounding structures. Lankester (8) terms the folds which actually wall in the atrial cavity "epipleural," and the projecting angles after these folds have united "metapleural." I shall use the term "atrial fold" to include the whole, of which both are parts.

It must already have struck the reader that the posterior ventral extensions of the collar cavities which I have described above occupied precisely the region where the atrial folds subsequently appeared ; hence it will not be surprising when I state that the cavity of the atrial fold, termed by Lankester and Willey "pseudocœlic," is nothing but the backward extension of the collar cavity. This I have succeeded in proving for the right collar cavity (comp. fig. 22, *a*, *b*, and *c*) ; and since the left collar cavity has precisely the course which Willey describes for the oral hood and left atrial fold, no one will doubt that this is the case for the left side also. From the walls of these two collar cavities the ventral muscles of Amphioxus are formed, and their lumen becomes occluded in the centre (fig. 24), but remains at the sides as the "metapleural lymph canal."

Lankester and Willey describe the atrial folds as first

appearing behind, and then growing forward; but the first recognisable trace of the future fold on the right side is an epithelial thickening (fig. 22, *a*) in the anterior region of the pharynx. This thickening, which later lines the outside of the fold, is recognisable even at the end of the embryonic period.

It will be remembered that the name "collar cavity" was given to the cœlomic pouches so denominated on account of their general resemblance in mode of formation to the collar cavities of Balanoglossus. The homology implied in this name is borne out by the subsequent history of the sacs in question; for (1) they remain distinct from the cavities derived from the cœlomic grooves or trunk cœlom, and (2) they swell out into ridges overhanging and protecting the gill-slits, just as the hinder edge of the collar region does in Balanoglossus, as Bateson (2) has pointed out,—only in that animal, of course, at most two gill-slits are protected.

Résumé and Conclusions.

The most important points established in this paper are as follow.

(1) The primitive gut or archenteron is formed in Amphioxus by a typical process of embolic invagination, the endoderm being at first not sharply marked off from the ectoderm. The blastopore is at first posterior, but subsequently becomes dorsal by the preponderant growth of the ventral lip of the blastopore.

(2) The mesoderm originates in Amphioxus as a series of true gut pouches, viz. one anterior unpaired pouch and two pairs of lateral pouches. Of these the first divides to form the two head cavities: the anterior pair give rise to the first pair of myotomes, and in addition to two long canals extending back ventrally: the posterior pair are gradually separated from the gut, and pari passu divided into a series of myotomes. The whole process of mesoderm formation is therefore referable to the type found in Balanoglossus, the main differ-

ence being that the pouch corresponding to the trunk cœlom of Balanoglossus becomes segmented.

3. Hatschek's nephridium is the persistent connection of the left of the pair of collar-pouches with the gut.

4. The metapleural "lymph canals" found in the atrial folds arethe persistent ventro-lateral extensions of the "collar-pouches."

The general conclusions which can, I think, be fairly deduced from the foregoing study are, in the first place, that all attempts to explain the formation of the nervous system of Vertebrates by the coalescence of the two halves of a nervous ring lying in the lips of a long slit-like blastopore must be given up, any appearances interpreted thus being due to secondary disturbances introduced by increasing food-yolk; for of any such process no trace is observable in the simple development of Amphioxus.

Secondly, that the theory of the descent of the Vertebrates from a form somewhat like Balanoglossus receives strong support from the early developmental history of Amphioxus. I say from a form somewhat like Balanoglossus advisedly, for it may not be superfluous to lay stress on this point, that we can no more suppose Vertebrates to be descended from Balanoglossus than from Amphioxus. The main stem of the Vertebrate phylum most probably continued throughout its whole history to lead an active predatory existence, and Balanoglossus and Amphioxus are to be regarded as degenerate offshoots from different levels of this stem. It is much more probable that the Tornaria larva of Balanoglossus gives the best idea of the remote ancestor of the Vertebrates, and in this respect the condition of the nervous system in the larval Amphioxus is of great interest. So far as we know central nervous systems are generally developed in close connection with prominent sense-organs. Now the Tornaria (in its later stages) has two main nervous centres—(*a*) at the apex of the præoral lobe, a sensory nervous plate with two eye-spots; (*b*) a short nervous tube in the collar region. Of these, the second has probably been developed in connection with a

series of sensory tentacles such as Cephalodiscus possesses in this region, and which probably correspond to the ambulacral tube-feet or tentacles of Echinoderms. The first is lost in Balanoglossus, owing no doubt to its burrowing life; but in the free-living Vertebrate ancestor this would not have occurred. As the præoral lobe became reduced in size (a process which may have been connected with the giving up of cilia as a means of progression and obtaining nutriment) the two nervous centres of the Tornaria-like ancestor would become approximated, and we should reach the condition which we actually find in the Amphioxus larva, viz. a sense-plate immediately followed by a nervous tube; for the part of nervous system under the neuropore becomes pigmented, and is sensitive to light. Figs. 27 and 28 are diagrammatic side views of Balanoglossus and an Amphioxus larva, and are intended to emphasise the immense diminution which the præoral lobe has undergone in the latter.

If these conclusions are well founded, Amphioxus would represent a more primitive offshoot from the Vertebrate stem than Ascidians, for the larvæ of the latter possess a large vesicular brain, which only retains a small pore leading into the stomodæum. This deduction is, however, supported by the fact that whereas the Ascidian larva possesses a long post-anal muscular tail (a feature which has become more and more accentuated in fishes), in the Amphioxus larva the anus is as Hatschek pointed out, and as I can confirm, at the extreme posterior end of the body on a vertical neurenteric canal, and becomes only slowly and to a small extent shifted forwards during development.

Zoological Laboratory, Cambridge;
August, 1897.

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EXPLANATION OF PLATES 43—45,

Illustrating Mr. E. W. MacBride's paper on "The Early Development of Amphioxus."

List of Abbreviations employed.

Al. Alimentary canal. *Ch.* Notochord. *End.* Endostyle. *gl.* Club-shaped gland. *Musc.* Longitudinal muscles of the somites. *N. c.* Nerve-cord. *Neph.* Persistent communication of the left collar cavity with gut, commonly called "Hatschek's nephridium." *V. Coll.* Ventral portion of collar cavity. *V. Tr.* Trunk body-cavity produced by the fusion of the ventral portions of the somites. *x* marks the dorsal lip of the blastopore in gastrulæ.

The last four figures are diagrams; the outlines of all the rest have been drawn with the camera lucida.

PLATE 43.

(All the figures drawn with magnification obtained by Zeiss C, oc. 2.)

Fig. 1.—Section of young blastula.

Fig. 2.—Sagittal section of blastula which is just commencing to flatten.

Fig. 3.—Sagittal section of blastula which has flattened on one side.

Fig. 4.—Sagittal section of gastrula in which invagination is just commencing.

Fig. 5.—Sagittal section of gastrula in which invagination is more advanced.

Fig. 6.—Sagittal section of gastrula in which invagination is still more advanced.

Fig. 7.—Sagittal section of gastrula in which invagination is well advanced; the first trace of the flattening which marks the dorsal side is visible.

Fig. 8.—Sagittal section of gastrula in which invagination is very advanced.

Fig. 9.—Sagittal section of gastrula in which blastopore is becoming narrowed.

Fig. 10.—Sagittal section of completed gastrula. The blastopore has been shifted to the dorsal side (in consequence of a slight obliquity a piece of the medullary fold is included in the section).

Fig. 11.—Transverse section of embryo about the age of that in Fig. 10 (7—8 hours from fertilisation).

Fig. 12.—Transverse section of embryo of about ten hours; first trace of neural plate and cœlomic groove.

FIG. 13.—Transverse section of embryo of about ten hours; shows first trace of collar cavity distinct from cœlomic groove.

FIG. 14, *a*, *b*, and *c*.—Three sections from a series through an embryo of from ten to twelve hours in age.

a. Shows the cœlomic groove.

b. Shows the cœlomic groove closed off in front so as to form the cœlom.

c. Shows the independent opening of the collar cavities.

FIG. 15, *a* and *b*.—Two sections from a series cut parallel to the sagittal longitudinal plane through an embryo of twelve to thirteen hours.

a. Shows the cœlomic groove and collar cavity opening into the archenteron.

b. Shows neurenteric canal and division of trunk cœlom into somites.

FIG. 16.—Transverse section of embryo of about thirteen hours, showing the left collar cavity only, opening into the archenteron.

PLATE 44.

(All the figures on this plate are drawn under the magnification of a Zeiss D, oc. 2.)

FIG. 17, *a*, *b*, *c*, *d*, and *e*.—Five sections from a series through an embryo of fourteen to fifteen hours.

a. Shows head cavities opening into gut and anterior pore of the nervous system.

b. Shows head cavities constricted off from gut.

c. Shows downward extension of right collar cavity and persistent communication of the left with the gut (*Neph.*) (Hatschek's nephridium).

d. Shows the last somite, which is still continuous with the cœlomic groove.

e. Shows the cœlomic groove.

FIG. 18, *a*, *b*.—Two sections from a series through an embryo of about twenty hours.

a. Shows the right and left head cavities becoming differentiated the one from the other, the collar cavities having shifted forward.

b. Shows that further back the two head cavities still communicate with one another. The open neuropore is still seen.

FIG. 19, *a*, *b*, and *c*.—Three sections from a series through an embryo of about twenty-four hours.

a. Shows complete separation of right and left head cavities and the ventral shift of the former.

b. Shows inequality of the two collar cavities; the left retains a narrow communication with the gut (*Neph.*).

c. Shows backward continuation of the ventral part of the collar cavity (*V. Coll.*). *V. Tr.* A solid wedge of tissue which represents the trunk cœlom formed by the fusion of the ventral ends of the somites.

Fig. 20, *a*, *b*, and *c*.—Three sections from a series through an embryo of the same age as that figured in Fig. 19, showing the dying out of the ventral extension of the collar cavity (*V. Coll.*).

Fig. 21, *a*, *b*, and *c*.—Three sections from a series through a pelagic larva showing relations of the fully developed nephridium of Hatschek.

a. Shows the opening into the cavity of the first muscular somite (left collar cavity).

b. Shows appearance of a section across the middle of the structure.

c. Shows the opening into the pharynx.

PLATE 45.

Fig. 22, *a*, *b*, and *c*.—The ventral portions of three sections through an old pelagic larva, showing the formation of the atrial fold and the continuation of the ventral part of the collar cavity into it. *gl*. Club-shaped gland.

Fig. 23, *a* and *b*.—The ventral portions of two sections through a still older pelagic larva, showing a further stage in the development of the atrial folds.

Fig. 24.—Ventral portion of a section through a young adult Amphioxus, showing the cavities of the metapleural folds (the same as the collar cavities), and the schizocœlic artefacts which appear to the outer side of them.

Fig. 25.—Diagram showing the origin of the various portions of the cœlom from the gut in Amphioxus.

Fig. 26.—Similar diagram to show the origin of the cœlom in Balanoglossus.

Fig. 27.—Diagrammatic lateral view of an Amphioxus larva, showing mutual relationships of the head cavities, collar cavities, and trunk cœlom.

Fig. 28.—Similar diagram of Balanoglossus.

(Fig. 22, *a*, *b*, and *c* are drawn with Zeiss F, oc. 2; Figs. 23 and 24 with Zeiss D, oc. 2).

N.B.—In Figs. 17 to 20 the hypoblast and mesoblast are coloured red, and the epiblast grey.

On the contrary, in Figs. 21 to 24 the epiblast is coloured red, the mesoblast blue, and the hypoblast, including the notochord, grey.

On Drepanidotænia hemignathi, a New Species of Tapeworm.

By

Arthur E. Shipley,

Fellow and Tutor of Christ's College, Cambridge, and University Lecturer in the Advanced Morphology of the Invertebrata.

With Plate 46.

The specimens of the above-named tapeworm, of which I received but ten, are all small; they vary in length from 10 mm. to 22 mm. The head is very small; immediately behind it, there being practically no neck, the body begins to broaden out, and in some specimens the proglottides attain a width of 2 mm. The segmentation of the body commences immediately behind the head, and is very well marked a little further back. The posterior border of each segment overlaps the succeeding one with a prominent edge or rim; this is well shown in longitudinal section (fig. 6). The number of segments varies from some fifty to sixty to over a hundred. The measurements given above are about the average, but, as is well known, tapeworms are extremely extensible animals, and this to a great extent diminishes the value of figures quoted in reference to their size. In some specimens the body is stretched, and the length of the segments equals one half or even two thirds of their breadth, but in the commoner forms the segments are very short and broad, sometimes eight or ten times as broad as long. They are flattened, as is seen in transverse section, and sometimes, especially towards the posterior

end, the whole body is hollowed so that each segment is curved. The most posterior segments, which are crowded with embryos well advanced in their development, are rounder, less flattened, longer, and they readily broke off.

I was not able to detect any genital pore on the exterior even with the aid of powerful lenses, but sections (figs. 4 and 6) and stained mounted specimens show that it is on the same side of the body in all the segments.

The head of the tapeworm bears four suckers, and in the midst of them is the rostellum (fig. 9). The shape of the head is very various: in some cases the suckers are, as it were, hunched up and lying at each corner of a square, the lateral diameter of which does not exceed the dorso-ventral (fig. 8); in other specimens the head is not separated from the body by a deep constriction, but is flattened and spread out (fig. 7), so that the lateral suckers are separated from one another by a space considerably wider than that which lies between the dorsal and the ventral suckers.

The rostellum is minute and sunk in a pit (fig. 3); it bears a wreath of ten hooks. In all the specimens which I cut into sections, and I think in the others as well, the rostellum was retracted, the points of the hooks folded in against the axis of the rostellum, and not reaching so far forward as the mouth of the pit. When the animal is fixed to the mucous membrane of its host this rostellum is doubtless protruded from its sheath, and the hooks are divaricated. Certain muscle-fibres which run from the base of the rostellum, and lose themselves in the parenchyma, probably serve to retract it.

The hooks are slightly curved, and the projection which corresponds with the inner fork of the more triradiate hooks of other genera is hardly, if at all, marked (fig. 2). Measuring in a straight line from the base to the tip the hooks are 18—23 μ in length, thus corresponding pretty closely with those of *Drepanidotænia tenuirostris*, which, according to Railliet,[1] measure 20 to 23 μ, and to those of *D. lanceolata*, which measure 25 to 31 μ.

[1] 'Traité de Zoologie médicale et agricole,' Paris, 1895.

The four suckers present no peculiarities; they are deeply cupped, with a small orifice to their lumen, but probably they are capable of considerable change of form (fig. 9). They are probably retracted by some muscle-fibres which cross one another and run into the parenchyma.

The segmentation of the body begins immediately behind the suckers; at first the segments are very short, but they gradually increase in size throughout the first three quarters of the length of the body. For the last quarter the segments are crowded with embryos; they become in this region much narrower, more cylindrical in shape, and longer, and are very easily broken off. The posterior free edge of the segments of the anterior two thirds of the body is sharp, and may overlap the segment behind, or may stand out clearly from it.

The water-vascular system is well developed; on each side of the body are two longitudinal canals,—one, the ventral, much bigger than the other, or dorsal. The lining of the former seems to be a structureless cuticle with no cells especially related to it, but the wall of the dorsal vessel is surrounded by a number of small deeply stained cells (fig. 4). I did not see any communication between the vessels of one side, but the larger vessels communicate as usual, one with another, by a transverse vessel running from side to side along the posterior border of each segment. In the head the vessels all communicate. In some of the better preserved sections such structures as are depicted in fig. 10 were seen: these may or may not be flame-cells; they look rather like them. No valves were seen in the course of the vessels.

The lateral nerve-cords are well marked, lying externally to the ventral excretory canals; they fuse together in the head, forming a ganglion which is indicated in fig. 3. No traces of the nerve-ring described by Tower[1] as running round the posterior end of each segment of Moniezia, or of the secondary nerves described by the same observer, were to be seen. But these, if present, probably require fresh material and

[1] 'Zool. Anz.,' vol. xix, 1896, p. 323.

special methods of preservation to make them manifest. Special nerve-cells, described below, are scattered through the parenchyma of the body.

The histology—at least in some specimens—could be fairly well made out, and agrees roughly with what Blochmann has described in Ligula monogramma.[1] The whole body is covered by a cuticle, the outer fifth of which stains more deeply than the remainder. Within this, with a high power, a number of dots or knobs become visible (fig. 10). These are the swollen terminations of certain strands or processes of the ectoderm cells. The cells themselves, as Blochmann has shown, lie removed to some distance from the cuticle they secrete, but are in contact with it by means of the above-mentioned processes ending in the knobs.

The ectoderm cells are not all at one level, but on the whole form a fairly well-marked layer. Each cell is fusiform in shape, and produced into two or three processes, which project both peripherally and centrally. They contain large and well-marked nuclei. Neither the cells nor their processes are laterally in contact; they are separated one from another to varying extents by the intrusion of some of the parenchymatous network which makes up so much of the body of a Cestode.

This parenchyma consists of a meshwork which permeates everywhere the body of the tapeworm, surrounding all the organs, and often, as is the case with the ectoderm and the muscles, passing in between their constituent cells. In the spaces of the meshwork there is believed to be a fluid. The meshwork itself is secreted and nourished by certain large star-shaped cells which are irregularly scattered through the parenchyma, and which give off processes in all directions (fig. 10).

Round the generative glands this parenchymatous network becomes condensed, the spaces disappear, and it forms a close sheath to the ovary, testis, &c. At the posterior end of each segment it is also somewhat condensed, and in section presents

[1] 'Die Epithelfrage bei Cestoden und Trematoden,' Hamburg, 1896.

the appearance of a well-marked double line, which is very characteristic, and is well shown in fig. 6.

Scattered amongst the parenchyma are certain faintly stained cells which seem to be bipolar, and which differ from the cells of the parenchyma both in shape and in their powers of absorbing the staining reagents. These I take to be nerve-cells which are in communication with the nerve-fibres of the lateral cords. The latter are entirely devoid of any nerve-cells on their course.

Muscle-fibres are scattered through the substance of the body, and one set of longitudinal muscles are most definitely arranged. This layer is situated just below the epidermis in the anterior part of the segment, but as the latter increases in size posteriorly, the cylinder of muscle-fibres, which retains the same diameter throughout, comes to lie more deeply in the tissues. These muscles, like the nervous system and excretory canals, run from segment to segment; some of them, if not all, end in the cuticle, where it is most bent in at the posterior end of each segment. Laterally the fibres are not in contact, being separated by considerable intervals. Their regular arrangement is shown in fig. 6.

In the posterior segments, which are so ripe that the slightest touch breaks them off, the parenchyma has undergone considerable degeneration, the cells are less clear, and the spaces of the meshwork are larger and more irregular.

The generative organs begin to arise very early in the series of segments. Already in the eighth or tenth segment clusters of cells are segregating, and their deep staining shows that they belong to the gonads. In the sexually ripe segments the ovary is centrally placed, and is supported on each side by a lobe of the testis. From the latter a fine vas deferens leads into an extensive vesicula seminalis, which is as a rule crowded with spermatozoa; from this a muscular duct leads to the unilateral genital pore. I was unable to make out the details of the penis, and similarly I failed to detect any yolk-gland amongst the female genitalia.

The vagina leads at once into a large receptaculum seminis,

whose walls were strengthened by a series of cuticular-looking rings, whose cut ends are shown in figs. 4 and 6. This communicates both with the oviduct and with the uterus. The latter presents no special points of interest; in the posterior segments it contains the typical three-hooked larvæ, each segment containing at least one hundred and probably more.

Systematic.

In his paper on tænias in birds, Dr. Fuhrmann[1] remarks that of the 240 odd species of tapeworm described from avian hosts, only twenty-one have been studied anatomically; the remainder are but little more than names, and probably many of the names are of doubtful validity.

A certain amount of order has been introduced into this mass of material by the establishment of certain sub-groups, and by the giving of a new generic name to the members of these subdivisions; thus in 1891 Blanchard and Railliet[2] established the genus Davainea; in 1892 Railliet[3] suggested two new generic names, Drepanidotænia and Dicranotænia, for certain tapeworms inhabiting, for the most part, domestic birds. These are characterised chiefly by the nature of the hooks. In the following year Diamare[4] founded the genus Cotugnia, in which the generative organs are double and have two pores, but which is distinct from the genus Dipylidium of Leuckart. All these genera are characteristic avian tapeworms, and are, with but very few exceptions, confined to birds.

There is little doubt that the tapeworm which I have described above from the intestine of Hemignathus procerus corresponds with a Drepanidotænia of Railliet,[5] who defines his genus as follows:

"Tapeworms provided with a simple crown of uniform hooks,

[1] 'Rev. Suisse Zool.,' tome iii, 1895–6, p. 433.
[2] 'Mem. Soc. Zool. France,' tome iv, 1891, p. 420.
[3] Ibid., tome xvii, 1892, p. 115.
[4] 'Boll. Soc. Napoli,' ser. 1, vol. vii, 1893, p. 9.
[5] 'Traité de Zoologie médicale et agricole,' Paris, 1895, p. 298.

which are usually few in number; the outer limb (manche) of the forked base of the hooks is much longer than the inner (garde), which is always slight; the point is directed backwards when the rostrum is withdrawn. The majority live in the intestines of aquatic birds. Their larva is a Cysticercoid, and is found encysted in the bodies of small fresh-water Crustacea."

Railliet describes eight species of Drepanidotænia; in one of these the genital pores are on alternate sides of the body in successive segments: the remaining seven species are unilateral in this respect, but they fall into two groups,—one, with three species, in which the number of hooks is eight; and the other, with four species, in which the number of hooks is ten.

It is to this latter group that we must add the tapeworm from H. procerus. The four species D. anatina, D. sinuosa, D. setigera, and D. tenuirostris differ inter se in several respects, but perhaps the simplest way of determining the species is by measuring their hooks. Of these four species, D. hemignathi most nearly resembles D. tenuirostris, which occurs in certain of the ducks; it differs, however, markedly in size, being when mature about $\frac{1}{5}$ to $\frac{1}{12}$ the length of the last named. It resembles D. tenuirostris in the length of its hooks in the head, which in the latter are 20—23 μ, in the former are 18 to 23 μ; but whereas the hooks of the embryo are about the same length in the new species, i. e. about 20 μ, in D. tenuirostris they are but 7 μ. The neck is short, not long as in the last-named species, and the eggs are small, about 40—50 μ in diameter, and spherical in shape, not cylindrical as Krabbe[1] figures them, with a length of 85 μ. The hooks also differ in shape; those of D. tenuirostris have a much more strongly developed process corresponding with the inner limb of the forked base than occurs in D. hemignathi.

The new species, which I have named after its host, may be characterised as follows:

[1] 'Danske Selsk. Skr.,' Bd. viii, 1870, p. 249.

Drepanidotænia hemignathi, n. sp.

Length 1—2·2 centimetres; breadth, in the middle of the body, 2 millimetres. Head flattened and compressed, rostrum with a crown of ten hooks; each hook 18—23 μ in length, and with but a slight trace of the inner limb of the forked base. Neck short. The first segments are short, but they very soon (eighth or tenth) show traces of reproductive organs. Genital pore unilateral. The posterior limit of each segment is sharply defined, and forms an angle of about 45 degrees with the sides. Egg spherical, diameter about 40—50 μ. The three pairs of embryonic hooks measure about 20 μ each in length.

Habitat: Hemignathus procerus, Sandwich Islands, in the intestine.

Note 1.—In a paper which I published in this Journal[1] last year on Arhynchus hemignathi I stated that the parasites were found "adhering lightly to the skin around the anus." I had this description from Mr. Perkins, and I understood it to imply that the parasites were outside the body. In this I find I was mistaken; and fearing that others may be under a similar misapprehension, I am writing this note to say that they occur inside the body-cavity in the angle where the rectum joins the external skin.

Note 2.—Mr. Perkins has also given me two or three specimens of a tapeworm from a Loxops, sp. This bird, like the Hemignathus, is a member of the family Drepanididæ, which is confined to the Sandwich Islands. Unfortunately the specimens are without their head, and I am unable to identify them. They differ markedly from the Drepanidotænia described above.

The Zoological Laboratory,
Cambridge;
July, 1897.

[1] 'Quart. Journ. Micr. Sci.,' vol. xxxix, 1897, p. 207.

EXPLANATION OF PLATE 46,

Illustrating Mr. Arthur E. Shipley's paper "On Drepanidotænia hemignathi, a New Species of Tapeworm."

List of Abbreviations.

cut. = Cuticle. *dors. ex. can.* = Dorsal excretory canal. *ectod.* = Ectoderm. *lat. nerve* = Lateral nerve. *long. muscs.* = Longitudinal muscles. *Nerv. syst.* = Junction of nerve-cords in head. *ov.* = Ovary. *par. cell* = Parenchyma cell. *Proc. of ectod.* = Knob-like ends of ectoderm cells under cuticle. *rec. sem.* = Receptaculum seminis. *Ros.* = Rostellum. *tes.* = Testis. *ut.* = Uterus. *ven. ex. can.* = Ventral excretory canal. *ves. sem.* = Vesicula seminalis.

Fig. 1.—A view of Drepanidotænia hemignathi, × 15. The dark patches in the anterior two-thirds of the body are caused by the generative organs; in the posterior third they represent the eggs in the uterus.

Fig. 2.—An isolated hook from the rostellum, × 500.

Fig. 3.—A longitudinal section through the head, × 100. The rostellum, *ros.*, is retracted. The point of fusion of the two lateral nerves is shown at *nerv. syst.* The section passes between the suckers.

Fig. 4.—A transverse section through a mature proglottis, × 70.

Fig. 5.—A longitudinal section, somewhat oblique, showing the regular arrangement of the longitudinal muscles, × 50.

Fig. 6.—A longitudinal section through several mature proglottides, × 50. This shows the transverse connection between the two ventral longitudinal excretory canals and the transverse lines formed by the concentration of the parenchyma at the posterior end of each proglottis.

Fig. 7.—A view of the head in an expanded, flattened-out state, × 60.

Fig. 8.—A view of another head in a contracted, bunched-up condition, × 40.

Fig. 9.—A transverse section through the head, showing the ten hooks on the rostellum and the four suckers.

Fig. 10.—A portion of a proglottis, highly magnified to show the minute anatomy, × 450.

Spengelia, a New Genus of Enteropneusta.

By

Arthur Willey, D.Sc.,
Balfour Student of the University of Cambridge.

With Plate 47.

Part I.—Diagnosis.

From a rock-pool on the weather side of Lifu (Loyalty Islands) last year I obtained a single specimen of an Enteropneust living in company with Ptychodera flava,[1] which has proved, on examination, to constitute the type of a very distinct new genus.

Having been informed by Mr. J. P. Hill that he had received two kinds of Enteropneusta from Funafuti, one of which was Pt. flava, and the other a new species, I sent my material to him for comparison. Mr. Hill saw at once that my form was quite distinct from the Funafuti species, and he had the goodness to leave it intact until my return to Sydney.

The genera of Enteropneusta, as defined by Spengel, fall naturally into three groups, for which it is to be hoped Professor Spengel will shortly create family names.

Group I, including the genus Ptychodera, briefly characterised by the presence of an outer layer of circular muscles

[1] In a former paper on Pt. flava I suggested to add the name "caledonica" until Eschscholtz's species should be re-examined. Meanwhile Mr. J. P. Hill has informed me that the same species occurs at Funafuti.

Under these circumstances it will be well to cancel the name "caledonica," and if the form from the Marshall Islands turns out to be different, then its name, i. e. the name given by Eschscholtz, must be changed.

in the integument of the trunk, the occurrence of dorsal roots putting the fibrous layer of the collar nerve-cord in connection with the fibrous layer of the epidermis, and the presence of liver saccules, and of synapticula between the branchial bars.

Group II, including the genera Schizocardium and Glandiceps, characterised by the presence of an inner layer of circular muscles (inside the longitudinal layer), and by the occurrence of a long vermiform process extending forwards from the anterior end of the notochord or proboscis cæcum.[1]

Group III, including the genus Balanoglossus, characterised by the absence of circular muscles in the integument of the trunk, and by the absence of synapticula.

Spengelia belongs to the second of the above groups, but exhibits features which render it a remarkably synthetic genus.

The following table (compiled from Spengel) will suffice to show the relation of Schizocardium and Glandiceps to one another, and will assist in the appreciation of the characters of Spengelia.

Schizocardium.	Glandiceps.
1. Ventral septum of proboscis extends to anterior end of vermiform process of notochord.	Ventral septum of proboscis stops short at base of vermiform process of notochord.
2. Pericardial auricles well developed.	Pericardial auricles rudimentary.
3. Peripharyngeal spaces present.	Peripharyngeal spaces absent.
4. Synapticula present.	Synapticula absent.
5. Œsophageal portion of branchial sac reduced.	Œsophageal portion of branchial sac well developed.
6. Liver saccules present.	Liver saccules absent.
7. Medial gonads absent.	Medial gonads present.
8. Anterior, unpaired, post-branchial intestinal pores (Darmpforten) present.	Ditto.

[1] It would be desirable to translate the German word "Eicheldarm" in such a way as not to involve an abstruse morphological conception, which some authors object to.

9. Posterior, paired, præhepatic intestinal pores present.	Ditto.
10. Accessory genital pores present, which, when they occur laterad from the main series, perforate the longitudinal musculature.	Ditto.

Spengelia agrees with Glandiceps in the characters mentioned in the above table under Nos. 1, 2, 3, 5, 7, and probably 6.[1] It only agrees specially with Schizocardium in the possession of synapticula (No. 4 in above table), this being also a marked Ptychoderoid feature.

A further most interesting reminiscence of the Ptychodera type in the organisation of Spengelia is the occurrence of vestigial roots arising from the dorsal side of the collar nerve-cord.

Spengelia porosa, nov. gen. et sp.

External Form. [See Pl. 47, fig. 1.]

The proboscis was longer than the collar, measuring, when extended, 10·5 mm.[2] It was pear-shaped, and of a rich yellow colour. The collar measured 6·25 mm., and was coloured a rich orange, especially in the middle region, while the posterior region of the collar was whitish yellow. The rest of the body had a dull yellow colour.

The branchial region was 30 mm. in length, and in this region the body was quite cylindrical and faintly annulated. The post-branchial portion of the body, present in the specimen, measured about 20 mm.

Apart from the absence of genital pleura, Spengelia was readily distinguished from among the multitude of Ptychodera flava by the length of the proboscis and the bright orange-coloured collar.

But what at once distinguishes Spengelia from any other Enteropneust hitherto described is the occurrence on each side of the dorsal middle line of a series of deep dermal pits

[1] Unfortunately the post-genital region was lacking from my specimen.

[2] After preservation in a picro-acetic mixture the proboscis measured 5·25 mm., and the collar 4 mm.

in the post-branchial genital region. The mouth of each pit is about 1 mm. in diameter.

At a glance the pits appear to be regularly paired, but a re-examination has shown that they are not quite so regular as represented in fig. 1.

In the fresh condition the sides of the genital region of the body were occupied by elongated, somewhat pyriform bodies, which caused definite ridges on the external surface. These projections were caused by the gonads. The individual was a mature male.

The dermal pits lie in the submedian line, in direct continuation from the branchial groove, and the most anterior pits invade the posterior extremity of the branchial region, in consequence of which a number of the outer pores of the posterior gill-slits do not open near the surface of the body, but deep down at the base of the dermal pits.

The last newly formed gill-slit on each side opens directly from the gut into the base of a dermal pit; and at first I thought they represented the intestinal pores (Darmpforten) described by Spengel (discovered originally by Schimkewitsch). Under some circumstances it might be very difficult to distinguish true gill-slits from intestinal pores.

Spengelia differs from Schizocardium and Glandiceps in the absence of anterior intestinal pores. I am unable to say anything about the posterior intestinal pores, as my specimen was incomplete.

Transverse sections show that the dermal pits of Spengelia are remarkably deep, extending through more than half the thickness of the body, and actually branching amongst the gonads. They appear to serve for the irrigation of the gonads. If anyone saw a single section passing through the middle of a dermal pit, he would say that Spengelia possessed genital pleuræ. Perhaps these pits owe their origin to an incomplete fusion of genital pleuræ with the body-wall; or they may merely represent local depressions of the floor of a branchio-genital groove.

Apart from their connection with the gut by means of the

posterior gill-slits, the dermal pits do not communicate with the intestine, although they extend very near to the wall of the latter.

Internal Structure.

1. Vermiform Process of Notochord.—This is a long, generally solid cord of cells, lying in the centre of the proboscis, and surrounded by a stout limiting membrane, which serves for the insertion of the median dorso-ventral muscles of the proboscis. Its diameter is not quite equal throughout its course. It agrees closely with the corresponding process in Glandiceps, particularly in the fact that the ventral septum of the proboscis does not accompany it, as it does in Schizocardium.

2. Collar Nerve-cord.—One of the most interesting and generically important characters of Spengelia is the occurrence of vestigial dorsal roots. They do not reach the epidermis, nor do they contain fibres or "Punktsubstanz." Otherwise their similarity to the roots of Ptychodera is complete. I have seen two such roots in Spengelia. The anterior root is the longer, and it runs obliquely, so that it appears in several sections separate from the nerve-cord. It is mostly solid, but contains a few minute disconnected cavities. The posterior root is hollow and much shorter than the anterior root, so that it does not appear in section separate from the nerve-cord.

3. Splanchnic Nerve-fibres.—A rather puzzling feature in the anatomy of Spengelia is the occurrence of a layer of nerve-fibres (Punktsubstanz) at the base of the epithelium of the buccal or throat cavity. Anteriorly it is a thick layer, and it becomes gradually thinner posteriorly. It may be traced as a very thin layer for a long distance beyond the opening of the notochord into the buccal cavity, and even at the base of the epithelium forming the œsophageal portion of the branchial sac. The occurrence of this well-defined layer of splanchnic nerve-fibres round the throat and œsophagus alone distinguishes Spengelia from all other Enteropneusta.

4. Synapticula.—By preparing out a piece of the wall of the branchial sac I became aware of the presence of synapticula in Spengelia before seeing them in section (fig. 3).

5. Gonads and Genital Ducts.—In the branchial region gonads occur both medially and laterally, that is on each side of the branchial groove (fig. 2). Their ducts open at the lips of the latter. In the post-branchial region where the dermal pits occur, the genital pores are numerous, and are not confined to the submedian line, so that several genital pores may be seen in one section. Some genital pores open into the dermal pits, while others open directly to the exterior near the dorso-lateral margins of the body. But in Spengelia, contrary to what obtains in other Enteropneusta (with exception of Balanoglossus canadensis and of the mediad accessory pores of Schizocardium brasiliense), the accessory genital ducts and pores do not perforate the longitudinal musculature. In the post-branchial region of Spengelia there is a very wide interval between the dorsal longitudinal muscles and the ventro-lateral longitudinal muscles, and all the genital pores occur in this interval.

6. Miscellaneous.—With regard to other points, it is only necessary to mention here that Spengelia agrees with Schizocardium and Glandiceps in having an unpaired asymmetrical proboscis-pore. The canal (Eichelpforte) leading to the pore swells out into a large vesicle before discharging to the exterior, and at the base of the vesicle there are muscle-fibres presenting the appearance of a sphincter muscle.

Spengelia further agrees with Glandiceps in the massive development of chondroid tissue in the neck of the proboscis and in the length of the posterior cornua of the proboscis skeleton.

In the preserved condition the gill-pores were clearly visible at the base of the branchial grooves, as they are in Glandiceps talaboti.

7. Summary.—If it were not for the presence of vestigial roots in the collar nerve-cord, Spengelia (apart from its own peculiar features, e. g. dermal pits, splanchnic layer of

"Punktsubstanz," accessory genital pores not perforating the longitudinal musculature, &c.) might almost be defined as a Glandiceps with synapticula. Spengel specially mentions the absence of synapticula as indicating the primitive character of Glandiceps. In a recent paper in the 'Quarterly Journal of Microscopical Science'[1] dealing with impressions of Ptychodera flava derived from an examination of fresh material, I expressed the opinion that Ptychodera presented a more primitive type of organisation than the other known Enteropneusta. The discovery of this new genus, Spengelia, which has so many points in common with Glandiceps (see above), and yet which has synapticula between the branchial bars and vestigial roots arising from the collar nerve-cord, goes a long way to prove that Ptychodera is relatively primitive, and that Glandiceps and Balanoglossus are derived forms.

I hope to supplement the above account by a second part containing an illustrated description of the anatomy of Spengelia. What has been said is enough to establish the genus.

My work, so far as it has gone on the preserved specimen, has been carried out in Professor W. A. Haswell's laboratory at the University of Sydney. I made some sections of Ptychodera flava for purposes of control, but in addition I have had the advantage of examining Mr. J. P. Hill's beautiful preparations of three species of Ptychodera, besides having his opinion on various points in my own preparations of Spengelia, notably on the vestigial roots and the splanchnic layer of "Punktsubstanz."

Sydney; May 10th, 1897.

[1] Vol. 40, p. 165.

EXPLANATION OF PLATE 47,

Illustrating Mr. Arthur Willey's paper, "Spengelia, a New Genus of Enteropneusta."

Fig. 1.—Dorsal view of Spengelia porosa, after a sketch from the living animal, showing the dermal pits behind the branchial grooves. *p.* Proboscis. *c.* Collar. *d. g.* Median dorsal groove. *br. g.* Branchial or branchio-genital groove. *d. p.* Dermal pits.

Fig. 2.—Macroscopic section through the mid-branchial region of Spengelia, showing the medial gonads and the œsophageal groove. *m. g.* Medial gonad. *d. l. m.* Dorsal longitudinal musculature. *br. g.* Branchio-genital groove. *v. l. m.* Ventro-lateral longitudinal musculature. *l. g.* Lateral gonads. *œ.* Œsophageal groove. *br. s.* Branchial sac.

Fig. 3.—Portion of branchial skeleton of Spengelia, to show synapticula. From a preparation treated with caustic soda and mounted in glycerine. Zeiss, 3 A, cam. luc. *t. b.* Skeleton of tongue-bar. *s. b.* Skeleton of primary or septal bar. *sy.* Synapticula.

On a Prorhynchid Turbellarian from Deep Wells in New Zealand.

By

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With Plate 48.

In 1892[1] I announced briefly the discovery of this remarkable Turbellarian, which was found by Dr. Charles Chilton in deep wells in Canterbury, New Zealand, among other animals, chiefly Crustacea, of which he has since published a valuable account.[2] At that time the general examination which I had made of the specimens had led me to the conclusion that the new form found its nearest allies in the Alloiocœla. The series of lateral diverticula of the intestine, the complex structure of the pharynx, the entire absence of a body-cavity, and other features led me to take this view. A more thorough examination, however, with the aid of sections of better preserved specimens, has led to the result that, while having certain points of affinity with the Alloiocœla, the new form finds its nearest allies by far in the family Prorhynchidæ, and is in many respects closely related to the genus Prorhynchus, while presenting some remarkable features in which it differs not only from that genus, but apparently from all the rest of the Rhabdocœla.

[1] "Jottings from the Biological Laboratory of Sydney University, No. 17, Three Zoological Novelties," 'Proc. Linn. Soc. New South Wales,' 2nd series, vol. vii.

[2] 'Trans. Linn. Soc.' (2), vol. vi, pp. 163—284.

Of the four specimens received three are sexually mature, and agree with one another in all essential points; the fourth, somewhat smaller than the rest, differs from them in the rudimentary condition of the reproductive apparatus. In none of them is the state of preservation very perfect in all respects, and the following description is necessarily incomplete in a good many points. I am also unable, owing to a good many gaps in our Sydney scientific libraries, to attempt to deal in any complete manner with the literature.

The total length of the largest specimen is 2·5 cm., and the greatest breadth 4 mm. No pigment is present in any part, and there are no eyes. The body (fig. 1) is elongated, considerably depressed, thick in the middle where the alimentary canal and other organs lie, thin at the sides; these lateral thin regions assuming the character of thin solid longitudinal flanges. The anterior extremity is broad and truncate, the antero-lateral angles produced into small compressed projections; the posterior portion of the body tapers to a blunt point. The mouth is a wide aperture at the anterior end. The openings of two ciliated sacs (*c. s.*) are situated on the ventral surface, a little behind the antero-lateral angles. The two excretory apertures (*ex.*) are situated on the ventral surface, some distance apart, a little in front of the middle of the body. The female generative aperture (♀) is also situated on the ventral surface in the middle line, some little distance behind the excretory pores.

Integument, Integumentary Glands, Muscular Layers, and Parenchyma.—The specimens are not in a sufficiently good state of preservation to permit of a detailed investigation of the structure of these parts, and the following account is necessarily incomplete. The presence of vibratile cilia on the surface cannot be determined with certainty; but elevations of the integument, the arrangement and structure of which remain uncertain, bear, singly or in groups, long straight cilia, probably of a non-motile and sensory character. The cuticle (fig. 3, *c.*) is ·002 mm. in thickness; it does not appear completely homogeneous, but exhibits indications of

structure. It is perforated by innumerable minute openings—the openings of the ducts of the integumentary glands. In the epidermis, which is about ·01 mm. in thickness, nuclei, though they doubtless exist, are not to be made out with certainty in any of the series of sections. The rounded clear spaces, the presence of which is so characteristic of the epidermis of the Turbellaria in general, occur abundantly. A distinct, though thin basement membrane (*b.*) lies below the epidermis.

All over the surface there occur at fairly wide intervals (on an average 0·5 mm. apart) remarkable unicellular glands (*gl.*), which for the sake of distinction I will term the superficial integumentary glands. These are pear-shaped when seen in vertical section, but in transverse section often present a stellate appearance, owing to the presence of processes given off from the basal part; the total length, including the duct, is ·07 mm. The broad end lies altogether beneath the epidermis and basement membrane, in the zone of ducts, to be presently referred to, while from the narrow end a short duct pierces the integument to open on the surface. The most striking feature of these glands is the enormous relative size of the nucleus, which almost completely fills the cell, so that sometimes only a very thin layer of cytoplasm is distinguishable around it. This cytoplasm, prolonged into the narrow neck of the cell, is of a very finely granular character, without any appearance of reticular or other structure; it has become stained by the eosin in the sections, and the substance of the deeper or basal part of the cell is much more intensely stained than the rest.

Immediately below the basement membrane is a thin layer of circularly arranged muscular fibres—the outer circular layer (*e. c.*), and in immediate contact internally with this again is a muscular layer of approximately equal thickness in which the fibres run longitudinally, the outer longitudinal layer (*e. l.*). Between this and the inner layers of muscle is, over all parts of the body, a broad stratum—the zone of ducts—closely packed with the dilated portions of the ducts (*dct.*) of the deep integumentary glands. These are so closely placed,

and are so strongly coloured by the hæmatoxylin, that scarcely any intermediate substance is distinguishable. It is in this zone that the wide portions of the superficial unicellular glands lie embedded. Here and there occurs a cell of stellate shape. Below this zone is the inner circular layer of muscular fibres, which is much thicker than the outer; and below this again is the inner longitudinal layer, which is of great thickness, and extends uniformly over both surfaces.

The deep integumentary glands lie below the muscular layers, scattered through the parenchyma in all parts of the body. Their ducts, which frequently branch and perhaps anastomose, pass from them with a sinuous course, perforate the muscular layers and the layers of the integument, and open on the outer surface. Each gland consists of a single cell of about ·1 mm. diameter, of evenly rounded outline without processes, except where the duct is given off. Enclosing the cell is a distinct capsule, in which lies a flattened nucleus as large as that of the cell itself. The nucleus of the gland, situated towards the middle, always has a lobed outline, and contains about six spherical nucleolar bodies of about equal size. The cytoplasm exhibits a strongly marked reticulum, the threads of which have a prevailingly radiate arrangement. The interfibrillar substance appears clear and homogeneous in the great majority of the glands, but in many the cell is full of well-formed rhabdites. The ducts appear as tubes with well-defined walls. When they enter the zone of ducts they become dilated, contracting again as they pass through the superficial layers. The ducts of the glands which contain rhabdites also contain rhabdites, while in the interior of the ducts of those glands in which no rhabdites occur is a reticulum similar to that of the cell itself. Special strands of ducts of the deep integumentary glands run forwards to open about the anterior margins of the body, and very many open on the extreme lateral margin. The parenchyma completely and closely fills up the interspaces between the organs, and a cœlom, such as is described as being characteristic of the Rhabdocœles in general, is not in any way represented. The

parenchyma is apparently syncytial, with a coarse reticulum continuous from cell to cell, and large nuclei. Strands of muscular fibres (parenchyma muscle) pass through it, mainly in a dorso-ventral direction.

No account is given by v. Graff[1] or by Vejdovsky[2] of the integument in Prorhynchus, so that I have no means of comparing it with that of the form under consideration. The superficial integumentary glands apparently correspond to the cells described by Vejdovsky (l.c., p. 144) as occurring on the ventral surface of P. hygrophilus, and figured in fig. 76.

Digestive System.—The pharynx (fig. 1, *ph.*) is of great relative size (about one third of the entire body in length) and high degree of complexity. It is capable, as shown in one of the specimens, of protrusion to some extent through the aperture of the mouth, when the free margin appears irregularly lobed. Its sheath extends back only a short distance from its anterior end. Its form is that of a thick-walled cylinder somewhat contracted posteriorly. The layers of muscle in its wall (fig. 4) have the following arrangement, taken from without inwards:—(1) External longitudinal, (2) external circular, (3) internal circular, (4) internal longitudinal. Between the external and internal circular layers is a broad zone occupied only by glands, nerves, and radial and arched fibres. The radiating fibres run from the inner surface of the external longitudinal layer to the basement membrane; they divide the internal longitudinal layer (which lies immediately beneath the epithelium and basement membrane) into numerous very regularly arranged bundles. The arched fibres are all directed in the transverse plane, so that they only appear in transverse sections. They are arranged in bundles running with a strong curvature from the external circular layer to the deeper part of the internal circular. The pharyngeal unicellular glands lodged in the zone above referred to send narrow ducts inwards to open into the internal cavity of

[1] 'Monographie der Turbellarien,' I. "Rhabdocœlida," p. 264 (1882).

[2] "Zur vergleichenden Anatomie der Turbellarein," 'Zeitschr. f. wiss. Zool.,' lx (1891).

the pharynx; the openings of the ducts are most abundant round the anterior aperture. A complex system of nerves extend through the wall of the pharynx.

Von Graff[1] refers to the pharynx of Prorhynchus as belonging to the same type as that of the Plagiostomidæ, and in his account of P. stagnalis states that it has the outward form and probably also the internal structure of the "pharynx variabilis" of that family. He had, however, not examined sections. Vejdovsky[2] states that the order of the layers in P. hygrophilus is that laid down by v. Graff as diagnostic of the "pharynx doliiformis;" but the description which he gives and his figure 92 do not bear out this statement. The order of layers in the "pharynx doliiformis," as given by v. Graff,[3] is, from without inwards, longitudinal, circular, longitudinal, circular; whereas the order which Vejdovsky describes and figures is longitudinal, circular, circular, longitudinal, as in the form now under consideration, though all the layers are very feebly developed, being each only about one fibre in thickness. This order of the layers occurs in none of the described types—that in the "pharynx variabilis" being circular, longitudinal, circular, longitudinal, —and would appear to be characteristic of the family Prorhynchidæ.

The intestine extends to very near the posterior extremity of the body. Anteriorly it gives off on the ventral side a short and wide diverticulum, which passes forwards for a short distance below the posterior extremity of the pharynx. Laterally it is divided throughout its entire extent by constrictions brought about by ingrowths of the investing fibrous layer of the intestinal wall. Of these there are about forty on each side. On the right side they correspond very closely with the windings of the ovary. The epithelium of the intestine consists of extremely long narrow cells of very irregular shape, many of which are filled with the large granules so characteristic of

[1] Loc. cit., pp. 87 and 265.
[2] Loc. cit., p. 148.
[3] Loc. cit., p. 84.

the intestinal cells of Turbellaria in general. The epithelium is supported by a thin layer of a fibrous character, apparently muscular, containing numerous spindle-shaped nuclei.

Nervous System.—The brain (fig. 1) consists of two large ganglia situated near the anterior extremity of the body above the mouth, and connected together by a thick commissure; the nerve-cells are mainly confined to the ganglia, to which they form a thick investment. Given off from the brain on each side is a large nerve passing to the corresponding ciliated sac. Two main longitudinal trunks (figs. 1 and 2) are given off on each side posteriorly. One of these, which is much the smaller, runs in the lateral flange of the body.[1] It gives off regularly arranged transverse branches, of which those on the inner side join corresponding branches of the larger trunk, thus giving rise to a number of commissures connecting the two trunks together, while those on the outer side given off opposite the commissures run outwards towards the lateral margin, giving off branches to the integument. In addition to the commissures which connect it with the smaller trunk, the larger nerve-cord gives off on its inner side many transverse branches running in the ventral wall of the body; whether these form complete commissures was not ascertained. The ciliated sacs are two deep excavations, each situated just behind the corresponding anterior angle of the body, and opening on the ventral surface near the lateral border by a wide orifice. The cavity is lined with a layer of regularly arranged, large, columnar cells, beset at their inner extremity with long cilia. Internally and posteriorly the ciliated sac is prolonged into a narrow cylindrical tube, which, after receiving the ducts of a number of unicellular glands similar to those that discharge on the outer surface, ends blindly. In close contact with the columnar cells of the sac, and probably in continuity with them, is a layer of nerve-cells, processes from which go to form the nerve already referred to.

[1] The lateral cords have been made too thick in fig. 1, and made to run too far back.

Excretory System.—There are two excretory apertures (fig. 1, *ex.*) situated on the ventral surface, just behind the ventral diverticulum of the intestine, and immediately external to the main nerve-cord. Each leads into a tubular sac with thick walls, and lined with a thick layer in which nuclei are not visible, though doubtless it is of the nature of an ectodermal epithelium. Spaces similar to those in the epidermis occur at intervals; and in the interior of the sac is a quantity of fibrillated substance, which may partly be the remnants of cilia, though of this there is no positive evidence. From this excretory sac runs dorsad a sinuous canal, which bifurcates to form an anterior and a posterior longitudinal trunk. The former is the smaller. It runs forwards, external and dorsal to the larger nerve-cord, to the anterior extremity of the body, where in the neighbourhood of the ciliated sac it becomes somewhat dilated and much convoluted. Its continuation, or a branch, passes transversely in front of the brain, but is not traceable as far as the middle line. The posterior longitudinal trunk is very wide, and, like the anterior, twists about in a sinuous manner. Some distance back it bifurcates, the two branches running back side by side for some distance. Both anterior and posterior trunks give off numerous branches. At some points they seem to unite for a short distance and diverge again.

In the main the disposition of the parts of the excretory system resembles that of the corresponding parts in Prorhynchus, as described by Schultze, v. Graff, and others; but there are some important points of difference. The position of the external openings is the same in both, and in both the short vessel into which the aperture leads bifurcates to form anterior and posterior trunks; but in Prorhynchus the posterior trunk does not bifurcate, and an internal longitudinal vessel running through the entire length of the body is given off from a transverse commissural vessel situated far forwards.

Reproductive Apparatus.—The organs of the two sexes are both mature in three out of the four specimens. The male

aperture is a small slit situated in a recess below the pharynx, this recess opening on the exterior through the mouth. The penis (figs. 1, 5, and 6) is a long and slender, pointed, chitinous spine. Contained within the lumen of the penis is a finely fibrillated material, which stains faintly with eosin. Investing it is a layer of fine fibres, most of which take a spiral course. This layer is continuous at the base of the penis with the wall of the ejaculatory duct, while at the apex it is continuous with the penis sheath; between it and the penis on the one hand, and the penis sheath on the other, are several large nuclei. The sheath of the penis consists of an outer layer of circular fibres, and an inner of longitudinal; the latter is a continuous layer in the greater part of its extent, but divided towards the apex of the penis into a number of distinct bundles. Supporting the sheath are ten slender chitinous rods, which, where the sheath is reflected in front to become continuous with the investing layer of the penis, bend sharply backwards for some distance.

The ejaculatory duct presents a slight bulbous dilatation at the base of the penis. It is a thick-walled tube with a muscular wall, composed mostly of circular fibres. The penis sheath is continued over it for a short distance, but stops short completely before reaching the vesicula seminalis. The vesicula seminalis (fig. 1, *v. s.*) is a very large sac of long oval form. Its walls are thick and muscular, the fibres taking for the most part an oblique direction round the wall. In the interior is a thick layer of a finely fibrillated substance bounding a relatively narrow central lumen. At the junction of the ejaculatory duct and vesicula open the ducts of a number of unicellular glands, which probably are the prostate or granule glands. From the vesicula runs a very greatly coiled narrow duct, the vas deferens. This becomes somewhat thicker posteriorly, and eventually is continued into a wide thin-walled spirally twisted sac, the sperm reservoir (*sp. r.*)—completely filled, in all three mature specimens, with ripe sperms. From this a short, straight, narrow efferent duct leads obliquely towards the left, where it enters the testis, and is

continued as an exceedingly fine tube connecting together the lobes of that organ.

The above account of the efferent part of the male apparatus differs in certain important respects from the accounts that have been published of the structure of those parts in Prorhynchus. The exceptional position of the male aperture is shared with that genus and certain of the Alloiocœla. The form of the penis corresponds, to some extent, with that of Prorhynchus stagnalis as described and figured by v. Graff.[1] The chitinous lamellæ of the penis sheath which he describes obviously correspond to the chitinous rods referred to above. But if Hallez's statement be correct, that in Prorhynchus stagnalis the sheath is continued back as the wall of the true ejaculatory duct, which encloses an inner duct continuous with the wall of the penis itself and passing back to the "secretion reservoir," then there is at least one important point of difference.

The testis (figs. 1 and 2, *te.*) is a long narrow body extending from a little behind the posterior end of the pharynx on the left side of the intestine between it and the main trunk of the excretory system to near the posterior end of the body. It is divided into a large number—over a hundred—of small lobes connected together by the efferent duct and its branches. Investing the lobes is a thin layer of connective-tissue fibres.

The female (fig. 1, ♀) aperture is situated in or near the middle line on the ventral surface. It leads through a short passage lined with ciliated cells into a rounded chamber (vagina) lined with an epithelium, the cells of which are large and similar in general shape to those of the epithelium of the intestine, but much shorter. From this there runs forwards a wide pyriform chamber, which may be provisionally termed uterus (fig. 1, *ut.*). It has a wall composed of a fibrous layer and a layer of large cells similar to those lining the vagina. From the first-mentioned chamber the oviduct, a wide sinuous tube with a lining of somewhat flattened granular cells, passes backwards and to the right to become continuous with the

[1] Loc. cit., p. 265, Taf. xv, fig. 19.

vitello-ovary. The latter (fig. 1, *ov.*) is long and narrow, thrown into numerous sinuosities, and occupies a position on the right side of the intestine corresponding exactly to that occupied on the left by the testis, though it does not extend so far back as the latter, stopping short some distance in front of the posterior end of the intestine. It is enclosed in a fibrous layer similar to and continuous with that enclosing the intestine, and is lined internally with a layer of flattened granular cells. Its interior is occupied, for the most part, by vitelline cells or follicle cells. These are large cells arranged, for the most part, in an epithelium-like manner, but sometimes collected into more irregular clumps. Each contains a large nucleus, and each has a distinct enclosing membrane. Throughout the greater part of the length of the vitello-ovary the follicle cells are loaded with large rounded yolk-granules, but in the posterior portion these granules are entirely absent, and the cells are much smaller.

Here and there is an ovum (fig. 7). Each of these is a large rounded cell with a large nucleus and very fine-grained protoplasm, enclosed in a follicle of regularly disposed follicle cells. The ova are smaller and more numerous in the posterior part of the ovary than they are in front, and here the follicle cells that enclose each are numerous, and have the appearance of a columnar epithelium. Further forward, where the ova are large, the follicle cells that surround them are much smaller than the rest. Towards the posterior end of the vitello-ovary there is, in two of the three mature specimens, a great mass of sperms distending the cavity, the wall of which is here very thin; there is thus formed from a portion of the ovary a distinct, though perhaps temporary, bursa seminalis. Further back again the vitello-ovary resumes its normal character. In the sexually immature specimen the penis is completely developed, but the various parts of the male duct have not yet become formed. The testis is represented by a narrow duct connecting together a chain of small cavities. In these and in the lumen of the connecting duct are a number of cells of a peculiar character, having the appearance of malformed

sperms of various stages. From this runs forwards an irregular median channel without well-defined walls; this contains bodies similar to those in the testis itself, and is traceable as far forwards as the base of the penis. The female aperture is present; it leads into a cavity from which runs backwards on the right side a wide irregular channel representing the ovary, but containing no cells that are recognisable as follicle cells or ova.

The remarkable disposition of the reproductive organs described above is one which is not paralleled, so far as I can ascertain, in any other Turbellarian, and is perhaps sufficient in itself, apart from the other points of difference, to render necessary the separation of the present form from Prorhynchus as at least generically distinct.

In Prorhynchus stagnalis, according to v. Graff,[1] the testis is not sufficiently known; the female opening is in the middle of the ventral surface; the ovary is an elongated body in the posterior blind portion of which are numerous germs of ova, while further forwards are the ripe ova surrounded by yolk-cells. In P. sphyrocephalus the same author states that a similar condition of things obtains.

None of the specimens of Prorhynchus hygrophilus obtained by Vejdovsky had the testes developed. He assumes that they were in a degenerate condition, and that Prorhynchus is a proterandrous hermaphrodite. He adds, however, that from the statements of v. Kennel and Braun it may be looked upon as determined that the testes of Prorhynchus are developed as small rounded follicles on both sides of the intestine. Of these the latter author has described two or three pairs, while v. Kennel characterises them as vesicles sometimes in close contact with one another, sometimes separate, at first in a single series, later arranged in several irregular rows extending not quite as far as the posterior end of the body.

Vejdovsky had found only three such follicles, and these not in a state of functional activity, in the form of longish round

[1] Loc. cit., p. 266.

vesicles situated close to the sides of the intestine. The vitello-ovary consists in its posterior portion of indifferent reproductive cells; further forwards it contains a row of ova surrounded by yolk-cells.

Impregnation.—Embedded in the parenchyma, close to the ovary, not far from its posterior end in the specimen, without a bursa seminalis, is a chitinous tube, which is without doubt the remains of the chitinous part of the penis of another individual. This circumstance affords very strong evidence in favour of the conclusion that the sperms of one individual are conveyed into the interior of the ovary of another, to form the seminal bursa by the penis piercing the body-wall and penetrating to the ovary. The structure of the penis and vesicula seminalis would of itself suggest such a mode of copulation; the whole apparatus has exactly the form of a hypodermic syringe, with a compressible ball instead of a cylinder and piston.

A mode of copulation in which a wound is inflicted in any part of the body by the penis, and masses of sperms are discharged into the wound, was long ago described by Lang[1] as occurring in many Polycladida. But, so far as I am aware, this is the first recorded instance of the direct injection of the ovary with sperms through a perforation of the body-wall by means of the penis.

In the incomplete condition of our knowledge of the genus Prorhynchus it is somewhat difficult to give a diagnosis which will clearly define the new form. Von Graff's definition of Prorhynchus is as follows:—"Prorhynchida mit Wimpergrübchen, Mund am Vorderende des Körpers, ein chitinoses copulationsorgan vorhanden, Korper fadenförmig gestreckt."[2] In all of these points, with the exception of the last, which is not important, the new form agrees with Prorhynchus. But it differs from it apparently in several material points not included in v. Graff's diagnosis. Such are the more complex

[1] "Der Bau von Gunda segmentata, &c.," 'Mitth. ans der Zool. Stat. zu Neapel,' Bd. iii, p. 222 (1882); 'Die Polycladen,' pp. 231 and 636 (1884).

[2] Loc. cit., p. 263.

pharynx, the bifurcate posterior vessel of the excretory system, the rod-like chitinous supports of the penis sheath, the laterally placed vitello-ovary, and the unpaired laterally placed testis. In view, however, of the necessity for a more thorough knowledge of the structure of the described species of Prorhynchus in these and other respects, I think it best to regard the New Zealand species provisionally as a member of the genus Prorhynchus, and propose for it the name P. putealis.

EXPLANATION OF PLATE 48,

Illustrating Mr. William A. Haswell's paper "On a Prorhynchid Turbellarian from Deep Wells in New Zealand."

Fig. 1.—Semi-diagrammatic view of the organisation of Prorhynchus putealis as seen from the ventral aspect. × 10. *a. l. v.* Anterior longitudinal trunk of excretory system. *cæ.* Diverticulum at the anterior end of the intestine. *com.* Commissure between the brain ganglia. *c. s.* Ciliated sac. *ej.* Ejaculatory duct. *ex., ex.* Excretory apertures. *int.* Intestine. *l. n*^{1}. Large longitudinal nerve-cord. *l. n*^{2}. Smaller longitudinal nerve-cord. *od.* Oviduct. *ov.* Ovary. *ph.* Pharynx. *s. b.* Bursa seminalis. *sp. d.* Anterior portion of sperm-duct. *sp. d*^{1}. Posterior portion of sperm-duct. *te.* Testis. *ut.* Uterus. *v. s.* Vesicula seminalis. ♀. Female aperture.

Fig. 2.—Semi-diagrammatic representation of a transverse section of the body in the middle of the intestinal region, to show the relations of the various organs. × 30. *ex.* Excretory vessels. *int.* Intestine. *l. n*^{1}. Large longitudinal nerve-cord. *l. n*^{2}. Smaller longitudinal nerve-cord. *ov.* Ovary. *te.* Testis.

Fig. 3.—Section of the integument. *b.* Basement membrane. *c.* Cuticle. *ep.* Epidermis. *e. c. m.* External circular layer of muscle. *e. l. m.* External longitudinal muscular layer. *dct.* Ducts of deep integumentary glands. *gl.* Superficial integumentary gland.

Fig. 4.—Portion of a transverse section of the pharynx. *e. c.* External circular layer. *e. l.* External longitudinal layer. *ep.* Epithelium. *gl.* Glandular zone. *i. c.* Internal circular layer. *i. l.* Internal longitudinal layer. *r.* Radial fibres.

Fig. 5.—Ventral view of the penis and its sheath reconstructed from horizontal sections. *c. m.* Circular layer of muscle of penis sheath. *ej.* Ejaculatory duct. *l. m.* Longitudinal layer of penis sheath. *p.* Chitinous tube of penis. p^1. Investing layer of penis. *pr.* Protractor muscles. *r.* Chitinous rods. *x. x.* Line of section of fig. 6.

Fig. 6.—A transverse section of the penis and its sheath at about the line marked *x. x.* in Fig. 5. Lettering as in Fig. 5. *sh.* Portion of the sheath reflected on the penis at the anterior end.

Fig. 7.—Section of vitello-ovary behind the vitelline region. *fol.* Follicle cells. *in.* Investing fibrous layer. *ov.* Ovum.

Note on the Development of the Atrial Chamber in Amphioxus.

By

E. Ray Lankester, M.A., LL.D., F.R.S.

Owing to the promotion of my friend Professor MacBride to a post in the University of Montreal in Canada, it has not been possible for me to confer with him personally as to the significance of certain passages in his interesting and valuable memoir on "The Early Development of Amphioxus" published in the present number of the Journal. I must therefore take the passages in question as they stand, and deal with them in print.

Professor MacBride has made the interesting discovery that the lymph-canals in the metapleura of Amphioxus are prolongations of the pair of cœlomic pouches which immediately follow the head cavity (bifid in its later growth). He identifies them with the collar cavities of Balanoglossus, and sees in this relation a confirmation of the view, advanced originally by Bateson, that the atrial chamber may be considered as morphologically identical with the small region overhung by the free posterior margin of the collar of Balanoglossus, and the wall enclosing the atrial chamber with the free projecting ring of the Balanoglossus collar. Whilst I by no means deny that there is a relationship between the metapleura of Amphioxus and the collar of Balanoglossus, it appears to me that MacBride has been led by theoretical bias into a serious misapprehension of the significance of the observations on the development of the atrial chamber in Amphioxus published by me in conjunction with Dr. Arthur Willey in 1890.

In referring to those researches Professor MacBride says (p. 591), "Lankester and Willey's paper on the development of the atrial chamber confirms in most points Kowalevsky's statements." Later (p. 605) he says, "Kowalevsky was the first to discover that the atrial cavity was formed by the meeting in the mid-ventral line of two long ridges or folds." And further, "Lankester terms the folds which actually wall in the atrial cavity 'epipleural,' and the projecting angles after these folds have united 'metapleural.' I shall use the term 'atrial fold' to include the whole, of which both are parts."

It appears to me that both in his references to Kowalevsky's observations and in his iteration of the word "folds" and proposal to call what I had called epipleur and metapleur by the term "atrial fold," Professor MacBride is in error, and that his statements and use of terms are inconsistent with the facts demonstrated by Willey and myself.

The region of the body of Amphioxus termed "epipleur" by me (at a time when I accepted Rolph's theoretical scheme of its development based on Kowalevsky's observations, and now shown to be erroneous) includes the whole of the atrial wall on each side from the level of the dorsal artery to the median ventral raphe. It seems to me absolutely unjustifiable to speak of this as a "fold" since my paper of 1890, the more so inasmuch as the erroneous view of its origin (that of Rolph) was that it arose as a horizontal down-growing fold. To call it and the metapleur resting on it "the atrial fold" at the present day, as Professor MacBride does, is simply to ignore Willey's and my results, and to perpetuate error.

It seems that Professor MacBride has somehow forgotten what Willey and I actually showed, since he declares that we confirmed Kowalevsky, and further that Kowalevsky discovered that the atrial cavity was formed by the meeting in the mid-ventral line of two long folds.

As a matter of fact, Willey and I did not confirm Kowalevsky. The whole point of our paper lay in a correction of a misinterpretation made by that most distinguished observer.

So far from confirming the existence of "folds," or admitting anything which Professor MacBride can justifiably term "atrial folds," we showed that there are no atrial folds. We showed that what Kowalevsky had mistaken for "atrial folds" are really the metapleura, and that these do not grow round and meet in the middle line, but that a very small in-sinking is formed between them, and is covered in by a minute horizontal growth right and left which we called the "subatrial ridges or folds," their union resulting in the formation of what is, at first, a very narrow "subatrial floor" lying between the two upstanding metapleura. We showed, then, that "the two long folds' of Kowalevsky, quoted by MacBride as though it were established that they are the rudiments of the epipleura (as in Rolph's scheme) do not form the atrial cavity, but are really the metapleura. MacBride says we confirmed Kowalevsky, and that Kowalevsky "discovered," the mode of formation of the atrial cavity. He did not do so, but, on the contrary, was misled as to the nature of what he thought to be coalescing folds. And Willey and I did not confirm him, but discovered a totally different mode of formation.

As to the continuity of the cavity of the metapleura with the cœlom, Willey and I showed that the space in the metapleura is not at any time freely open above into the adjacent cœlomic cavity, as was figured by Kowalevsky, who mistook the metapleural cavity for the lateral division of the cœlom, in which the gonads develop. We reproduced Kowalevsky's figure, and showed that it was defective. MacBride confirms us in denying a continuity of the lymph-space of the metapleur with the adjacent cœlom. We suggested, with reserve and caution, that the lymph-space of the metapleur might form as "pseudocœl,"—that is, as an intercellular space,—having no relation to enteric pouches. MacBride has now shown that the most anterior pair of enteric pouches (the collar-pouches) are the sources of the lymph-spaces in the metapleura. This is certainly altogether a different relation to that indicated by Kowalevsky's transverse section (fig. 1, p. 449, of Willey's

and my paper, 'Quart. Journ. Micr. Sci.,' 1890, vol. xxxi) and in no way justifies MacBride's implication that Kowalevsky was right in considering the metapleural canals as cœlomic in nature, and that Willey and I were wrong in denying their connection with cœlom.

I will conclude this note by a quotation from the paper by Willey and myself above cited (p. 455), in which the difference between the actual state of things made known by us and the theory of "atrial folds" so strangely resuscitated by MacBride, is set forth.

"It is important to point out that the mode of formation of the atrium as a narrow groove, which closes and sinks (as it were) into the body of the Amphioxus, is really different in important respects from the enclosure of a space by down-growth of large folds, though ultimately, no doubt, the two contrasted modes of formation come to the same thing, so far as the more obvious morphological relations are concerned. The mode of formation which really occurs in Amphioxus is readily harmonised with the existence of the post-atrioporal extension of the atrium, which gradually tapers to a fine cæcal canal. It also gives us an essentially different view of the region called "epipleur" by Lankester, and generally so designated, from that which Rolph's theory necessitated. That portion of the epipleur into which the myotomes of the body-wall extend is seen now to be no downgrowth, no extension or fold. It is the original unchanged body-wall, which bounds the sides of the animal's body in front of the atripore, just as much as it does behind. The only new growth in the atrial region which takes part in the limitation of the surface is the subatrial growth formed by the two little horizontal folds which floor in the atrium when it is a mere canal. These in the adult are represented by the region of longitudinally pleated ventral wall between the two metapleura."

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NEW SERIES.

PRINTED BY ADLARD AND SON,
BARTHOLOMEW CLOSE, E.C., AND 20 HANOVER SQUARE, W.