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Outlines of the Development of the Tuatara, Sphenodon (Hatteria) punctatus.

By

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With Plates 1—10.

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1. Preface.

Shortly after my arrival in New Zealand my friend Professor G. B. Howes, LL.D., F.R.S., urged me to undertake the investigation of the development of the Tuatara (Sphenodon punctatus), but for various reasons I decided at that time not to do so. Some time afterwards, however, while examin-

ing the parietal eye in some embryo Australian skinks, I was struck with the existence of an optic cup, apparently similar to that of the paired eyes. This seemed so startling that I resolved to attempt to obtain Tuatara embryos for the purpose primarily of studying the development of the parietal eye.

Knowing that Stephens Island, in Cook Straits, on the recommendation of the Australasian Association for the Advancement of Science, had been proclaimed a reserve for the Tuatara, it occurred to me by a happy inspiration to address a letter to the lighthouse keeper, then entirely unknown to me even by name, asking his assistance in the matter. I wrote to him first on July 3rd, 1896, and most fortunately I found in Mr. P. Henaghan, the principal keeper on Stephens Island, an ardent enthusiast, who threw himself heart and soul into the work in the interests of science; and I cannot sufficiently express my gratitude to him for the magnificent supply of material which he obtained for me, and also for the valuable information which he gave me from time to time in his interesting letters regarding the habits of the Tuatara.

Stephens Island being now a reserve, it was of course necessary to obtain permission from the New Zealand Government to collect, and this, through the kind assistance of Sir James Hector, F.R.S., was successfully accomplished. To the Hon. the Colonial Secretary and to Sir James Hector I also wish to express my gratitude for their courtesy.

I had proposed visiting Stephens Island myself during the breeding season, which was supposed to begin about January, but pressure of other business made it very difficult for me to do so, and I ultimately resolved to trust entirely to Mr. Henaghan for the supply of eggs. There is regular communication between Stephens Island and the mainland only once in six weeks, so that considerable difficulty and delay were experienced in sending the eggs, in consequence of which a considerable number perished. Several lots of the eggs were sent packed in moss or lichen in tin cans, but they were very liable to go bad in this packing if delayed

on the voyage, and subsequently we found that much the best way was to pack them, only a few together, in tin cans filled with the coarse brown sand which occurs on the island. Thus packed they travel admirably. I found it quite possible to keep the eggs developing, buried in damp sand after their arrival, but this was attended with considerable risk, as they are very subject to the attacks of mould if kept too damp and without sufficient ventilation, while they readily shrivel up if allowed to get too dry.

The first consignment of eggs was received about the end of January, 1897. Unfortunately the embryos all died, apparently from drying up of the eggs, before they reached Christchurch. Nevertheless one advanced embryo, the only one as yet obtained of Stage Q, was removed from the egg in sufficiently good condition to be of considerable value.

I obtained no more eggs that summer, my arrangements being interfered with by a German collector, who visited Stephens Island about the new year, having obtained a recommendation to the keeper from the authorities. The only other eggs found that summer were forwarded to him, but I am informed that they perished in transit.

The next eggs which I received arrived, greatly to my surprise, in July, 1897, a season of the year at which we did not at all expect them. They were only three in number, and contained very advanced embryos (Stage R) in excellent condition.

In November, 1897, the work began in earnest, and from that time to the present I have received consignments of eggs at more or less frequent intervals. Latterly I have requested Mr. Henaghan, when he finds a nest, to keep the eggs on the island, to continue their development, and send them on to me at intervals in small quantities, a plan which we find to work admirably, and which I regret did not occur to me before, as all the embryos thus obtained have been in much the same advanced stage in development, and some more of the earlier stages would have been more useful.

The result of our operations, however, has been to secure a magnificent series of embryos, the majority of which have been

hardened in Kleinenberg's picric acid after removal from the egg, and preserved in alcohol. Those which were required for sections were stained with borax carmine, and cut in paraffin with Jung's sliding microtome. I found it desirable to use oil of cloves for clearing the embryos previous to embedding in paraffin, as this allowed of more advantageous examination of them as transparent objects than the turpentine method which I usually employ.

It would, of course, be impossible in the time which I have had at my disposal to give anything like a complete account of the development of the Tuatara; and in this communication I propose only to give a general outline, with special reference to the formation of the germinal layers, the fœtal membranes, the modelling of the body, the foundation of the principal systems, and the classification of the embryos into stages; which will, I hope, be useful in future investigations either by others or by myself.

This general account may be followed by more special memoirs dealing with the development of the particular organs. Already, indeed, I have sent home for publication one such memoir "On the Development of the Parietal Eye and adjacent organs."

In concluding this preface I must express my sincere gratitude to Professor G. B. Howes for his kindly interest and encouragement, and for revising the proof sheets, and superintending the execution of the plates in my absence from England.

At my request he has undertaken to investigate the development of the skeleton, and for this purpose I am forwarding to him a supply of material.

2. Introductory Remarks.

(*a*) On the Habits of the Tuatara.

Since I have not myself visited Stephens Island, it will be as well to give the following account of the habits of the Tuatara as nearly as possible in Mr. Henaghan's own words.

"Our island," writes Mr. Henaghan,[1] "is densely covered with scrub of various kinds; the soil is good in most places, especially on the ridges; in the gullies the soil is of a light brown colour, largely composed of oxide of iron. The birds and lizards burrow into this soft soil, and one can often find both living peaceably in the same hole. There are three or four kinds of petrel frequenting the island, and if you were here now, which is their breeding season, you would be surprised at the numbers of them,—there is hardly a foot of soil but is undermined with their holes. Insects of various kinds are also well represented, and I think the lizards feed largely on them, especially beetles. I believe they also eat young birds,—in fact, I have seen them do it.[2] They, however, live a large part of the year without any food, keeping constantly to their holes. There are three or four kinds of lizards here altogether, the Tuatara being the largest; the others are very small. There have never been many lizards' eggs got here yet, though the Tuataras are very numerous."

The breeding season apparently commences in November on Stephens Island, and each female lays about ten (10) eggs.[3] On November 1st, 1897, Mr. Henaghan removed ten eggs from an individual which had probably been accidentally crushed by a cow. These eggs, which were forwarded to me, were apparently quite ready to lay, but in the one which I opened on their arrival I found no embryo, and the others unfortunately went bad subsequently, instead of continuing their development, as I hoped they might.

The ground, Mr. Henaghan informs me, is so full of holes that one has to dig ever so many before finding a Tuatara, and then the probability is that it will turn out to be a male, these being far more numerous than the females. The females are much smaller than the males, but otherwise they do not appear to be distinguishable externally.[4]

[1] Letter dated October 28th, 1896.

[2] Compare Thomas (1) for a similar observation.

[3] Probably up to fifteen sometimes (cf. infra).

[4] According to Thomas (1) the male has the crests on the neck and back far more strongly developed.

On December 11th, 1897, Mr. Henaghan wrote me:

"I was out to-day searching for eggs, and I discovered two nests. One of them was probably laid late in the last season, as I noticed the embryos were fully developed. This I discovered through one of the eggs getting broken, and the other three I have sent you. You will observe that the advanced ones are much larger than the new ones,—in fact, double the size.[1]

"There were a lot more eggs in the same nest, but they were shrivelled up and consequently no use. I think it takes them several months to develop to maturity. All the eggs we found this season, with the above exception, were in the earlier stages of development. I notice a good many of the eggs are shrivelled up, owing to the dry state of the ground. We are having a long spell of drought just now, and I am afraid if it continues much longer a lot of the eggs will perish. It is only fair to give the lizard credit for a large amount of sagacity in the way she selects places for depositing her eggs, for it must be observed that there are plenty of enemies to contend with; as I have before mentioned, the birds are very numerous, and continually scratching out holes. The lizard, as a rule, shares the same hole with the bird, but never lays her eggs there, so that when searching for lizards' eggs one has to select a place with a sunny aspect and free from birds' holes."

In reply to questions as to the holes in which the eggs are laid, I was informed as follows:—"First, the holes are small cavities made chiefly in the surface soil; the entrance is about one and a half or two inches vertical height, and about three inches horizontal width. The chamber is continued at about these dimensions for five or six inches into the ground on a level surface, when it is then slightly increased to receive the eggs. The eggs are packed close together in layers of two or three, and owing to expansion in developing,[2] it is with dif-

[1] It is doubtful if the size of the egg has anything to do with the state of development of the embryo. This question is discussed subsequently.

[2] See previous note.

ficulty the eggs can be got out of some of them without injury. The eggs, in the majority of cases, must be laid outside of the chamber and carried in by the lizard, either by its mouth or claws, and placed in position; not a vacant space is left in the cavity where the eggs are deposited. The most of the nests found were only a few inches underneath the surface. The eggs are covered up with the soil removed from the chamber in excavating, and well pressed in on the eggs, then the entrance to the hole is stuffed with grass or leaves, and left to appear as like the surrounding locality as possible."

"A few nests have been found at the extreme end of tunnels extending into the earth for a distance of two or three feet. The main tunnel is used by the lizard as a place of abode, and is left open at the entrance. At the extreme end a small chamber is scratched out at right angles from the main tunnel, and in this are the eggs packed and covered up with soil. The eggs in this case were probably laid in the main chamber and then conveyed to the nest, as described in the first kind of nests. The soil where the eggs were deposited is chiefly composed of a mixture of clay and sand, but a good many of the nests were found in surface soil, and, as before stated, only a few inches in the ground. I may, however, say that no nests were found in loose soil, and a very favourite place for them is underneath a footpath. The ground being hard on top would no doubt cause the rain to run off in the winter. From ten to fifteen eggs is the average number found in any nest. In the deep holes some of the eggs were scratched outside the entrance, and it was owing to this that these were found. If you will picture to yourself the slope of a hill, you will easily understand the above description of their operations. I very much regret that your suggestion about retaining some of the eggs in the nests for further development did not reach me sooner; some of the nests, at all events, could be dealt with in the manner you describe."

On February 4th, 1898, I received another letter from my indefatigable correspondent, containing most valuable information. He tells me that they had been searching all the cleared

places on the island nearly every day since the first of January, and were beginning to give up all hope of obtaining any more eggs for this season, when it occurred to him to try and find out where the Tuataras laid their eggs before any clearings were made for lighthouse purposes. "Remember," writes Mr. Henaghan, "that all the eggs found previously were got on the clearings made for tracks, &c. In order to solve this problem I went down a steep cliff about 400 feet, or in other words about 200 feet above the sea. There is no vegetation in this place, and to-day I started grubbing out some loose earth; to my joyful surprise I found some eggs, good ones. There was plenty of evidence that they used to lay here in years gone by, for there were a lot of old egg-shells in the soil. This place was so steep that it was with difficulty I could keep my footing while working. Now I intend to keep these eggs as you proposed in a previous letter; I have put them in a hole in the ground close to my house, and in as favourable a situation for their development as there is in the island. I will be able to examine them occasionally, and at the same time send you a few every time there is a chance."

I may conclude these quotations from my correspondent's interesting letters with the following important note:—"On the 12th November one of my assistants excavated a track on the side of a slope leading down to a sheep-pen. In making this track he evidently cut into a lizard's nest, but did not notice it at the time. One day about the middle of January, when we were carrying a sheep up this track to be slaughtered, one of my children noticed an egg sticking out at the side of the cutting. On examination it was seen that a nest had been there. Some of the eggs, those that were farthest in, were empty, showing that the young lizards had escaped, while the eggs that were exposed to the sun had the skeletons in them. It is therefore quite plain that the eggs take about twelve (12) months to develop, and it is with the object of proving this that I send you the samples, hoping they may be useful."

One of the most important facts brought out by the valuable observations of Mr. Henaghan, above quoted is that the

Tuatara lays its eggs in special holes carefully concealed, and not as a rule in its ordinary dwelling-holes. This fact doubtless accounts for the failure of other collectors to find the eggs. As far as I know Mr. Henaghan is the only collector who has yet obtained the eggs in the natural breeding-places.

(*b*) On the Time occupied in Development.

I quite agree with Mr. Henaghan that the eggs take at least twelve months to develop,—indeed, I think they take rather more, being laid in November of one year and hatched about December of the year following. With a view to establishing this belief I subjoin the following table, in considering which it should be noted that those embryos which have not yet been stained and cleared are only referred approximately to their respective stages, while of course the different stages are connected by intermediate ones, so that it is sometimes a mere matter of choice to which stage a particular embryo should be referred.

It must also be noted that, in the earlier stages of development at any rate, the sequence of events is not always quite the same. Thus a feature which appears comparatively late in one embryo may appear comparatively early in another; and this inconstancy greatly increases the difficulties of classification.

The classification is, however, quite sufficiently accurate to establish a most remarkable coincidence between the sequence of dates and that of stages in development. The eggs were numbered consecutively as they came in, and the numbers missing from the following list belong to eggs which, for one reason or another, yielded no embryos. Except where otherwise stated, the date given is that on which the egg was opened.

List of Embryos obtained, classified in Stages and arranged in Chronological Order.

Dates.	Stages.	Numbers.
End of January, 1897	Q	1
„ July, 1897	R	2, 3, 4
(Removed from female on November 1st, 1897)	(Ready for laying)	(28 to 37)
November 22nd, 1897	C	5, 6, 7, 8, 9
December 9th, 1897	D	58
	E	56, 64
	F	61
	G	59
	J	40, 42, 43, 44, 46, 60, 62, 63
	K	38, 39, 41
December 10th, 1897	F	72
	J	14 ?, 73, 74, 75
	K	45
December 16th, 1897	H	78
	J	48, 79
	K	49, 80
	L	47
December 27th, 1897	L	50, 82, 84
	M	81
(Died between Nov. 12th, 1897, and middle of January, 1898)	S	149, 150
January 3rd, 1898	M	51
January 6th, 1898	N	93, 94, 95, 96, 97
	O	89, 90
	S	138, 139, 140 (all arrived dead)
January 10th, 1898	O	92
January 25th, 1898	O	103
	P	87, 106 ?
March 8th, 1898	R	141, 142
March 9th, 1898	R	143
March 12th, 1898	R	144, 145, 146
March 14th, 1898	R	147, 148
April 5th, 1898	R	151 to 158
May 12th, 1898	R	159, 160, 161
June 14th, 1898	R	169, 170
June 24th, 1898	R	162

I think that a careful examination of the foregoing table, taken in conjunction with the observations of Mr. Henaghan already quoted, establishes satisfactorily the following conclusions:

(1) On Stephens Island the Tuataras lay their eggs in November, and probably only at about this time.

(2) The eggs take about thirteen months to develop, during

which period it appears that a considerable number perish naturally from drought.

(3) The earlier stages in development are passed through much more rapidly than the later, and, Stage R having been reached in March, the embryo makes little further progress during the winter months, and does not hatch until about the middle of the following summer (January).

There is only one other animal known to me, the eggs of which take such a long time to develop, from the time of laying to the time of hatching, and that is the oviparous Victorian species of Peripatus, described by me under the name of Peripatus oviparus (2).[1] As I have noticed elsewhere, an egg of this animal which was laid in my vivarium at Melbourne took no less than seventeen months to develop before hatching. Sphenodon and Peripatus are both doubtless extremely ancient types, which have persisted with but little modification for a very long period, and one is strongly tempted to connect this fact with the extraordinarily long period occupied in their development from the egg.

(*c*) On the Structure of the Egg.

The eggs of the Tuatara are oval in shape, usually about equally rounded at the two ends, but sometimes rather narrower at one end than at the other. They vary very considerably in dimensions,[2] and the size of the egg seems to bear no relation to the stage of development of the contained embryo. Thus, taking the measurements of eight eggs numbered consecutively as they came in, and all containing embryos belonging to the same stage (R), I found them to be as follows :

[1] Compare the observations of Mitsukuri and Ishikawa (5) on the similar variation in size of the eggs of Trionyx.

[2] Cf., however, a note in 'Nature' (vol. lviii, p. 619) by Mr. G. A. Boulenger, F.R.S., on the embryo of Emp. orbicularis, contributed while this paper was in the printers' hands. Although most probably due to a different cause, the "suspended gestation" of the roe deer may be cited as a kindred phenomenon (Bischoff, 'Entwick. des Rehes. Giessen,' 1854, and the 'Zoologist,' 1889, p. 86).

Number 141, size 31 × 23·5 mm.	Number 145, size 24 × 21 mm.
„ 142, „ 28 × 21·5 „	„ 146, „ 28 × 21·5 „
„ 143, „ 27 × 22·5 „	„ 147, „ 33·5 × 22·5 „
„ 144, „ 33 × 22·5 „	„ 148, „ 30·5 × 23 „

Eggs containing embryos of other stages vary within about the same limits, and it will be seen that their measurements agree closely with those of Thomas (1), who gives the length as varying from 2·5 to 3·35 cm.

The colour of the eggs is dirty white, stained and mottled with brown by the brown sand or earth in which they are deposited.

The shell is flexible, tough, and elastic, rather rough externally, and contains in its outer portion a considerable amount of calcareous matter. This calcareous deposit occasionally gives rise to beautiful dendritic or stellate crystallisation patterns on the surface of the egg. The inner surface of the shell is smooth and white, and faintly opalescent. There is no distinct shell membrane, but the shell itself can be readily separated into layers which can be peeled off from one another.

In the recently laid egg the yellow yolk almost completely fills the shell. Thus the blastoderm when formed is separated from the shell by only a very small amount of watery material. Indeed, it is very difficult to open the shell without at the same time piercing the blostoderm and allowing some of the yolk to run out. I have, however, succeeded in puncturing the shell so that only the clear liquid, evidently corresponding to the "white" of a hen's egg, escaped. At a much later stage in the development there is a very large quantity of clear semi-gelatinous material present, which escapes when the egg is opened. This, though closely resembling the "white" of a hen's egg, does not at all correspond to it, but lies inside the allantois, the outer layer of which, being pressed close against the shell, is almost unavoidably punctured with the latter. No air-chamber is present even at a late stage in the development. This fact was specially determined by opening eggs of Stage R under water.

I have not been able to detect any vitelline membrane; at the earliest stages observed (*c*) it appears to have already disappeared, or become fused with the blastoderm.

The yolk, lying within the blastoderm, is of the usual yellow colour. In the earlier stages of development (*c*) it appears to be pretty uniformly soft and liquid, except where it is adherent to the under surface of the blastoderm. It is full of large transparent spheres and small granular spheres, floating freely. The small granular spheres nearly all contain bodies which may be termed "crystalloids" (fig. 107); otherwise they closely resemble the yellow yolk-spheres of the hen's egg. The crystalloids are clear, transparent, rod-shaped or spindle-shaped bodies with truncated ends. The sphere appears to be at first simply granular, and about 0·029 mm. in diameter (fig. 107, *a*); then the crystalloid appears within it (fig. 107, *b*), and gradually increases in size, stretching the sphere as it grows, while the granules of the latter gradually disappear until finally the crystalloid is left without any trace of the original sphere (fig. 107, *c*). Occasionally two crystalloids appear in the same sphere (fig. 107, *d*); they may then give rise to irregular four-sided bodies (fig. 107, *e*). I am inclined to think that ultimately the crystalloids lose their form, and run together to constitute the large transparent spheres which I regard as oil globules. Already at Stage C the crystalloids in some parts are aggregated apparently around the large transparent spheres, this aggregation appearing to take place first just beneath the blastoderm, where the yolk is already to some extent coherent.

As development proceeds the absorbent blood-vessels dip into the yolk from the yolk-sac, and the large transparent spheres, each surrounded by a layer of crystalloids, become attached to these vessels like onions on a string. This ropy or radially columnar character of the yolk in the later stages of development is very striking even to the naked eye (fig. 106).

The closeness of the blastoderm to the shell, and the elastic inward curling of the latter as soon as it is cut through, render the successful removal of the embryo a matter of considerable difficulty in the earlier stages of development. The embryo is sometimes found adhering to the inner surface of the part of the shell removed, and its remarkable transparency in the earlier stages makes it still more difficult to manipulate.

3. Systematic Account of the Stages in Development.

Stage C[1] (figs. 1—5).

This is the earliest stage which I have yet obtained, and to it I assign embryos numbered 5, 6, 7, 8, 9. The eggs from which these embryos were removed were collected on Stephens Island on November 14th, 1897, and the embryos were removed by me on November 22nd, the day on which I received them. They were therefore at least eight days old.

Already the blastoderm appears to have spread completely around the yolk, the epiblast forming over the greater part of its extent a thin transparent membrane, to the naked eye resembling a vitelline membrane, but composed of a layer of polygonal, nucleated, flattened, or in some parts columnar cells. This blastoderm lay so near to the shell that I found it impossible to open the egg without puncturing it and allowing some of the very liquid yolk to escape.

Beneath the superficial epiblastic layer of cells, and lying in the outermost part of the yolk, are numerous nucleated stellate cells which form a loose network holding the outer part of the yolk together, and causing it to adhere to the under surface of the epiblast, whereby the blastoderm outside the area pellucida acquires a thick, blanket-like character. These cells beneath the superficial epiblast may be termed "lower-layer" cells (fig. 4, *L. L. Y.*).

Over a certain area, corresponding approximately to the area pellucida, a large cavity has made its appearance in the lower layer; this is the segmentation cavity (fig. 4, *S. C.*), whereby the true embryonic portion of the blastoderm is widely separated from the underlying yolk. The floor of the segmentation cavity is formed by a very thin and delicate membrane, which I propose to call the sub-embryonal membrane (fig. 2, *S. E. M.*, and fig. 4, the dotted line). The sub-embryonal membrane is composed of yolk-spheres held together by a

[1] I commence with the letter C in order to make allowance for earlier stages which may be forthcoming later on.

meshwork of stellate cells; it forms part of the lower layer, and is continuous round the margin of the segmentation cavity with the similar but much thicker layer which elsewhere lies immediately beneath the epiblast. The roof of the segmentation cavity is formed by the area pellucida, which, however, is not at all sharply distinguished from the more opaque area which surrounds it.

In the hinder part of the area pellucida the embryo appears as a cap-shaped structure (fig. 1) projecting above the general surface of the blastoderm, from which it is marked off by a deep fold shaped like a horseshoe, very pronounced at the broad anterior end, but gradually dying out to the general level of the blastoderm behind. This is evidently the head-fold (figs. 1, 3, 5, *H. F.*).

Towards the close of this stage a distinct blastopore (figs. 1, 3, 4, *B. P.*), with a well-defined crescentic anterior margin, appears at the posterior narrow end of the embryo.

The embryo at this stage is very difficult to remove and harden satisfactorily. Kleinenberg's picric acid does not seem to be altogether suitable for the purpose. Owing, doubtless, to contraction, embryos 6 and 8 became cracked on top, while No. 7 appears to have shrunk inwards, so that it is now concave above and convex below, instead of just the opposite. Nos. 5 and 9, however, were satisfactorily preserved. The former was mounted whole in Canada balsam, and the latter cut into longitudinal sections.

A median longitudinal section passing through the blastopore has the appearance shown in fig. 4. It will be seen that in the area pellucida around the embryo the epiblastic cells gradually lose their flattened character and become elongated radially, prismatic, with very conspicuous, very darkly staining nuclei. Beneath them the lower-layer cells, here free or nearly free from yolk, form a fairly compact multiple layer. The lower limit of this inner layer is perfectly definite, but no very distinct hypoblast can yet be said to exist, though it will be formed later on from the deepest of the cells. (The cells of the sub-embryonal membrane, in the floor of the segmentation

cavity, doubtless belong also to the lower layer, as already observed, but they take no part in the formation of the embryo, and may in future be disregarded.)

In the embryo itself the epiblast and a lower layer can still be distinguished, but with a totally different histological structure. Immediately behind the blastopore both layers merge into a thick, dense mass of cells of very characteristic appearance. Each cell contains a very large but faintly staining oval nucleus, containing a few small, darkly staining granules. Under a low power the nuclei look as if they were the actual cells. This mass of cells doubtless represents the primitive streak (figs. 3, 4, 5, *Pr. S.*). The blastopore (*B. P.*) appears as a pitting in of its upper surface, as yet extending downwards and forwards only for a short distance, but destined later on to form a very distinct neurenteric passage opening below. The cells immediately surrounding the blastopore show a slight trace of radial arrangement.

In front of the blastopore the general cellular mass of the primitive streak passes into two perfectly distinct layers—an outer epiblast composed of several tiers of radially elongated cells, and an inner layer of cells not differing in any way from those of the primitive streak itself. This inner layer, which I propose to consider as at any rate chiefly mesoblastic, appears to be simply a forward prolongation of the primitive streak beneath the epiblast.

The epiblast of the embryo itself differs much in appearance from that of the surrounding blastoderm; the outlines of the cells are much less distinct, while their nuclei stain with much less intensity, and are coarsely granular instead of comparatively homogeneous; moreover they are arranged in several layers. Just in front of the blastopore the epiblast, though thick, is not nearly so thick as the layer beneath it; but as we trace it forwards its thickness greatly increases, attaining its maximum about the anterior margin of the head-fold, and then somewhat suddenly diminishing again as it turns downwards and backwards to join the epiblast of the area pellucida. In the region of the head-fold the epiblast forms a darkly stain-

ing granular mass, in which it is impossible to make out the outlines of the cells at all. Between this and the primitive streak its cells show a prismatic arrangement, and may be regarded as forming a medullary plate (fig. 4, *M. P.*). Just in front of the blastopore, especially a little to each side of the middle line, it shows a strong tendency to separate from the layer beneath it.

The inner layer (*P. S. M.*) of cells (mesoblast), continued forwards from the primitive streak beneath the epiblast, gradually thins out till it reaches a point a little in front of the middle of the embryo, where it is actually thinner than the overlying epiblast; it then thickens again considerably in the neighbourhood of the head-fold, and finally contracts once more to become continuous with the lower layer (*L. L.*) of the area pellucida.

Behind the blastopore the primitive streak passes gradually into a thick mass of precisely similar cells, which can be traced backwards for some distance beneath the epiblast of the area pellucida. This mass of mesoblastic cells (fig. 4, *Si.*) forms an ill-defined transverse thickening behind the blastopore, and evidently represents the so-called "sickle" of other reptilian embryos.

It will be observed that no amnion is yet present. It is true the section represented in fig. 4 shows an uprising of the blastoderm (pro-amnion) in front of the head-fold, but nothing was visible in the living embryo, and I have no doubt the appearance shown is simply due to contraction.

Stage D (figs. 6—14).

I have obtained only a single example of this stage—viz. embryo 58, removed on December 9th, from an egg found by Mr. Henaghan about the end of November.

The external appearance of this embryo, when viewed from above as an opaque object without staining, is shown in fig. 6. No trace of the amnion is yet visible. The head-fold still projects conspicuously above the blastoderm, and has grown forwards, while its anterior end has become considerably nar-

rowed, so that the embryo is now broadest in the middle about where the lower limb of the head-fold joins the underlying blastoderm (fig. 7, *F. Sp.*). The hinder half of the embryo is narrower than the broadest part of the head-fold, and forms a well-defined elevation above the surrounding blastoderm, with the conspicuous blastopore (*B. P.*) lying in the mid-dorsal line near the posterior end. The most posterior portion of the embryo is formed by the primitive streak behind the blastopore, which rises up, and is very sharply defined from the surrounding blastoderm (fig. 7).

In the mid-dorsal line of the embryo, between the blastopore and the anterior extremity, a faint indication of the medullary groove was visible even in the opaque, unstained embryo (fig. 6, *M. G.*). After staining and clearing the medullary groove was more conspicuous (fig. 7, *M. G.*), but owing to a certain amount of crumpling in the head-fold its course could not be exactly followed except in sections.

A selection from the series of transverse sections into which this embryo was cut is represented in figs. 8 to 14, the sections being arranged in order from in front backwards.

Fig. 8 represents a section through the area pellucida, just in front of the head-fold, and it will be seen at once that it presents a very curious appearance. In and about the middle line the three germinal layers are all clearly differentiated. The epiblast (*Ep.*) lies above, composed of a single layer of short columnar cells, with very large, darkly staining nuclei. The hypoblast (*Hyp.*) lies below, and is formed of short columnar cells clearly derived from the lowermost cells of the original lower layer. These hypoblast cells present an appearance of ciliation, but this may be due to post-mortem changes. Between the epiblast and hypoblast the remainder of the original lower-layer cells form the mesoblast (*Mes.*), which has already split into two perfectly distinct layers, an outer somatopleuric, which cleaves to the epiblast, and an inner splanchnopleuric, which cleaves to the hypoblast. Between these two a wide cavity (*P. C.*) has appeared, the development of which causes the somatopleure to bulge slightly upwards, and the splanchno-

pleure strongly downwards. Traced backwards this cavity appears to divide into two irregular fissures (fig. 9), lying one on either side beneath the head-fold.

The development of a large cœlomic cavity at this stage, and in this situation, seems very remarkable. That it is not in any way due to accident seems to be proved by the marked differentiation of the hypoblast cells beneath it, which acquire their characteristic short columnar form earlier in this particular region than anywhere else. Comparison with later stages seems to me to prove pretty conclusively that it is due to a very early development of that part of the cœlom which will later on form the pericardium, being carried backwards into the body of the embryo with the lower limb of the head-fold (compare figs. 24, 50, *P. C.*).

Fig. 9 represents a section passing through the head-fold, which lies quite freely above the blastoderm. In the blastoderm beneath the head-fold are seen the backward prolongations of the cœlomic space above mentioned, and the epiblast has become flattened. In the embryo itself the epiblast on the dorsal surface forms a medullary plate, in the middle of which lies the medullary groove (*M. G.*). The alimentary canal (*Al. C.*) is completely enclosed in this region, forming a wide space lined by slightly flattened hypoblast cells, not at all sharply marked off, especially below, from the mesoblast cells. The latter, for the most part, form a loose network of irregular cells lying between epiblast and hypoblast, but denser and apparently undergoing more rapid division on the ventral surface.

Fig. 10 represents a section just behind the head-fold, showing the alimentary canal still widely open below. The epiblast on the dorsal surface on each side of the medullary groove is widely separated from the underlying mesoblast, but the floor of the medullary groove itself remains in close contact with it. The mesoblast and hypoblast of the embryo are clearly seen to be here formed primarily by infolding of the original lower layer of the blastoderm before it has become clearly differentiated into two layers. It seems likely, how-

ever, that part of the mesoblast in this region is derived from the forward growth of the primitive streak described in the preceding stage.

Passing backwards from the last section the medullary groove is seen to disappear, and give place to a continuous flat medullary plate, composed of radially elongated, columnar epiblast cells, and extending for some distance. As it approaches the blastopore the epiblast thins out in the middle (fig. 11), and a groove (*P. G.*) appears on its upper surface, which can be traced back almost to the blastopore. This groove has prominent lips in the hinder part of its course (fig. 12, *P. G.*). It probably corresponds to the primitive groove of the chick.

For some distance in front of the blastopore the mesoblast forms a very dense mass of cells, which appear to be rapidly proliferating, and this is continued as a thick layer right across the middle line, and also outwards, on either side, as two thin lateral sheets (figs. 11, 12). This mass of cells is clearly derived from the forward prolongation of the primitive streak described at Stage C (compare fig. 4). It is perfectly clear that the embryonic mesoblast originates from two sources, viz. (1) from the original lower-layer cells of the area pellucida after separation of the definite hypoblast (compare fig. 8), and (2) from the cells of the primitive streak. The limits between the two different kinds of mesoblast I have not been able to determine.

Fig. 12 represents a section just in front of the blastopore, through the primitive streak. It will be noticed that the columnar or prismatic epiblast of the embryo passes into the undifferentiated mass of the primitive streak a little way in front of the blastopore, so that the section under consideration shows no differentiated epiblast on the dorsal surface at all. The mid-dorsal line is occupied by the primitive groove (*P. G.*) with its prominent lips. Beneath this, deeply embedded in the dense mass of the primitive streak, lies a small cavity (*N. En.*). This is the cross-section of the anterior ventral end of the neurenteric canal, which passes downwards and forwards from the blastopore, but does not yet open below.

On either side the cells of the primitive streak divide into two distinct layers, an upper layer which joins the columnar epiblast of the area pellucida, and a lower layer which forms part of the lateral sheet of mesoblast running out into the surrounding blastoderm.

Between this lateral sheet and the columnar epiblast, especially adherent to the under surface of the latter, a few irregular mesoblast cells are clearly recognisable. These cells are, I think, undoubtedly derived from the original lower layer. It will be seen by reference to figs. 12—14 that the epiblast, with these few adherent mesoblast cells, is separated by a distinct interval from the deeper or primitive-streak mesoblast. This separation appears to be the commencement of the splitting off of the serous envelope (*S. En.*) which will be described in later stages (compare figs. 30, 31, 55).

The hypoblast (*Hyp.*) forms an ill-defined and possibly imperfect layer of flattened cells, closely adherent to the under surface of the primitive streak, and probably also derived, as in the front part of the embryo, from the original lower layer of the blastoderm.

The section represented in fig. 13 passes actually through the opening of the blastopore (*B. P.*), which is seen as a wide funnel-shaped depression on the upper surface of the primitive streak, lined by distinctly columnar cells.

Fig. 14 represents a section through the primitive streak behind the blastopore, and embraces a somewhat wider area than the preceding figures, so as to show a portion of the area opaca and its junction with the area pellucida. The yolk-laden, sub-embryonal membrane, which forms the floor of the segmentation cavity beneath the area pellucida, is not represented. It will be seen that the blastoderm of the area opaca (*A. O.*) is very much thicker than that of the area pellucida (*A. P.*) owing to the enormous development of the lower layer with its contained yolk-spheres. In this region of this particular embryo the thickened lower layer of the area opaca passes quite suddenly into the thin lower layer of the area pellucida, forming a kind of "germinal wall" (*G. W.*), but the transition

from the one to the other is not always by any means so abrupt. Just outside the margin of the area pellucida blood islands (*B. I.*) are making their appearance. These seem to me to be derived from the sheet of mesoblast which grows out from the primitive streak, and which extends about as far as the germinal wall.

Stage E (figs. 15—34).

I refer to this stage embryos numbered 56 and 64, found on Stephens Island about the end of November, and removed from the eggs on December 9th. The two agree very closely with one another in all essential features, and differ very conspicuously from the preceding stage. This difference is due chiefly to the development of the pro-amnion and amnion, which has taken place in such a manner that the anterior end of the embryo completely enveloped in its pro-amniotic covering, now lies freely beneath the general surface of the blastoderm, instead of above it as in the preceding stages, while a large crescentic aperture, situated on the surface of the blastoderm in front of the primitive streak, leads into the amniotic cavity behind (vide figs. 15—20).

Although all the stages in the process have not been actually observed, it is evident that the pro-amnion and amnion develop in the following way :—The anterior end of the embryo sinks down, carrying with it the part of the blastoderm (pro-amnion) which lies beneath. The blastoderm (pro-amnion) thus pushed downward seems to become stretched at the same time, and thus forms a very thin sac (figs. 22—27, *Pro-Am.*) whose lips meet together and unite in the mid-dorsal line above the head. The pro-amnion thus formed around the anterior end of the embryo is composed of two layers, which must be regarded as epiblastic and hypoblastic respectively, with no distinct mesoblast between them. The cells of both layers have become flattened out, and so closely pressed together that it is difficult, if not impossible, to distinguish them except where they pass into the epiblast and hypoblast of the embryo itself (figs. 24, 25). The thin pro-

amniotic membrane thus formed around the head becomes completely separated from the overlying blastoderm (area pellucida), which lies at some little distance above, and exhibits no special peculiarities (fig. 22).

As we trace the pro-amnion backwards we find that at the posterior limit of the anterior closure of the alimentary canal its inner layer becomes continuous with the epiblast of the embryo proper, while its outer layer passes into the hypoblastic lining of the alimentary canal (fig. 25). A little further back the outer or hypoblastic layer of the pro-amnion becomes continuous above with the ill-defined hypoblast of the overlying blastoderm, to which the embryo is thus attached in the mid-dorsal line (fig. 27). Still further back the amnion is formed by an uprising of the epiblast on each side of the mid-dorsal line, forming two folds which meet and fuse above the medullary groove, and thus arch it over, while mesoblast cells extend in between the two layers of the epiblast (figs. 28, 29). Further back again the arching over of the medullary groove by the amnion is as yet incomplete, so that the narrow amniotic cavity opens widely to the exterior (fig. 30), and behind this the amnion has not yet begun to develop (figs. 31, 32).

In embryo 64 the formation of the amnion has extended back as far as the region of the primitive streak, which, however, is still incompletely arched over by the uprising folds of the somatopleure (fig. 34).

A comparison of transverse sections through the posterior portions of embryos of Stages C and D shows that the formation of the amnion is here also accompanied by a downsinking of the body of the embryo, but not nearly so marked as in the anterior part (compare figs. 9—14 with figs. 22—32).

The separation of the serous envelope, commenced already in the preceding stage, is progressing in the hinder part of the area pellucida. Figs. 29—32 show clearly that it consists of the superficial epiblast of the area pellucida, with a few adherent irregular mesoblast cells (derived from the original lower layer), which splits off from the deeper mesoblast derived partly from outgrowth of the primitive streak.

The rudiment of the pericardium, observed in the preceding stage, appears to be almost obliterated by stretching in the formation of the pro-amnion. It seems to be represented by the split, containing a few mesoblastic cells, which lies between the epiblast and hyboblast, where they turn up to join the pro-amnion just in front of the opening into the anterior closed-in part of the alimentary canal (fig. 24).

Having thus described the condition of the fœtal membranes, we may consider next the external features of embryos of this stage.

The appearance of embryo 56, when viewed from above as an opaque object without staining, is represented in fig. 15. The embryo lies close to the hinder end of the pear-shaped area pellucida, the outline of the anterior portion being somewhat dimly seen through the overlying blastoderm. At the hinder end, just in front of the primitive streak, lies the large crescentic opening into the amniotic cavity (*Am. O.*) On each side the primitive streak runs out into a wing of mesoblast (*L. W.*), which at once turns sharply forward and runs for a while almost parallel with the long axis of the embryo, but dies out before reaching the middle of its length. Of course the mesoblast also extends backwards from the primitive streak, and the latter, with its posterior and lateral mesoblastic outgrowths, forms a crescentic thickening around the hinder end of the embryo which corresponds more or less closely to the "sickle" of other reptilian embryos.[1]

Fig. 16 represents the same embryo viewed from beneath after removal of the sub-embryonal membrane, a fragment of which, however, remains just behind the embryo (*S. E. M.*). The anterior half of the embryo is now, of course, very much more distinct, and is seen as a finger-shaped body, curving somewhat downwards from its junction with the posterior half, and projecting freely forwards beneath the blastoderm enveloped in its thin transparent pro-amnion. The mesoblastic outgrowth from the primitive streak appears now to form a conspicuous thickening all round the posterior half of the

[1] Compare Mitsukuri and Ishikawa (5).

embryo. On the ventral surface of the embryo the alimentary canal is outlined by two parallel grooves, united in front by a deep crescentic transverse fold which marks the limit to which the anterior enclosure of the alimentary canal has as yet extended (fig. 16, *F. Sp.*).

Fig. 17 represents the same embryo seen from above as a transparent object after staining and clearing.

Figs. 18—20 represent three corresponding views of embryo 64. The sub-embryonal membrane not having been so completely removed in this case, the hinder end of the embryo could hardly be made out at all as an opaque object.

Fig. 20 is especially worthy of note, as it shows very clearly the spreading of the lateral wings of mesoblast (*L. W.*) derived from the primitive streak. It will be seen that they spread outwards and forwards as two irregular sheets, following mainly the line of junction between the area pellucida and area opaca. Reference to transverse sections (figs. 30, 31) show that these sheets (*L. W.*) lie in the floor of the developing pleuro-peritoneal space which separates the serous envelope from the future yolk-sac. They lie, in fact, in the yolk-sac itself, where they undoubtedly give rise to a portion at any rate of the vitelline vessels (compare figs. 20 and 58).

Turning now to the more minute structure of these embryos, the following features seem worthy of note.

The enclosed portion of the alimentary canal extends further back than in the preceding stage, and is considerably narrowed, while the hypoblastic cells forming its wall have assumed their characteristic short columnar shape (compare figs. 9 and 23). The change from flattened to columnar in the shape of the hypoblast cells extends also for some distance behind the limit of closure (except in the mid-dorsal line), but gradually ceases as we approach the primitive streak (figs. 25—33).

The notochord has made its appearance in the middle line beneath the medullary groove. It appears to me quite clear that it has been formed by separation of a solid rod of cells from the sheet of mesoblast which has already been noticed as growing forward from the primitive streak, and which, it will be re-

membered, was continuous in the preceding stages across the middle line beneath the medullary plate. This mode of origin is very clearly shown in the section represented in fig. 33, and also in the sections represented in figs. 31 and 32. As we trace it towards the anterior end the forward growth of primitive-streak mesoblast gradually dies out, and so also does the notochord, which gradually narrows and finally disappears somewhere between the point where the head end of the embryo becomes free from the overlying blastoderm, and the point to which the anterior closure of the alimentary canal has extended (vide figs. 25—32).

Beneath the notochord the hypoblastic lining of the alimentary canal has not yet assumed its characteristic short columnar form, even anteriorly.

The medullary groove at this stage has extended backwards as far as the primitive streak, and has become continuous with the neurenteric canal or blastoporic passage, which, however, has not yet acquired its ventral opening (see figs. 31, 32). The columnar cells lining the neurenteric canal appear to be ciliated, but this appearance may be due to post-mortem changes. The blastopore is no longer recognisable as a distinct structure, its lips having become confluent with those of the medullary groove in front of it. Posteriorly, however, in embryo 64 a longitudinal furrow, not lined by columnar cells, is continued backwards from the end of the medullary groove for some distance over the surface of the primitive streak (fig. 34). This furrow, like the similar one noticed in front of the blastopore at the preceding stage (fig. 12), probably represents a portion of the primitive groove, which would thus seem to extend both in front of and behind the blastopore.

Tracing the medullary groove forwards (figs. 21—22) we find that although it has become very deep, and its lips are approaching one another, yet it is still open for either the whole or the greater part of its length. In both embryos it is greatly dilated in front (figs. 17, 20, 21) to form the rudiment of the brain, and in No. 64 this rudiment is already differentiated into fore-, mid-, and hind brain, the latter being repre-

sented on the ventral surface by two distinct dilatations (fig. 21), while about the region of the mid-brain the lips of the medullary groove are already united by a great proliferation of cells.

In embryo 56 the medullary groove exhibits a sudden contortion or curvature in the region of the hind brain (fig. 17), but as this is more conspicuous in some later embryos I shall postpone any further account of it, as well as of the closure of the medullary groove, until I come to deal with them.

Before leaving this stage it may be worth while to note certain differences exhibited by the blastoderm in the neighbourhood of the primitive streak, especially in embryo 64, as compared with the corresponding parts at the preceding stage (*D.*), although these differences are probably due rather to individual variation than to progressive development. The differences in question are strikingly brought out by comparison of figs. 14 and 34, representing transverse sections through the primitive streak of embryos 58 and 64 respectively. In the former, as already observed, there is a distinct germinal wall, due to the sudden diminution in thickness of the lower layer of the blastoderm at the juncture of the area opaca with the area pellucida, so that only a very thin stratum of lower-layer cells is continued across the area pellucida and beneath the primitive streak. In the latter (fig. 34) the lower-layer cells form a thick stratum both at the sides of and beneath the primitive streak, though in the front part of the embryo they thin out suddenly on meeting the margin of the area pellucida as in embryo 58. Thus the primitive streak is surrounded, except above, by a network of stellate cells with yolk-spheres entangled in their meshes (compare also fig. 35, Stage F). In transverse sections of embryo 64, taken a little in front of the primitive streak, the twofold origin of the mesoblast is very clearly recognisable (fig. 33). The two lateral sheets of mesoblast (*P. S. M.*), derived from the proliferation of the compact rounded cells of the primitive streak, are seen thrusting themselves in between the loose stellate cells of the original lower layer (*L. L. M.*), and dividing the latter into two parts,—an upper very thin layer which adheres to the epiblast, and a

lower, thicker layer, from the lowest cells of which the hypoblastic lining of the alimentary canal is differentiated.

Stage F (figs. 35—46).

To this stage I refer embryos 61 and 72, collected about the end of November, and removed from the egg on December 9th and 10th. Though on the whole closely agreeing with the last stage, these embryos differ in certain noteworthy respects, so that it seems desirable to deal with them separately. They illustrate well the great difficulty which I have experienced in classifying the embryos, owing to the fact that events do not always take place in exactly the same order in different individuals.

The external characters of the embryos themselves agree so closely with those of the preceding stage that it seems unnecessary to do more than refer the reader to figs. 37—41. Both embryos, however, differ from those described in the last stage, in the remarkable fact that the amniotic cavity is continued backwards for some distance as a narrow canal (the posterior amniotic canal), which opens to the exterior through a crescentic aperture on the surface of the blastoderm at some distance behind the primitive streak (figs. 37, 39, 40, 41). This tubular prolongation of the amniotic cavity may either lie in a straight line with the long axis of the body (fig. 41), or obliquely to it (fig. 39). It is formed by invagination of a strip of modified epiblast continued backwards from the primitive streak, combined with an uprising of the edges of the strip, which unite and close it in above (figs. 45, 46). The modification of the epiblast in question consists in a loss of definition on the part of its cells, the nuclei of which stain less intensely than those of the normal epiblast of the area pellucida. These characters are shown in fig. 46, which represents a portion of a transverse section passing through the posterior amniotic opening, where the amnion is still in process of formation by the uprising of the epiblast on either side of the modified strip. When the posterior amniotic canal has become closed in, it lies altogether below the superficial epiblast,

and a few mesoblast cells make their way in between the two (fig. 45). It is very narrow, and the cells of its roof are much more clearly differentiated from the surrounding mesoblast than those of its floor.

In both embryos of this stage the medullary groove has become closed in the region of the mid-brain by the union of its two very prominent lips to form a thick mass of cells, which projects backwards so as to overlap the hind brain. The thickening of the lips of the medullary groove in this region is already prominent at the preceding stage, as shown in fig. 27. Fig. 42 shows the thickened lips united to form the roof of the mid-brain (*R. M.*). Fig. 43 shows this roof overlapping the hind brain, and fig. 44 is through the hind brain a little further back, showing the medullary groove still widely open. Fig. 35, representing somewhat diagrammatically a median longitudinal section of embryo 61, shows that it is only in the region of the mid-brain that the closure of the medullary groove has become completed.

In both these embryos also, but especially conspicuous in No. 72 (fig. 39), there is a sharp bend in the region of the hind brain. This bend appears to be due to the restraining influence of the pro-amnion, which attaches the head to the under surface of the blastoderm in the region of the mid-brain, and seems to prevent the embryo from elongating in a straight line. The result is that the head appears to be pulled upwards and backwards, as a horse's head is pulled back by the reins (compare fig. 36, and Stage G, fig. 50). This pulling back of the head probably accounts for the overlapping of the hind brain by the roof of the mid-brain (compare figs. 39 and 43, *R. M.*).

In embryo 72 the neurenteric canal is still closed below, but in No. 61 it opens widely into the future alimentary canal just behind the notochord, and in front of the great mass of the primitive streak. This opening is clearly shown in the longitudinal section represented in fig. 35, which also shows very clearly how the notochord appears as a forward growth from the region of the primitive streak, or, to speak more

accurately, as the axial portion of this forward growth separated from the lateral portion; while the hypoblastic lining of the alimentary canal assumes its characteristic columnar form from in front backwards.

The rudiment of the pericardium, which should lie just in front of the letters "*F. Sp.*" in fig. 35, appears in this section to be quite obliterated, but it is seen again in the corresponding position at the next stage (compare fig. 50, *P. C.*).

Stage G (figs. 47—50).

I have only a single embryo of this stage, numbered 59, which was removed from an egg found on Stephens Island about the end of November, and opened on December 9th.

When examined as an opaque object this embryo was seen to differ from those of the preceding stage chiefly in the appearance of the ventral surface (fig. 47), which showed the as yet unenclosed portion of the alimentary canal as a shallow, elongated trough, distinctly bounded all round by a thick bolster-like rim. Close to the hinder end of the oblong area thus enclosed the aperture of the neurenteric canal was conspicuously visible (fig. 47, *N. En.*). The backward tilting of the head (fig. 50) is more pronounced than in the preceding stages, and at the same time the head is turned to one side (figs. 48, 49).

The structure and relations of the amnion and pro-amnion remain much as before. The serous envelope has progressed greatly in development (figs. 47 and 50, *S. En.*), having been formed almost entirely by the splitting off of the superficial epiblast, accompanied by a thin layer of mesoblast, from the underlying portion of the blastoderm. The latter may now be termed the yolk-sac (figs. 47 and 50, *Y. S.*). In the removal of the embryo from the egg the fœtal membranes were considerably disturbed, and the yolk-sac to a large extent torn off around the embryo; the result of this was to bring out very clearly its relations to the overlying serous envelope, as shown in fig. 47. Dorsally, of course, where the pro-amnion joins the superficial blastoderm, the yolk-sac is reflected for-

wards to form the outer layer of the pro-amnion, as shown in fig. 50. Behind this point the serous envelope remains united with the true amnion in the mid-dorsal line to form a single membrane, composed of three layers, viz. cubical epiblast on the outside, mesoblast in the middle, and flattened epiblast on the inside (fig. 50, *L. A. S.*). The space developed elsewhere beneath the serous envelope is, of course, the pleuro-peritoneal space, and beneath this space the yolk-sac is not yet, at any rate in most parts, clearly differentiated into mesoblast and hypoblast.

The posterior amniotic canal is still clearly traceable in sections; though, owing perhaps to the crumpling of the membranes in removal, I failed to observe it in the embryo before cutting. It lies embedded in the thickness of the serous envelope above the pleuro-peritoneal space, and it opens in front into the hinder end of the much wider amniotic cavity above the embryo, while behind it still opens to the exterior as before. In this specimen it lies obliquely to the long axis of the embryo, and its anterior opening lies to one side of the middle line. It is, therefore, not shown at all in the median longitudinal section represented in fig. 50.

The alimentary canal, except as regards the development of the thickened rim already noticed, has scarcely progressed further than at Stage F. The short columnar or cubical epithelium which lines it is well marked throughout the greater part of its extent, but gradually thins out and becomes flattened behind (fig. 50).

The notochord also has progressed no further. On the contrary, it seems to be scarcely so far advanced as in embryo 61 (Stage F), appearing to end in a mass of undifferentiated mesoblast cells beneath the hind brain (compare figs. 35 and 50).

The pericardium is now again represented by a distinct cavity lying beneath the anterior enclosed part of the alimentary canal, just where the epiblast and hypoblast are reflected upwards to join the pro-amnion around the head (fig. 50, *P. C.*). Just where the bend takes place the epiblast and hypoblast are separated from one another by a considerable space. This space I believe to be the pericardium, although in this embryo

the mesoblast does not appear to extend so far as this, but ceases somewhat abruptly beneath the fore-brain. It certainly seems to correspond to the spaces which I have identified as pericardial in earlier stages. In front of the pericardium the epiblast and hypoblast unite together closely to form the very thin pro-amnion of the head region (fig. 50, *Pro. Am.*).

The medullary canal is now closed in dorsally, except in the region of the fore-brain, and for a considerable space at the hinder end, above and in front of the neurenteric canal, where it still opens widely into the amniotic cavity. Owing to the peculiar backward tilting of the head, the mid- and hind brains are bent in a vertical plane somewhat into the form of the letter S, as shown in fig. 50. The fore-brain is slightly twisted to one side, so that its long axis lies obliquely to the long axis of the spinal cord, and the right and left halves of the roof of the fore-brain overlap one another slightly in the middle line (fig. 48).

Stage II (figs. 51 and 52).

To this stage I refer embryo 78, removed on December 16th from an egg found on Stephens Island about the end of November. Unfortunately the hinder half of this embryo was accidentally injured after the first drawing (fig. 51) had been made, so that I am unable to give as full a description of it as I could wish.

When viewed as an opaque object the embryo was seen to differ from the preceding stage chiefly in the much greater extent to which the closure of the alimentary canal has extended backwards. Thus the ventral transverse fold (fig. 51, *F. Sp.*) caused by the turning forward of the splanchnopleure lies only a short way in front of the middle of the body. The enclosed portion of the alimentary canal (fig. 52, *Al. C.*) is filled with yolk particles, whereby it is rendered very conspicuous when the embryo is examined as a transparent object after staining. Beneath the hinder part of this enclosed portion lies the pericardium (fig. 52, *P. C.*) in the form of a somewhat quadrangular sac, narrower in front than behind. In the pericardium lies the heart (fig. 52, *Ht.*), in the form of

a short pear-shaped sac. The posterior and narrower end of the heart receives the two vitelline veins (fig. 52, *V. V.*) running in the folds of the splanchnopleure. From its broad anterior end a very short bulbus arteriosus (fig. 52, *B. Ar.*) runs forward beneath the throat.

The head has straightened out again, and there is no trace of the upward and backward tilting noticed in the preceding stages. The fore-brain lies at the anterior extremity, and has not yet begun to bend downwards. The overlapping of the two lateral halves of the roof of the fore-brain, already slightly indicated in the last stage, is now much more pronounced, and gives rise to the very curious appearance shown in fig. 52, from which it will be observed that it is the left half which overlaps the right.[1] On either side of the fore-brain a prominent optic vesicle has grown out, and projects somewhat backwards (fig. 52, *O. V.*).

The mesoblast on either side of the medullary canal has become segmented into about a dozen not very distinct mesoblastic somites. It was impossible to count them exactly, partly because of the injury to the posterior half of the embryo, and partly because, on their first appearance, the mesoblastic somites are not nearly so distinct as they are in the chick.

Stage J (figs. 53—73).

This stage is represented by a fine series of embryos, numbered as follows:

14?	Collected on Stephens Island	Nov. 14,	removed from egg	Dec.	10.
40, 42, 43	„	„ 30,	„	„	9.
44, 46	„	„ 30,	„	„	10.
48	„	„ 30,	„	„	16.
60, 62, 63	„	end of Nov.,	„	„	9.
73, 74, 75	„	„	„	„	10.
79	„	„	„	„	16.

[1] Fig. 52 is taken from the ventral surface and reversed by the microscope, so that the left side of the figure corresponds to the left side of the embryo.

Of course these embryos exhibit a certain amount of variation amongst themselves, but not in my opinion sufficient to make it necessary to separate them into distinct stages. I have accordingly selected No. 44 as typical, and propose to describe it in some detail, referring afterwards to such deviations from the type as seem to deserve special mention in the others.

General Relations of the Blastoderm and Embryo. —The living embryo was still very transparent, but a red-coloured sinus terminalis was conspicuous in the blastoderm surrounding the hinder part of the body (fig. 58, *S. T.*). The sinus terminalis forms the margin of the posterior half of a clear-looking area in which the embryo lies. This clear area exhibits a strong constriction in the middle, where the vitelline veins are developing (fig. 56). In some embryos (e.g. No. 46, fig. 54, and No. 60, fig. 53) the clear area thus acquires a very characteristic 8-shaped outline at this stage; in No. 44, however, the shape is less regular.

The part of the blastoderm which forms the front half of the 8, corresponding to part of the original area pellucida, lies entirely above the anterior half of the embryo, which projects freely beneath it, and in front of the vitelline veins. This part of the blastoderm is thin, and contains but little yolk, hence its transparency. It is composed of epiblast and lower-layer cells. Some of the latter (mesoblastic) adhere to the epiblast to form with it the serous envelope, which, however, is as yet only partially separated from the underlying yolk-sac composed of the remainder of the lower-layer cells. At the margin of this anterior half of the clear area the lower layer (yolk-sac) thickens suddenly and becomes heavily laden with yolk, which accounts for its greater opacity. At the level of the vitelline veins the serous envelope and yolk-sac join the amnion and pro-amnion, thus giving rise to the transverse crease across the back of the embryo (fig. 56, *Tr. L.*; compare also fig. 50, Stage G).

In the hinder part of the 8-shaped clear area, bounded by the sinus terminalis, the serous envelope is separated from the vascular yolk-sac by a wide pleuro-peritoneal space on each

side of the body of the embryo, forming a kind of blister (compare figs. 69—71). Along the mid-dorsal line, however, the body of the embryo is attached by the true amnion to the under surface of the serous envelope (compare figs. 68—70), except just above the primitive streak, where the two have completely separated (fig. 71).

The Fœtal Membranes.—As we have just seen, the serous envelope (fig. 69, *S. En.*) has completely split off from the underlying portion of the blastoderm (yolk-sac) throughout the posterior half of the 8-shaped clear area, while in front of this it is not yet widely separated. It is composed as before of the highly characteristic but now somewhat flattened cells of the superficial epiblast, with their very darkly staining nuclei, and of a number of adherent mesoblast cells derived from the lower layer, some of which appear to be specialising as a distinct epithelioid layer on the under surface of the thin transparent membrane thus formed (figs. 72, 73, *Mes. E.*). In the mid-dorsal line of the embryo, from about the level of the vitelline veins to the primitive streak, the serous envelope remains continuous with the true amnion (figs. 68—70). Above the primitive streak, however, it lies quite separate above the true amnion, so that the two lateral halves of the pleuro-peritoneal space communicate freely above the embryo in this region (fig. 71). They also extend backwards behind the embryo as a single wide cavity, roofed over by the serous envelope and bounded by the sinus terminalis. On the floor of this pleuro-peritoneal cavity, formed by the yolk-sac, the vitelline vessels are rapidly developing in the form of blood islands (figs. 69, 72, *B. I.*), which together with the sinus terminalis (*S. T.*) are, as already noticed, probably derived from the sheet of mesoblast which spreads out from the primitive streak.

The pro-amnion, completely enveloping the anterior end of the embryo in front of the vitelline veins, has exactly the same structure and relations as before, being a thin transparent membrane composed of two layers,—an outer hypoblastic, which is really a portion of the yolk-sac, and an inner epiblastic,

which is really part of the somatopleure. Both these layers are clearly shown in figs. 63 and 64, from which it will also be seen that there is no recognisable mesoblast between the two. Behind the vitelline veins, however, the true amnion envelops the dorsal portion of the embryo, and is composed of somatopleure alone, with distinct epiblast and mesoblast, the latter becoming continuous with the mesoblast of the serous envelope in the mid-dorsal line (figs. 68—70).

Although it was no longer conspicuous externally, the posterior amniotic canal is still clearly recognisable in sections behind the embryo. It appears as a very narrow canal (figs. 72, 73, *P. A. C.*), embedded in the mesoblast of the serous envelope, and extending obliquely backwards from the point where the latter becomes disconnected from the true amnion to a short distance beyond the sinus terminalis, where it still opens to the exterior on the surface of the blastoderm. The lumen of the canal is distinct in the hinder part of its course (figs. 72, 73), but in front it is closed, so that it is no longer possible to trace it into connection with the amniotic cavity above the embryo, as in earlier stages. The epiblast cells lining the posterior amniotic canal seem to become gradually assimilated to the surrounding mesoblast cells, so that it is difficult, if not impossible, to recognise it at its extreme anterior end after its lumen has disappeared.

External Characters of the Embryo.—In this particular specimen the head end of the embryo projects beyond the anterior margin of the clear area of the overlying blastoderm, so that when viewed from above as an opaque object part of the head is concealed from view by the yolk-laden opaque area (fig. 56). When viewed from below as an opaque object the embryo exhibits the appearance shown in fig. 57. The fore-brain, with the optic vesicles, has been bent downwards at right angles to the long axis of the body, so that what was formerly its anterior extremity now lies ventrally. Immediately behind the fore-brain a dark-looking triangular depression, with its apex pointing backwards, marks the position of the stomodæum (fig. 57, *Stom.*), where the ventral

wall of the alimentary canal has already begun to thin out, preparatory to perforation by the mouth. A short way behind this is seen the heart (fig. 57, *Ht.*), lying in the pericardium, a little in front of the middle of the body. Just behind the heart lies the opening of the anterior enclosed part of the alimentary canal. The remainder of the ventral surface is occupied by the as yet unenclosed portion of the alimentary canal in the form of a shallow trough with prominent margins, and with the aperture of the neurenteric canal (fig. 57, *N. En.*) lying near its posterior end.

The Alimentary Canal.—The extreme anterior end of the alimentary canal lies in the angle between the mid-brain and the downwardly turned fore-brain, its blind extremity being in the same transverse plane as the now posterior limit of the fore-brain beneath it. Fig. 62 represents a transverse section taken just behind the anterior extremity of the alimentary canal, which in this region occupies only about one third of the total width of the head. Beneath the hind brain (fig. 63) it widens out until its side walls meet and fuse with the lateral epiblast, preparatory to the formation of the first visceral cleft. Its floor also here becomes extremely thin, the epiblast of the stomodæum (fig. 63, *Stom.*) lying in close contact with the hypoblast, and both being attenuated preparatory to perforation by the mouth. On approaching the pericardium, however (figs. 64, 65), the lateral and ventral walls of the alimentary canal retreat from the epiblast and assume their normal aspect, which is maintained until, on reaching the opening to the exterior behind the heart, the folds of the splanchnopleure diverge, and leave the alimentary canal widely open below (fig. 67). This open condition is continued to the posterior extremity behind the conspicuous aperture of the neurenteric canal (fig. 70). The anterior enclosed part of the alimentary canal is densely filled with yolk particles, conspicuous both in sections (figs. 62—66), and when the whole embryo is viewed as a transparent object (fig. 59). Its hypoblastic lining is now composed almost throughout of the characteristic short columnar cells, flattening

more or less, however, in the mid-dorsal and mid-ventral lines towards the anterior extremity.

The Primitive Streak and Neurenteric Canal.—The primitive streak, or at any rate the posterior mass of mesoblast derived therefrom, forms a dense mass of cells behind the neurenteric opening, somewhat triangular in transverse section, covered by flattened epiblast, and arched over by the true amnion (fig. 71). The blastopore has disappeared from view externally, being included in the closure of the hinder part of the medullary groove; but the neurenteric passage still leads downwards through the floor of the medullary canal, and places the cavity of the latter in free communication with the cavity of the unenclosed portion of the alimentary canal below (fig. 70).

The Notochord.—The notochord can now be traced forwards to a point just in front of the anterior extremity of the alimentary canal, where it lies above the latter in the angle between the mid- and fore-brain. Posteriorly it can, of course, be traced back to the neurenteric aperture, with the margin of which it is continuous. In the trunk region it is now widely separated from the underlying hypoblast (figs. 57—69).

Mesoblastic Somites, Cœlomic Cavities.—The mesoblast on each side of the spinal cord has become segmented into fourteen mesoblastic somites, the first of which lies just behind the auditory pit, and the last some distance in front of the neurenteric aperture. These somites are cubical blocks, whose cells have the usual columnar form and radial arrangement (figs. 65—68, *M. S.*). Anteriorly they are connected with the peritoneal epithelium by the intermediate cell mass (figs. 55, 67, 68, *I. C. M.*), beyond which the somatopleure and the splanchnopleure diverge, leaving between them only a narrow cœlomic space in the body of the embryo, which is continuous with the very large pleuro-peritoneal space outside (fig. 68). Posteriorly, about the region of the twelfth mesoblastic somite (fig. 69), the cœlomic split can still be traced into the somite itself, the latter having not yet completely separated off from the lateral plates of mesoblast.

In the head region, just outside the aortic dilatation on either side of the front end of the alimentary canal, lies the commencement of the head cavity. This is shown in fig. 62 as a small space in the mesoblast (*H. C.*). Anteriorly it extends forwards for a short distance, dilating somewhat and becoming irregular, while posteriorly it soon disappears.

The pericardium is now very largely developed, forming a great quadrangular sac on the ventral aspect of the embryo (figs. 59, 64—66, *P. C.*). The cavity of the pericardium is continuous above with the narrow cœlomic space appearing between the somatopleure and splanchnopleure in the body of the embryo on either side of the alimentary canal (fig. 66).

In the region of the heart (figs. 65, 66) the splanchnopleuric and somatopleuric mesoblast on either side both turn inwards, and unite ventrally with the corresponding layers of the opposite side, the splanchnopleuric mesoblast thus enclosing the heart, and the somatopleuric helping to enclose the pericardium. Behind the pericardium the somatopleuric mesoblast turns upwards with the epiblast to form the true amnion, while the splanchnopleuric mesoblast runs out with the hypoblast to join the yolk-sac (figs. 55, 68—71).

The Wolffian Bodies and Ducts.—These organs are only just beginning to appear, and are as yet very ill-defined. From about the fifth to the eleventh mesoblastic somites the intermediate cell mass is thickened on its outer aspect (fig. 68, *I. C. M.*). Behind the eleventh somite this thickening seems to be growing freely backwards between the peritoneal epithelium and the epiblast as a solid rod of cells, the rudiment of the Wolffian duct (fig. 69, *W. D.*), which can be traced backwards about as far as the thirteenth somite. Anteriorly, opposite somites 6 and 7, segmental vesicles appear to be developing in the thickened intermediate cell mass (fig. 68, *S. V.*). It would be beyond the scope of the present paper to trace the development of these organs in detail, but it probably takes place very much in the same way as described by Weldon for Lacerta (6).

Vascular System.—The heart (fig. 59, *Ht.*) has now the

form of a wide tube slightly bent on itself, made up behind by the union of the two vitelline veins running across from the yolk-sac in the folds of the splanchnopleure just where the latter diverge, and ending in front in the short bulbus arteriosus (fig. 59, *B. Ar.*). In transverse sections (figs. 65, 66) the heart appears as a saccular diverticulum of the splanchnopleuric mesoblast, here composed of short columnar cells, beneath the front part of the alimentary canal, hanging down into the pericardium, and containing two epithelioid tubules. These tubules (figs. 65, 66, *Ep. T.*) are continuous behind with the vitelline veins, while in front they unite to form the bulbus arteriosus. The bulbus arteriosus very soon divides into two primitive aortæ (figs. 62 to 69, *P. Ao.*), which run forward beneath the alimentary canal for some distance, and then, curving upwards, turn back and run above the alimentary canal towards the posterior end of the body, giving off the vitelline arteries (fig. 69, *V. A.*) in the trunk at the level of the twelfth mesoblastic somite. Where the two primitive aortæ turn upwards in front to reach the dorsal aspect of the alimentary canal they dilate to form a large blood-space (fig. 62, *P. Ao.*) on each side of the extreme anterior end of the alimentary canal; like the rest of the blood-vessels this space is lined by a distinct epithelium.

In the splanchnopleuric mesoblast of the yolk-sac, forming the floor of the large pleuro-peritoneal cavity posteriorly, numerous blood islands have made their appearance (figs. 58, 69, 70, *B. I.*), and the posterior half of the sinus terminalis is distinctly visible (figs. 58, 69, 71, *S. T.*), marking the limit to which the separation of the serous envelope from the yolk-sac has extended. These vessels of the yolk-sac, as already noticed, appear to be formed, at any rate in part, from the sheet of mesoblast which grows out from the primitive streak.

Nervous System and Sense-organs.—The medullary canal has completely closed in above, except on what is now the ventral aspect of the fore-brain, where the margins of the medullary groove overlap one another as in the preceding stage, but are not yet fused. Reference to fig. 60, in which the

right and left sides are reversed, again shows that it is the left half of the roof of the fore-brain which overlaps the right. Posteriorly, the medullary canal still communicates freely with the as yet unenclosed portion of the alimentary canal through the conspicuous neurenteric passage (fig. 70, *N. En.*).

The fore-brain (figs. 59—61, *F. B.*), as already observed, is bent down at right angles to the long axis of the body, and presents two conspicuous hollow lateral outgrowths, the optic vesicles of the ordinary paired eyes (figs. 59—61, *O. V.*).

The mid-brain (figs. 58, 60, 61, *M. B.*), nearly spherical when seen from above, occupies the extreme anterior end of the body. It now lies in the same straight line with the hind brain, from which it is separated by a very strongly marked constriction, while the curvature which existed in this region at preceding stages has disappeared.

The hind brain (figs. 58, 63, 64, *H. B.*) shows indications of transverse segmentation into at least four neuromeres, and its roof is thinning out in the usual manner (fig. 63). It tapers very gradually behind, and passes insensibly into the spinal cord.

On either side of the posterior part of the hind brain the auditory pits (figs. 58, 64, *Au.*) have made their appearance as shallow depressions of the superficial epiblast, widely open to the exterior and lined by elongated columnar cells.

Other Embryos.—Amongst the other embryos which I have referred to Stage J, the following seem worthy of special mention.

In No. 40 the posterior amniotic canal is still distinctly visible in the unstained embryo when viewed as an opaque object. It runs from the hinder end of the embryo obliquely backwards, inclining towards the left, as in embryo 72 (Stage F, figs. 37, 39), and opens to the exterior on the edge of the clear area of the blastoderm. I have not detected this canal externally in any other embryo of Stage J, though, as noted above, it was easily demonstrable in sections of No. 44.

[1] This overlap was not observed until the sections were cut, and is therefore not shown in Fig. 59.

No. 46 exhibits an extremely interesting peculiarity with regard to the development of the amnion. Towards the hinder end of the body, but in front of the neurenteric canal, a longitudinal slit-like opening in the serous envelope was clearly visible in the unstained embryo viewed as an opaque object (fig. 54, *Am. S.*). Transverse sections (fig. 55) demonstrate that this opening leads into the true amniotic cavity above the embryo, and is due to the two uprising folds of the somatopleure not having yet united in the middle line, although the amnion is completely formed both in front of and behind the slit, and the posterior amniotic canal is also present behind the embryo, and no longer communicates with the true amniotic cavity. A similar opening into the amniotic cavity in this region is, of course, a feature very characteristic of the chick at a certain stage in its development, but it appears to be abnormal in Sphenodon, as I have seen it in no other embryo. It is interesting to notice just within the lips of the slit the sudden transition from the large epiblastic cells of the serous envelope, which become markedly columnar at the lips of the slit, to the flattened epiblast cells lining the amniotic cavity (fig. 55).

The downward curvature of the fore-brain may be greater than in No. 44, as in No. 60, where the fore-brain is inclined downwards and backwards, so that it comes to lie very close to the heart. In some embryos the cleft, where the two halves of the fore-brain have not yet united in the middle line, is still clearly visible externally.

The development of the mesoblastic somites does not appear to be nearly so definite and regular as in the chick, so that these are of comparatively little use for the purpose of determining stages in the development. It appears that a number of mesoblastic somites are differentiated almost or quite simultaneously, but very indistinctly. In No. 60 only about four pairs were recognisable in the stained embryo viewed as a transparent object, and in No. 73 they were very indistinct.

I have already stated that some embryos (e. g. 46 and 60, figs. 54 and 53) show much more clearly than No. 44 the

characteristic figure-of-8 form of the clear-looking area of the blastoderm surrounding the embryo.

In No. 48 the heart was observed to be beating in the living embryo, but very slowly.

From this stage onwards I propose to describe only those features which seem to be characteristic of each stage, marking a definite advance on the preceding stage, or which otherwise seem to demand special attention, as the organisation has now become so complex that to describe all the remaining stages in full detail would be far beyond the scope of the present paper, especially as the development of the various organs conforms very closely to that of already well-known types.

Stage K (figs. 74—82).

To this stage I refer six embryos, as follows:

38, 39, 41	Collected on Stephens Island	Nov. 30,	removed from egg	Dec.	9.
45	„	„	„ 30,	„	„ 10.
49	„	„	„ 30,	„	„ 16.
80	„	„	end of Nov.,	„	„ 16.

Embryo No. 39 may be taken as typical of the stage. The general relations of the embryo and fœtal membranes remain as before (vide figs. 74, 75), but the posterior amniotic canal has almost, if not quite, disappeared, having been only doubtfully recognised in a single section.

The body of the embryo is still straight as far forward as the mid-brain, but the anterior part of the head, including the fore-brain, is bent downwards at more than a right angle, so as to point backwards, thus considerably reducing the interval between the head and the pericardium on the ventral aspect (figs. 74, 75). There is as yet no distinct tail, but the hinder end of the embryo has become, to a certain extent, folded off from the yolk-sac, and projects backwards for a short distance in the pleuro-peritoneal space between the yolk-sac and the serous envelope (fig. 82).

The alimentary canal has begun to close in behind (figs. 75, 82), and the neurenteric canal opens into the short enclosed

portion behind the notochord (fig. 82), so that it is no longer visible from below. The stomodæum has become widely perforated by the mouth (fig. 78). Externally one pair of visceral clefts is visible (figs. 74, 75). Transverse sections (fig. 78) show them as a pair of outgrowths of the pharynx which do not, however, as yet perforate the epiblast in the specimen of which sections were cut. Just behind the mouth, and lying above the front part of the pericardium, a median ventral groove in the floor of the alimentary canal is conspicuous in transverse sections (fig. 79, *Th.*). It is lined by columnar epithelium, and doubtless represents the commencing thyroid gland.

The two halves of the fore-brain have now completely united, but the line of union can still be recognised in transverse sections as a shallow median furrow between the paired eyes (fig. 77, *L. U.*).

The auditory pits (figs. 74, 75, 79, *Au.*) are now well defined and deep, but still widely open to the exterior.

The optic vesicles of the paired eyes have begun to invaginate to form the optic cups (fig. 77, *Op. C.*), and opposite the mouth of each the superficial epiblast is thickening to form the lens (fig. 77, *Le.*).

The parietal eye makes its first appearance at this stage as a small rounded diverticulum (primary parietal vesicle) of the roof of the fore-brain just to the left of the middle line and, owing to the cranial flexure, on the apparent ventral surface (vide fig. 76, *P. V.*, in which right and left sides are reversed). The number of mesoblastic somites is about twenty-three.

The head-cavities are conspicuous behind the paired eyes even in the opaque embryo (figs. 74, 75, *H. C.*), and sections show that each has begun to divide into two parts (fig. 77, *H. C.*).

Wolffian ducts and tubules are both present in the anterior part of the trunk (fig. 80, *W. D.*, *W. T.*); passing backwards the tubules first disappear, then the ducts also.

In the front part of the trunk a longitudinal blood-vessel is

present on each side in the mesoblast of the amnion, just above the line where the latter joins the body of the embryo (fig. 80, *B. V.*). These vessels probably join the vitelline veins in front, but of this I was unable to make certain, owing to imperfection of some of the sections. In two embryos of this stage the heart was observed to be beating.

One histological feature at this stage seems to deserve notice. In the hinder part of the body the epiblast (fig. 81, *Ep.*) is very conspicuously thickened on the flanks of the embryo, being divided into distinct inner and outer layers, widely separated but connected by strands of protoplasm containing a few nuclei.

Stage L (figs. 83—91).

To this stage I refer four embryos, viz.:

47	Collected on Stephens Island	Nov. 30,	removed from egg	Dec. 16.
50	„	„	„ 30,	„	„ 27.
82	„	„	end of Nov.,	„	„ 27.
84	„	„	„	„	„ 27.

The separation of the serous envelope has extended widely, so that it can be readily torn off nearly all round the yolk-sac as a thin transparent membrane, but it is still adherent to the true amnion above the embryo about the region of the shoulders, at the spot marked with an X in fig. 83.

The living embryo is still almost transparent except for the presence of the red blood, and the beating of the heart was observed in three cases.

The external characters of the embryo are shown in figs. 83 and 84. Not only is the down-bending of the fore-brain very strongly marked at this stage, but there is also a strong ventral flexure in the region of the neck and shoulders, so that the head end of the embryo hangs down into the yolk, enveloped in the pro-amnion. A well-marked flexure in the opposite direction has also made its appearance in the lumbar region.

At the hinder extremity of the body the short tail (figs. 83, 85, *T.*) projects freely into the pleuro-peritoneal cavity between the yolk-sac and the serous envelope, and in No. 50 (fig. 85)

it has already begun to turn downwards and acquire its characteristic spiral twist.

The Wolffian ridges (figs. 83, 85, *W. R.*) have made their appearance, and are quite conspicuous at the hinder end of the body.

The cerebral hemispheres (figs. 83, 90, 91, *C. H.*) have begun to grow out from the fore-brain, causing a marked elongation of the head in front of the eyes.

The primary parietal vesicle (fig. 83, *P. V.*) is prominent, still in the form of a simple hollow outgrowth of the roof of the thalamencephalon, causing a projection of the external epiblast between the paired eyes. The cavity of this vesicle still communicates freely with that of the brain; its wall is composed of a layer of columnar cells, like those forming the brain roof, and is not yet thickened to form a lens.

The auditory vesicles (figs. 83, 84, 91, *Au.*) are almost if not quite closed.

In the paired eyes, pigment is just beginning to be visible in living specimens, but it is not yet noticeable externally in the preserved embryo.

The nasal pits (fig. 83, *Na.*) are just beginning to appear.

The first three visceral clefts are visible externally (figs. 83, 84), and four are recognisable in sections (fig. 91).

Perhaps the most characteristic feature of this stage is the first appearance of the allantois, which is visible externally as a small rounded or finger-shaped outgrowth (figs. 83—86, *All.*) on the ventral aspect of the body beneath the root of the tail and between the two Wolffian ridges, and projecting into the pleuro-peritoneal space above the yolk-sac. It originates as a very thick-walled outgrowth from the posterior enclosed part of the alimentary canal, its walls being continuous behind with the Wolffian ridges. The transverse sections figured (figs. 87—89), of which that represented in fig. 89 is the most anterior, show this mode of origin sufficiently without further explanation.

The closure of the alimentary canal posteriorly has extended forwards for some little distance, and the enclosed portion

opens to the exterior by a narrow aperture (fig. 86, *O. P. A.*) a short way in front of the allantois. No proctodæum is yet recognisable, and the neurenteric canal no longer opens into the alimentary canal below.

No. 47 has about thirty mesoblastic somites recognisable in the stained embryo viewed as a transparent object, but they are very ill-defined at the hinder end, so that it is impossible to count them exactly.

Sagittal sections through the head at this stage are very instructive. It would be out of place to describe them in detail here, but the following features, shown in figs. 90 and 91, are perhaps worthy of mention. In the fore-brain the cerebral hemispheres (*C. H.*) and infundibulum (*Inf.*) are clearly recognisable, the latter projecting from the thalamencephalon towards the pituitary body (*Pit.*). The hind brain (*H. B.*) is partially divided transversely into at least six neuromeres. The notochord extends about as far forward as the pituitary body, its extremity lying above and just in front of the stomodæum and below the anterior part of the hind brain. The pituitary body (*Pit.*) is just commencing its development as a thickening of the front wall of the pharynx, composed of columnar cells and extending forwards between the infundibulum and the front end of the notochord. The two head-cavities (*H. C.*) on each side are very conspicuous. The anterior pair are connected across the middle line by a short transverse canal (fig. 90, *H. C. C.*) which lies just in front of the end of the notochord, while each of the posterior pair gives off a conspicuous branch (*H. C. M.*) into the mandibular arch.

Stage M (figs. 92, 93).

To this stage I refer two embryos, viz. No. 51, collected on Stephens Island on November 30th, 1897, and removed from the egg on January 3rd, 1898; and No. 81, collected about the end of November, and removed from the egg on December 27th, 1897.

No. 51 (figs. 92, 93) may be taken as typical of the

stage. The appearance of the living embryo as seen from above is represented in fig. 93. The embryo beneath the thin transparent serous envelope lies lengthwise in a well-defined, oblong, clear area of the yolk-sac, corresponding more or less closely in extent to the original area pellucida. Outside this clear area the yolk-sac is very heavily laden with yolk, which disappears abruptly at its margin.

The sinus terminalis (*S.T.*) now lies at a considerable distance outside the clear area of the yolk-sac, enclosing an oval space about 10 mm. long by 8·8 mm. broad. Anteriorly its two halves meet to form a large vein which runs backwards and somewhat to the left, turning inwards at the left-hand end of the fold (*Tr. L.*) along which the yolk-sac is invaginated around the anterior end of the embryo to form the outer layer of the pro-amnion. In this vessel the blood flowed in a steady stream towards the heart, which was beating about thirty-eight times per minute. The larger vitelline vessels on the right and left sides can be traced inwards as far as the edge of the clear area of the yolk-sac, where they become lost to view from above, dipping down to follow the splanchnopleure inwards along the floor of the pleuro-peritoneal cavity.

Fig. 92 represents the same embryo seen from below as an opaque object after hardening. The anterior half of the embryo, up to a point just behind the fore-limbs, is pushed into the yolk-sac, and in life hangs freely down into the yolk, enveloped in a thin transparent membrane (the pro-amnion), composed of the true amnion inside, and an outer layer which really belongs to the yolk-sac but which is not vascular. (In hardened specimens the head is turned either to the right or to the left, according to the way in which the embryo happened to lie in the hardening fluid.)

The following features in the embryo itself may be regarded as characteristic of the stage :

The ventral flexure of the body in the region of the shoulders is more strongly marked, so that the head almost touches the allantois (*All.*), which has increased considerably in size.

There are (in No. 51) forty-one mesoblastic somites.

The limbs appear as outgrowths of the Wolffian ridges, the fore-limb (*F. L.*) opposite to mesoblastic somites 9—13 inclusive, the hind limb (*H. L.*) opposite to somites 28—32.

Still only three visceral clefts are visible externally in the opaque embryo, but the visceral arches between them are much more prominent than in the last stage, and the superior maxillary process (*S. M.*) has begun to grow out.

The nasal pits (*Na.*) are much larger and more distinct.

The liver (*Liv.*) begins to form a conspicuous projection just behind the heart.

The proctodæum has formed as an invagination of the epiblast on the ventral surface immediately behind the allantois.

The primary parietal vesicle still remains as a simple sac opening into the cavity of the fore-brain, slightly to the left of the middle line. Its position is again marked, *P. V.*, fig. 92, by a projection between the paired eyes.

Stage N (figs. 94, 95).

To this stage I refer four embryos, numbered 93—97, all collected on Stephens Island about the end of December, 1897, and removed from the eggs on January 6th, 1898.

No. 96 (fig. 94) may be taken as typical of this stage, which is distinguished from the preceding by the following features:

The curvature of the body has increased somewhat.

The limbs are much more conspicuous, and the distal extremity of the fore-limb (*F. L.*) has become flattened to form the hand.

The tail is larger, and more distinctly twisted into a spiral.

The allantois is larger, and has become vascular.

All four visceral clefts are visible externally when the embryo is examined as an opaque object.

The superior maxillary process (*S. M.*) is much larger.

The pigment in the paired eyes is for the first time con-

spicuous externally, forming a broad rim around the lens, interrupted by the choroid fissure.

The parietal eye and its "stalk" are both distinct, the eye lying upon the roof of the brain, in front of and to the left of the forward-pointing "stalk," the cavity of which still communicates widely with that of the brain. The eye is almost, if not quite, separated from the "stalk," though still in close contact with it, and its front wall has begun to thicken to form the lens.

The paraphysis appears as a simple backward-pointing diverticulum of the roof of the fore-brain, some distance in front of the parietal eye, the two being widely separated by the greater part of the thin roof of the thalamencephalon.

Another noteworthy feature of this stage is the condition of the anterior part of the notochord, which is twisted into a spiral beneath the hind brain, while its narrowed tip bends sharply down and ends just in front of the pituitary invagination, as shown in fig. 95.[1]

About this stage also a feature which becomes very conspicuous later on in the yolk-sac outside the embryo begins to make its appearance. This consists in the formation of radial corrugations of the yolk attached to the under surface of the yolk-sac just outside the clear area (compare Stage O, fig. 96, *R. C.*), due to the development of the absorbent vessels in connection with the vitelline circulation. Around these vessels the yolk particles are aggregated as described in the Introduction.

Stage O (figs. 96—100).

To this stage I refer four embryos, viz.:

89, 90 } Collected on Stephens Island about the end of December, 1897, and removed from the eggs on January 6th, 1898.

92. Collected about the same time, and removed from the egg on January 10th.

103. Collected about the same time, and removed from the egg on January 25th.

The following features may be taken as more or less characteristic of this stage:

[1] Cf. Parker, W. R., on the notochord in the embryo of the green turtle 'Challenger Reports,' Zoology, vol. i.

The anterior half of the embryo begins to withdraw from the invaginated yolk-sac, but the extent to which this takes place is not at all constant. Thus in No. 103 (fig. 96) the withdrawal seems hardly to have commenced, while in No. 92 (figs. 97, 100) it has progressed so far that the fore-limb has become completely extricated.

In No. 92 the yolk attached to the under surface of the yolk-sac appears to be spreading inwards, so as to reduce the clear area surrounding the embryo (fig. 97); but in No. 103 (fig. 96) the clear area is as large as in the preceding stage. The attached yolk is more or less strongly corrugated.

The embryo, as its anterior end withdraws from the yolk-sac, comes to lie on its left side, and the allantois, now large and with a well-developed circulation, passes up on the right side and comes to lie above the embryo, between it and the serous envelope (fig. 100).

The alimentary canal has now become completely closed in ventrally, except for a small aperture (figs. 97, 98, *O. Al.*) where the splanchnic stalk passes out to the yolk-sac.

The limbs have elongated, and both manus and pes have become evident (fig. 98), but no digits are yet visible, at any rate externally.

The tail has lengthened considerably, and is coiled inwards in a close spiral on the ventral surface between the hind limbs (fig. 98, *T.*).

The visceral clefts have begun to close up, but the hyomandibular (figs. 96, 98, *H. M.*) is still conspicuous externally.

On the under surface of the head a broad fronto-nasal process (fig. 99, *F. N. P.*) is present, but it does not yet meet the large superior maxillary process (*S. M.*) on either side, so that the external nares (*Na.*) are not yet closed in behind.

The mid-brain is very prominent (figs. 98, 99, *M. B.*), and just in front of it the parietal eye and its "stalk" are clearly visible in the unstained embryo examined as an opaque object, though as yet without pigment. The "stalk" appears in the middle line at the hinder end of the roof of the thalamence-

phalon as a round white spot with a darker centre. The eye itself lies just in front and to the left of this spot, and appears as another round white spot, distinguished from the one behind it by its double outline, the inner circle representing the outline of the lens. The eye still lies close upon the roof of the thalamencephalon, and closely pressed against the "stalk" behind. Its position is indicated by the letters *Pa. E.* in fig. 98.

The paraphysis is still a simple diverticulum of the front part of the roof of the thalamencephalon, but it has begun to be folded.

Stage P (fig. 101).

Of this stage I have only a single specimen, numbered 87, collected on Stephens Island about the end of November, 1897, and removed from the egg on January 25th, 1898. I suspect that this embryo was already dead before the egg was opened, though apparently still in good condition.

The fœtal membranes were evidently somewhat injured, presenting at the time when the drawing was made the arrangement shown in fig. 101.

The embryo has completely extricated itself from the yolk-sac, and lies entirely above it on its left side, so that the pro-amnion has ceased to exist. It remains attached to the yolk-sac, however, by the now greatly elongated splanchnic stalk (*Sta.*) containing the main trunks of the vitelline vessels. The clear area of the yolk-sac around the embryo is in this specimen still very extensive. The amnion (*Am.*) has been ruptured and shrunk away from the front part of the embryo, still, however, partially enveloping the posterior part of the body. Owing doubtless to the rupture and shrinkage of the amnion, the junction between the latter and the serous envelope (*S. En.*) has been pulled backwards instead of lying in its normal position above the shoulders. Later on, at Stage R, the true amnion was again found continuously enveloping the entire embryo, so that its rupture and absence from the anterior portion in this specimen is doubtless due to accident.

The allantois (*All.*) is also in an abnormal condition, being collapsed and shrivelled.

In the body of the embryo itself little advance is visible, at any rate as regards external characters; and, having only a single specimen in a doubtful state of preservation, I have not cut any sections. In the paired eyes the choroidal fissure is still just recognisable. No pigment is yet visible externally in the parietal eye. The fronto-nasal process is still widely separated from the superior maxillary processes, but has developed a small median projection which becomes much more prominent at the next stage. The external opening of the hyomandibular cleft (*H. M. C.*) has become very small, but is still plainly visible. No trace of digits is yet present externally in manus or pes.

In short, the only important difference between this stage and the preceding lies in the complete extrication of the embryo from the yolk-sac, and the accompanying elongation of the splanchnic stalk.

Stage Q (figs. 102, 103).

This stage is again represented by only a single embryo, No. 1, collected on Stephens Island on January 18th, 1897. Unfortunately the eggs sent in this first consignment all dried up in transit, the sand in which they were packed not having been sufficiently moist, and the embryo represented in figs. 101 and 102 was the only one of any service. This embryo was dead when received, but still in a sufficiently good state of preservation as regards external characters.

It exhibits a marked advance upon the preceding stage in the following features:

(1) The fronto-nasal and superior maxillary processes have united so as to complete the upper margin of the mouth, in the middle of which a prominent beak-like projection is visible (fig. 103).

(2) In consequence of this the external nares (fig. 103, *Na.*) have become delimited as a pair of small crescentic apertures.

(3) Five digits appear as blunt projections on the margins of both hand and foot.

(4) The hyomandibular cleft has completely closed.

I am inclined to believe that pigment first appears in the parietal eye at this stage, but the sections are not sufficiently well preserved to be conclusive.

Stage R (figs. 104—107).

Owing to its long duration this stage is represented in my collection by a large number of embryos, viz.:

2—4, collected on Stephens Island on July 1st, 1897, and removed from the eggs about a week later.

141—148, collected on Stephens Island on February 4th, and removed from the eggs on March 8th, 9th, 12th, and 14th, 1898.

151—158, removed from the eggs on April 5th, 1898.

159—161, removed from the eggs on May 12th, 1898.

162, removed from the egg on June 24th, 1898.

169, 170, removed from the eggs on June 14th, 1898.

I have also a number of eggs, doubtless containing embryos of this stage, as yet unopened.

In this stage the embryos pass the winter, entering upon it in February or March, and developing very slowly through the winter months. Embryos removed from the eggs in March almost exactly resemble in external characters those removed in July, the differences not being, in my opinion, sufficient to necessitate a separation into distinct stages. The external differences concern chiefly the appearance of the head as viewed from above, the younger embryos showing distinctly the optic lobes and cerebral hemispheres through the integument, while in the older embryos (fig. 105) these are no longer visible. As regards internal differences I have not made a sufficiently detailed study to say much, but it is noteworthy that while in the younger embryos the parietal eye still lies close up against its so-called "stalk," in the older ones it is separated by a considerable interval from the blind end of the latter, and the nerve of the parietal eye has made its appearance. It is also noteworthy that whereas at the commence-

ment of this stage little or no ossification has taken place, this process has progressed very considerably before its close.

This stage is a very good one at which to describe the condition of the fœtal membranes at what is probably almost, if not quite, the acme of their development. The following account is based chiefly upon dissection of the membranes in Nos. 143 and 149, verified by dissection of others.

The embryo lies on its left side and lengthwise in the shell, and by opening the eggs carefully under water, in which they sink, it was found that there is no air-chamber.

On piercing the shell carefully a small quantity of a thin colourless liquid is squirted out with some force, showing that there must be considerable tension within the egg. This liquid corresponds to the "white" of a hen's egg, but in the Tuatara egg, as already observed, it forms only an extremely thin layer between the shell and the serous envelope.

The serous envelope extends all round inside the shell. Above the embryo it is still connected with the true amnion over a small area in the region of the shoulders, and opposite to this, on the other side of the egg, it becomes continuous with the yolk-sac over another small area.

The true amnion closely envelops the entire embryo as a thin transparent investment.

The allantois is very largely developed, and extends almost completely around the embryo inside the serous envelope. It is filled with a viscid, semi-gelatinous, transparent, colourless liquid, closely resembling the white of a hen's egg. By the presence of this liquid it is greatly distended, so that its outer limb is pushed closely against the serous envelope, while its inner limb is pushed close against the true amnion above and the yolk-sac below. I found it impracticable to pierce the egg-shell without piercing the serous envelope and the outer limb of the allantois at the same time, so that the emission of the thin liquid "white" is immediately followed by a copious oozing out of the viscid contents of the allantois. The tension being thus relieved, it is possible to remove the shell without further injury to the fœtal membranes.

The short stalk of the allantois passes out on the right side of the embryo, and the allantoic vessels at once divide into two sets. One set passes upwards just in front of the right arm in that part of the allantois which adheres closely to the true amnion, while the other passes downwards in that part which adheres to the yolk-sac. The vessels of the upper set are reflected back above the embryo into the outer limb of the allantois, under the serous envelope.

As already stated, the allantois extends almost completely round inside the serous envelope, but it is of course interrupted by the attachment of the latter, on the one hand, to the true amnion above, and on the other to the yolk-sac below.

The yolk-sac is now attached to the ventral surface of the embryo by a slender stalk about 5 mm. long. The vitelline circulation embraces nearly the whole of the yolk, but there is a rounded area, about 6 mm. in diameter, on the side opposite to the embryo, over which the vitelline circulation has not yet extended. Although this non-vascular area is well defined, there is no longer a distinct sinus terminalis. It is in this region, of course, that the serous envelope is still united with the yolk-sac.

The radially columnar structure of the yolk, due to the aggregation of the yolk particles around the absorbent vessels, as described in the Introduction, is now becoming well marked.

For external characters the embryos of July may be taken as typical. They present the following strongly marked advances on the preceding stage:

(1) The head has acquired a markedly Chelonian form, which may be better realised by reference to figs. 104 and 105 than from any description which I can give.

(2) A very conspicuous, sharp-pointed "shell-breaker" (fig. 104, *S. B.*) has been developed on the snout by thickening and cornification of the epidermic cells.

(3) The external nares are represented by a pair of small round white spots (fig. 104, *Na.*), having been completely blocked up by a dense growth of cells which fills the outer part of each nostril, and which is already present at the com-

mencement of this stage. This growth forms a darkly staining mass of tissue, apparently derived from proliferation of the epiblast lining the nostrils, into which it gradually merges.[1] The epiblastic lining of the nostrils is still perfectly sharply defined on its outer aspect from the surrounding mesoblast.

(4) Owing to development of the eyelids the paired eyes have acquired their proper elongated appearance.

(5) Pigment is present in the parietal eye (figs. 104, 105, *Pa. E.*), rendering it conspicuous externally as a small black circle surrounding a white spot. In the younger embryos of this stage, where the outlines of the optic lobes and cerebral hemispheres are still visible externally, the parietal eye is now seen to lie above the apparent centre of the diamond-shaped roof of the thalamencephalon. As far as one can judge, it has become median in position.

(6) Teeth have appeared in both upper and lower jaws, including the palatine teeth, but I have not succeeded in detecting any vomerine teeth.

(7) The tail is to a large extent uncoiled, and lies with its apex pointing backwards against the animal's right side, as shown in fig. 104. A distinct corrugation, indicating the future crest, already appears on its dorsal aspect.

(8) The digits have become greatly elongated, and the limbs have much the same proportions as in the adult.

(9) Pigment has made its appearance in the general integument, forming a very conspicuous and remarkable pattern, as shown in figs. 104 and 105. On the back and sides of the body and tail this pattern takes the form of narrow, discontinuous, longitudinal stripes of white on a grey ground, combined with a less strongly marked development of much broader, transverse bands of white, especially distinct about the root of the tail. The limbs also are marked with longitudinal streaks and dashes of white. In the mid-dorsal line of the body there is a very characteristic narrow moniliform stripe, composed of a row of small white spots which seem to indicate the position of the spines of the future crest. On the dorsal aspect of the head

[1] Cf. Addendum at the end of this paper.

there is very little pigment except for a broad transverse band, incomplete in the middle, which runs inwards from in front of each eye. Around the eyes are well-marked radiating bands of pigment, and on the sides and under surface of the throat and chin are seen irregular longitudinal bands of grey on a white ground. Numerous very minute round white dots are scattered over the upper surface of the head.

In embryo No. 2 the total length measured along the curve of the back from the tip of the snout to the tip of the tail was about 55 mm., of which the tail occupied about 22 mm.

The only internal structures which I propose to speak of at this stage are the parietal eye and its immediate surroundings, and as these will be fully dealt with in a special memoir they need not detain us long here.

By the approximation of the cerebral hemispheres and optic lobes in the straightening out of the cranial flexure the roof of the thalamencephalon has become thrown into folds, and at the same time strongly arched upwards.

At the commencement of this stage the parietal eye lies close over the end of its so-called "stalk" and above the hinder part of the roof of the thalamencephalon. In July embryos, however, it has shifted forwards till it is separated from the end of its "stalk" by a considerable interval, and comes to lie slightly in front of the middle of the thalamencephalon and above the paraphysis, which is now represented by a mass of convoluted tubules, intermingled with blood-vessels, lying between the roof of the thalamencephalon and the parietal eye.

The "stalk" of the parietal eye has elongated considerably, and its lumen has become obliterated at its point of attachment to the brain. Immediately in front of this point the superior commissure[1] has developed.

In the parietal eye itself the "lens" has become very sharply marked off from the rest of the wall, which latter now forms an optic cup clearly differentiated into two layers. In the inner layer, next to the cavity of the eye, pigment has been deposited between the cells, while towards the close of the stage

[1] Erroneously termed the "posterior" in my preliminary notes (4, p. 442).

the nerve of the parietal eye makes its appearance in connection with the outer layer.

A small quantity of pigment also appears on the floor of the hollow distal portion of the "stalk."

Stage S.

To this stage I refer embryos numbered 138, 139, and 140, which reached me on January 6th, 1898, having unfortunately died on the voyage, still enclosed in the shell; and 149 and 150, which are the "skeletons" referred to by Mr. Henaghan[1] as having been found dead on Stephens Island about the middle of January. All these embryos were evidently very nearly ready to hatch when they died; in fact, in the case of Nos. 149 and 150 the shell was already ruptured and the embryo partially extricated. The following description is based upon Nos. 138, 139, and 140, which seem to be a little less advanced.

The animal now closely resembles the adult, except in point of size. The total length from the tip of the snout to the tip of the tail in the specimen measured was 92 mm.; from the cloacal aperture to the tip of the tail 50 mm.; length of head from the tip of the snout to the level of the jaw-angles 15 mm.; width of head between the angles of the jaw 12 mm.

A yolk-sac about 12 mm. in diameter, densely packed with yolk, is still attached to the embryo by a narrow stalk a short way in front of the cloacal aperture, which now forms a large transverse slit.

Scales and claws are present as in the adult. The nuchal and caudal crests of spines are also present, the former as yet very small, the latter well developed; while the dorsal crest is not yet recognisable.

The "shell-breaker" is still present, but relatively small as compared with the preceding stage. It appears to be represented in the adult by the especially large scale at the apex of the snout.

The palatine, maxillary, and posterior mandibular teeth are

[1] Vide Introduction.

present as in the adult. In the front of the mandible the two large cutting teeth of the adult are represented each by three distinct conical, pointed teeth, a larger one behind and two smaller ones in front. These three teeth probably fuse together in later life. Similarly in the premaxillæ each of the two large cutting teeth of the adult is represented by three distinct conical, pointed teeth, of which the outermost is much larger than the other two. These also probably fuse together in later life.[1] I could find no vomerine teeth in the specimen specially investigated as to this particular.

The parietal eye is no longer visible externally, but its position seems to be indicated by a small median tubercular scale lying a short way in front of the first nuchal spine.

The colour of the dead animal is dirty white, irregularly mottled, and banded with grey. The longitudinal striping has disappeared except under the throat and chin, where it still remains distinct, but with the white stripes [2] now narrower than the grey. The transverse banding is still clearly recognisable on the back and tail. The whitish or yellowish spots, so conspicuous in some adult specimens, have not yet appeared; they would seem to be formed by gradual encroachment of the grey pigment over the paler parts of the integument.

4. Summary and Discussion of Principal Embryological Observations.

(*a*) The Formation of the Germinal Layers.

The question of the origin of the germinal layers, and especially of the hypoblast, is one of great difficulty, and I have slightly altered my views on the subject since writing my summary of results (4) some time ago.

The blastoderm spreads around the yolk at an extremely early

[1] As was surmised by the late G. Baur ('Anat. Anz.,' Bd. xi, p. 436); cf. also Günther, 'Phil. Trans.,' 1867, pt. ii, p. 8.

[2] These stripes appear to be represented by longitudinal rows of light-coloured scales on the under surface of the head of the adult animal.

stage, so that already at Stage C, and probably before, the yolk is completely enclosed. This rapid spreading of the blastoderm appears also to be characteristic of lizards[1] and chelonia,[2] and perhaps of reptiles generally as compared with birds.

At Stage C, the earliest examined, the blastoderm is somewhat vaguely divided into area pellucida and area opaca, with the cap-shaped embryo lying in the former (figs. 1, 2, 4).

In the area opaca the blastoderm consists of two distinct layers, an upper epiblast, consisting of a single well-defined layer of flattened or columnar polygonal cells (fig. 4, *Ep.*), and a lower multiple layer of irregular stellate cells with numerous yolk particles entangled in their meshes (fig. 4, *L. L. Y.*). This lower layer evidently corresponds to the lower layer of the chick, though it seems to be thicker and less sharply defined from the yolk. A similar layer in Chelonians is spoken of by Mitsukuri and Ishikawa (5) as "the yolk" containing nuclei; but in Sphenodon it separates readily from the underlying yolk, which is distinguished from it by its want of coherence.

In the area pellucida the epiblast cells gradually become more columnar in character as they approach the embryo, but outside the embryo itself they still remain as a single layer (fig. 4).

The lower layer in the area pellucida becomes divided into two, which are separated from one another by a large cavity extending right across beneath the embryo (fig. 4, *S. C.*). This cavity evidently corresponds to the similar cavity in the chick, termed by Foster and Balfour (9) the segmentation cavity, and by Marshall (7) the subgerminal cavity. According to Weldon (6) it appears to be doubtful whether or not such a cavity occurs in Lacerta.

The floor of the segmentation cavity in Sphenodon is formed by a thin membrane which I have termed the subembryonal membrane, and which is represented by the dotted line in fig. 4. In the earlier stages of development it is

[1] Compare Balfour (8).

[2] Compare Mitsukuri and Ishikawa (5).

conspicuous beneath the embryo after the blastoderm has been removed from the yolk (figs. 2, 16, 19, *S. E. M.*), but it takes no part in the development of the embryo itself. As far as I can judge from the scanty literature at my disposal, it corresponds to the secondary endoderm or yolk layer of Will (13) in the gecko and other vertebrates.

Above the segmentation cavity the lower layer outside the embryo is a multiple layer of irregular cells with little or no yolk in their meshes (fig. 4). At Stage C it appears to pass gradually into the lower layer of the area opaca, but in some sections of later stages the transition appears very sudden, there being a distinct germinal wall at the edge of the area opaca (fig. 14).

In the embryo itself the epiblast is much thickened, and its cells are arranged in a multiple layer. It is especially thick in the region of the head-fold (fig. 4, *H. F.*), where the epiblastic cells appear to be rapidly proliferating. Further back it is thinner, and its cells distinctly prismatic, forming a medullary plate (fig. 4, *M. P.*), which passes behind into the very thick, undifferentiated cellular mass of the primitive streak. Beneath the epiblast of the embryo there is a thick layer of small rounded cells which also passes into the primitive streak, from which, indeed, it cannot be histologically distinguished, and from which it appears to have been derived by forward growth and proliferation (fig. 4). In the region of the head-fold this layer passes into the lower layer of the area pellucida, but whether the cells of the latter actually extend into the embryo at this stage (C) is very doubtful.

Posteriorly the cells of the primitive streak pass into the prismatic epiblast of the area pellucida above, while below this they are continued backwards almost to the edge of the area opaca as a thick compact layer of small rounded cells, in no way distinguishable from the primitive streak itself. This layer in turn becomes continuous with the original lower layer of the blastoderm behind the embryo.

It appears to me that we must regard the lower layer of the embryo itself at this stage (C) as the mesoblast derived from

the primitive streak. It agrees closely in histological character with the primitive-streak mesoblast of the chick as described by Marshall (7), but differs in its much greater forward extension, and in the much larger share which it takes in the formation of the embryo. In this connection it is interesting to note that, according to Foster and Balfour (9), Kölliker holds that the mesoblast of the region of the embryo is derived from a forward growth of the primitive streak.

In Lacerta muralis Balfour states (8) that a layer of mesoblast spreads out in all directions from the primitive streak, and is stated by Kupffer and Benecke to be continuous across the middle line in the region of the embryo. This latter statement Balfour regards as highly improbable, but it certainly agrees with what I have observed in Sphenodon (compare fig. 11).

"The chorda-entoblast" of Hertwig and Mitsukuri and Ishikawa in Trionyx (5), the "axial strip of invaginated hypoblast" of Weldon in Lacerta (6), and the "head-process" of Haswell in the emu (10), all seem to correspond to some extent to the layer under discussion.

Posteriorly the primitive streak passes insensibly into a transverse thickening formed of precisely similar cells, and representing the so-called "sickle," as described by Mitsukuri and Ishikawa (5) in Trionyx. From the sides of the primitive streak, or one might say from the ends of the transverse "sickle," two lateral wings of similar cells (mesoblastic) grow forwards and slightly outwards (at Stage E), outside the embryo proper, and give rise to the vitelline vessels in the vascular area (fig. 20, *L. W.*).

In the region of the primitive streak the dorsal surface of the embryo sinks to the general level of the blastoderm, and here the blastopore is established at Stage C by invagination, some little way behind the medullary plate (fig. 4, *B. P.*). This invagination, in the form of a pit lined by columnar cells, gradually extends downwards, and at Stage F comes to open by a single well-defined aperture into the space (the so-called segmentation cavity) beneath the embryo, which will presently

be enclosed, at any rate in part, in the alimentary canal (cf. fig. 35).

A primitive groove may be recognised both in front of and behind the blastopore (figs. 11, 34, *P. G.*).

The hypoblastic lining of the alimentary canal is probably formed in Sphenodon from flattened cells of the original lower layer of the blastoderm. These cells, at first absent, at any rate in the middle line of the embryo (fig. 4), appear to gradually spread inwards from each side and meet beneath the notochord (figs. 8—14). The layer thus formed corresponds to the "Darm-Entoblast" of Hertwig and Mitsukuri and Ishikawa (5). The latter authors state that in Chelonia (Trionyx) also it passes gradually under the so-called "chorda-entoblast," and fuses in the middle line to complete the upper wall of the digestive cavity.

Mitsukuri and Ishikawa (5) make much of the fact that in Chelonia the mesoblast in front of the blastopore arises as a paired mass from the junction of the "chorda-entoblast" with the "Darm-Entoblast" on each side. I have seen nothing of such a mode of origin in Sphenodon, where, as already noted, it seems to spread forward from the primitive streak as a broad and perfectly continuous sheet (vide fig. 11), the axial portion of which presently separates as the notochord from the lateral portions (vide fig. 33).

As in other cases, however, the mesoblast in Sphenodon certainly has two distinct origins; (1) from outgrowth of the compact rounded cells of the primitive streak, and (2) from loose stellate cells of the original lower layer left between epiblast and hypoblast after the formation of the latter. The first mode of origin is especially conspicuous in the posterior part of the body, but the exact share taken by each in the subsequent development of the embryo I have not determined.

The hypoblastic lining of the digestive cavity, at first consisting of flattened cells, gradually takes on a columnar character. It is noteworthy that this change commences at the anterior end of the body,—in fact, in front of the embryo altogether (fig. 8), and gradually extends backwards (compare

figs. 22—32 and fig. 35). It also takes place later in the middle line, beneath the notochord, than it does laterally.

(*b*) The Formation of the Fœtal Membranes.

The formation of the fœtal membranes in the Tuatara presents several very peculiar features, and appears to me to be by no means of a primitive nature; at the same time it presents no features which are not met with in a more or less highly developed state in other Vertebrate types.

The Pro-amnion.—At Stage C, it will be remembered, the blastoderm of the area pellucida, forming the roof of the large segmentation cavity (or subgerminal cavity) some little way in front of the embryo, is thin and almost free from yolk particles (fig. 4, *Pro. Am.*). This part of the blastoderm is evidently to be regarded as the rudiment of the pro-amnion, but we can hardly say that it consists, as in the chick, of epiblast and hypoblast alone, with no mesoblast, for the lower-layer cells still form a multiple layer, the lowest of which are only just beginning to be doubtfully distinguishable as hypoblastic, while the cells between these and the epiblast might certainly be regarded as mesoblastic, though perhaps it is best to consider the lower layer as being still undifferentiated into mesoblast and hypoblast at all. We may therefore say that the pro-amnion consists at this stage of epiblast and lower layer, the latter being several cells thick.

At Stage D this condition is still maintained, but behind the pro-amnion, though still in front of the embryo, the hypoblast has become clearly differentiated, and a large cœlomic space has appeared in the mesoblast (fig. 8); this, however, does not concern us now.

Between Stages D and E there is somewhat of a gap in the series as regards the formation of the pro-amnion, but it is evident that the head end of the embryo sinks down into the yolk, carrying the pro-amnion with it. It thus comes to project freely beneath the blastoderm, enveloped in a very thin transparent membrane, the pro-amnion (fig. 22). This mem-

branous investment of the head undoubtedly consists really of two layers, epiblast and hypoblast, which have become so stretched and flattened that they can be distinguished from one another in parts only, especially behind (figs. 24—27, 64), while there is no trace of mesoblast, at any rate in the more anterior portion of the pro-amnion. The pro-amnion is, perhaps, best shown in the diagrammatic longitudinal sections of somewhat later stages represented in figs. 35 and 50.

Thus the pro-amnion in Sphenodon forms a far more conspicuous feature than it does in the chick, owing to the much greater extent to which the front end of the embryo sinks down into the yolk. It is much more nearly approached by the condition of the rabbit, as described and figured by Marshall (7), but even here the pro-amnion does not appear to form nearly so prominent a feature as it does in the Tuatara.

According to Mitsukuri (14), as I learn from an abstract in the 'Journal of the Royal Microscopical Society,' in Chelonia also the head-fold sinks in the yolk below, but as I have not been able to see the original memoir I do not know how far this process agrees with what takes place in Sphenodon.

In Sphenodon it is only at a comparatively late stage in the development (Stage O) that the pro-amnion ceases to exist. At this stage the front end of the embryo, enveloped in the true amnion (inner part of the pro-amnion), withdraws from the pocket in the yolk-sac formed by the outer part of the pro-amnion, and thus comes to lie entirely above the yolk-sac.

The Amnion.—In the anterior part of the body the true amnion is formed, as we have just seen, by separation and withdrawal of the somatopleuric portion of the pro-amnion from the splanchnopleuric portion. From about the region of the shoulders backwards it is formed by the uprising of two folds of somatopleure alone, accompanied by a downsinking of the body of the embryo. These two somatopleuric folds (figs. 30 and 55, *Am.*) meet and coalesce in the mid-dorsal line above the embryo in a perfectly normal manner. This process of amnion formation, however, does not cease at the hinder extremity of the embryo, but is continued backwards for some distance behind

the primitive streak so as to give rise to a long narrow canal or tunnel, the posterior amniotic canal, which for some time opens on the surface of the blastoderm behind, and thus maintains a free communication between the amniotic cavity above the embryo and the very small space beneath the shell containing the "white" of the egg (figs. 35, 37, 39, 40, 41, 45, 71—73, *P. A. C.*). The posterior amniotic canal is formed partly by invagination of a specially modified linear strip of epiblast, and partly by uprising of two somatopleuric folds which meet and unite above it (fig. 46). It makes its appearance at Stage F, and disappears by obliteration of its lumen at about Stage K. After the serous envelope has split off from the underlying yolk-sac it lies entirely in the thickness of the former, embedded in the mesoblast (figs. 71—73).

So far as I am aware, the only other type in which anything comparable to the posterior amniotic canal of the Tuatara has been found is the Chelonian type, in which, according to Mitsukuri, the amniotic folds continue to grow backwards, and thus produce a tube which extends back from the posterior end of the embryo and connects the amniotic sac with the exterior. Although my knowledge of Mitsukuri's observations is derived solely from the abstract in the 'Journal of the Royal Microscopical Society' (15), I suppose there can be little doubt as to their close agreement with what I have myself observed in the Tuatara, and we thus have a striking embryological confirmation of the view so strongly insisted upon by Boulenger (16) as to the close relationship of Sphenodon with the Chelonians, a view which has hitherto been based entirely upon the structure of the adult animal.

As to the functions of the posterior amniotic canal I have no suggestions to make, but I understand that Mehnert has published a paper (18) on the subject, to which, unfortunately, I have been unable to obtain access.

The Serous Envelope.—The serous envelope is formed only to a very slight degree by the outer limb of the uprising amnion folds. Throughout nearly its whole extent it is formed by splitting off of the superficial epiblast, accompanied by a

thin layer of mesoblast derived directly from the original lower layer of the blastoderm, from the underlying yolk-sac (cf. figs. 29—32, 47, 50, 55, 68—74). It is doubtful if the separation of the serous envelope from the true amnion is ever quite complete, for at the latest stage at which it was possible to examine the fœtal membranes (Stage R) the two are still connected above the shoulders over a small area. A similar connection has been described by Hirota (**17**) in the case of the chick; while in Chelonians, according to Mitsukuri (**15**), this "sero-amniotic connection" remains much more extensive, and separates the "extra-embryonic cavities of the two halves of the amnion" over the dorsal region of the embryo to the end of development.

The Yolk-sac.—The yolk-sac is formed primarily from what is left of the original lower layer of the blastoderm after the serous envelope has split off from it. It appears, however, that a large portion of the mesoblast derived from the primitive streak spreads into or over it, and gives rise to, at any rate, the commencement of the vitelline vessels (cf. figs. 15—17, 20, 30, 31, *L. W.*). The vitelline circulation (figs. 58, 93, 100, 101) closely resembles that of the chick, though perhaps the absorbent vessels which dip down into the yolk are more strongly developed, and the arrangement of the yolk-spheres around them, like onions on a string, is certainly very striking (fig. 106).

The splanchnopleuric portion of the pro-amnion must also doubtless be regarded as forming part of the yolk-sac, but this has been sufficiently described already.

The Allantois.—The allantois originates at Stage L in a perfectly normal manner as an outgrowth of the ventral wall of the alimentary canal close to the posterior end (figs. 83—89). As development goes on it extends upwards on the right side of the embryo, and comes to lie beneath the serous envelope (fig. 100). Its walls become vascular, and it is very greatly distended by the accumulation within it of a clear, semi-gelatinous liquid. Thus the outer wall of the allantois becomes closely pressed against the serous envelope, and the

inner wall against the amnion above and the yolk-sac below, the two being separated by a wide cavity containing the semi-gelatinous liquid above mentioned. Before the close of development the allantois spreads almost completely around both embryo and yolk-sac, being interrupted at Stage R only by the sero-amniotic connection above the embryo, and by a small area at the opposite side of the egg where the serous envelope has not yet split off from the yolk-sac.

Marshall (7) describes the amniotic cavity of the chick as forming a water-bath in which the embryo can move freely in any direction. In the Tuatara this description is far more applicable to the allantoic cavity, which vastly exceeds that of the amnion in extent.

(*c*) The Modelling of the Body and the Foundation of the Principal Systems.

The Modelling of the Body.—The body of the embryo is first recognisable (Stage C) as a cap-shaped structure (figs. 1, 3, 5), the broad anterior end of which is raised up by the head-fold, while the narrow posterior extremity, formed by the primitive streak, lies at the general level of the area pellucida. The general form of the embryo at this stage thus closely resembles that of Trionyx, as figured by Mitsukuri and Ishikawa (5). The anterior end of the embryo then elongates and becomes at the same time narrowed (Stage D, fig. 6), and as it sinks into the yolk, enveloped in the pro-amnion, it comes to project freely beneath the overlying area pellucida (Stage E, figs. 15—20). It remains in this position until Stage O, when the front end of the embryo is withdrawn from the yolk-sac, and comes to lie on its left side above it.

A distinct tail-fold is not formed until much later than the head-fold, but at Stage J (and perhaps even at Stage H) the whole body of the embryo is clearly outlined by the head-, tail-, and side-folds (figs. 56—58), and by the time Stage K is reached the tail has begun to grow freely backwards between the yolk-sac and the serous envelope (fig. 82). As the tail elongates it becomes coiled inwards in a spiral between the

hind limbs (cf. fig. 98,), but at Stage R this spiral has become to a great extent straightened out, and the hinder part of the tail lies against the animal's right side (fig. 104).

The cranial flexure takes place in the usual way, commencing at Stage J, when the front part of the head, including the fore-brain, is seen to be bent down at right angles (fig. 57). Later on the whole of the front half of the body becomes curved ventrally in a spiral, best seen about Stages M and N (figs. 92, 94), while in the lumbar region there is a slight curvature in the opposite direction (figs. 83, 84, 92, 94). By the time Stage R is reached the curvatures of the body have to a large extent disappeared, and the cranial flexure has straightened out, the head acquiring a marked Chelonian aspect, with a conspicuous "shell-breaker" on the snout (fig. 104). A quite temporary flexure of the head in an upward and backward direction is conspicuous in some of the earlier stages of development, especially Stage G (figs. 48—50); it is caused apparently by the restraining influence of the pro-amnion, and very soon disappears.

The limbs make their appearance as outgrowths of the Wolffian ridges at Stage M (fig. 92), and develop in a perfectly normal manner, five digits appearing on each at Stage Q (figs. 102, 103).

The development of the visceral arches, superior maxillary, and fronto-nasal processes appears to be perfectly normal (cf. figs. 92, 94, 99).

The Central Nervous System.—The development of the central nervous system, so far as investigated, takes place quite in the ordinary manner, except perhaps for the temporary modifications in the form of the brain due to the restraining influence of the pro-amnion.

Already at Stage C the medullary plate (fig. 9, *M. P.*) is recognisable as a flattened area in front of the blastopore, where the epiblast cells are prismatic and arranged in two or three layers. At Stage D (figs. 6, 7, 9, 10) the medullary groove appears as an invagination of the cells of the medullary plate in the mid-dorsal line. At Stage E the front end of the

medullary groove is widened out and constricted into fore-, mid-, and hind brains (figs. 20, 21), the hind brain already exhibiting traces of further segmentation. At Stage F the medullary groove begins to close in the region of the mid-brain (figs. 35, 42). At Stage G the closure has extended backwards and forwards, leaving the medullary groove open above only in two places, viz.—posteriorly, above and in front of the now conspicuous neurenteric aperture; and anteriorly, in the region of the fore-brain (fig. 50). The mid and hind brains are now bent somewhat into the form of an S by the backward and upward tilting of the head due to the restraining influence of the pro-amnion (fig. 50), and, owing doubtless to the same cause, the two halves of the fore-brain are beginning to overlap one another in the middle line (fig. 48). At Stage H the mid- and hind brains are straightened out again, but the overlapping of the left half of the roof of the fore-brain is more conspicuous, while the optic vesicles commence to grow out on either side (fig. 52). At Stage G the medullary canal is completely closed in above, though the left half of the fore-brain still overlaps the right (fig. 60), and the margins of the two do not actually unite until the next stage (fig. 77). At Stage J, also, the fore-brain bends down at right angles, so that the mid-brain comes to occupy the anterior end of the body (figs. 58, 59), and the segmentation of the hind brain is much more strongly marked (fig. 58). The roof of the hind brain also is thinning out in the usual manner (fig. 63). At Stage K the primary parietal vesicle is formed as an outgrowth of the roof of the fore-brain a little to the left of the middle line (fig. 76). At Stage L, by the closure of the neurenteric canal, the central nervous system becomes completely shut in; the cerebral hemispheres begin to grow out from the fore-brain, and the infundibulum and pituitary invagination almost meet one another beneath the thalamencephalon, while the hind brain has become segmented into about six neuromeres (fig. 90). At Stage M the mid-brain has become extremely prominent, and the cerebellum begins to be conspicuous just behind it (fig. 92). At Stage N the paraphysis appears as a

simple, backward-pointing diverticulum of the roof of the fore-brain some considerable distance in front of the parietal eye. At Stage O the cerebral hemispheres and optic lobes are both prominent externally, with the thin roof of the thalamencephalon stretched out between them. Just in front of the optic lobes lie the parietal eye and "stalk," and just behind the cerebral hemispheres lies the pineal gland, with its wall just beginning to be folded. At Stage R the cranial flexure has become straightened out, and the cerebral hemispheres and mid-brain much more closely approximated to one another, so that the roof of the thalamencephalon has become shortened and folded, giving rise to the choroid plexus; while the paraphysis and parietal eye, which develop quite independently of one another and are never really connected, are brought close together. The paraphysis has now given rise to a convoluted mass of tubules, intermingled with blood-vessels, which comes to lie beneath the parietal eye and in front of its so-called "stalk." Meanwhile the superior commissure has made its appearance just in front of the point where the stalk of the parietal eye joins the brain. The details of the development of these organs, however, are beyond the scope of the present paper, and will be dealt with in a later memoir.

The Ordinary Paired Eyes.—The development of the paired eyes, so far as investigated, is shown in fig. 77, from which it will be seen that it takes place in the normal vertebrate fashion. The optical vesicle is invaginated to form an optic cup, and the lens is formed as a thickening of the superficial epiblast opposite to the mouth of the cup.

The Parietal Eye.—The development of this organ will form the principal subject of the special memoir already referred to, but it may be worth while to summarise the principal facts in this place. It is formed from the primary parietal vesicle, the origin of which as an outgrowth from the roof of the fore-brain slightly on the left of the middle line has been noted. The front part of the wall of this vesicle thickens to form the lens, while the deeper part gives rise to the retina. The latter forms a deep optic cup which holds the lens in its mouth, the

two being sharply distinguished from one another though remaining in close contact. The wall of the optic cup divides into two primary layers, in the inner of which pigment is deposited, while the outer becomes connected with the brain by the special nerve of the parietal eye, which is not formed from the so-called stalk. The so-called "stalk" of the parietal eye probably represents the right parietal eye retarded in development by the overlapping of the left half of the roof of the fore-brain, and in an extremely degenerate condition.

The Ears.—The development of the auditory organs, so far as followed, is quite normal. The auditory pits appear about Stage J as shallow depressions of the superficial epiblast on either side of the hind brain, the epiblast being here composed of a single layer of elongated columnar cells (figs. 58, 64). These pits gradually deepen (fig. 79), and about Stage L they entirely close up, forming two sacs, each still lined by a single layer of columnar cells (fig. 91, *Au.*). Further than this their development has not been followed.

The Olfactory Organs.—The nasal pits first appear as shallow depressions of the superficial epiblast at about Stage L (fig. 83, *Na.*). They have the usual crescentic outline, open towards the mouth (figs. 92, 94, *Na.*). As development goes on they gradually deepen, and with the downgrowth of the fronto-nasal process, commencing about Stage O (fig. 99), the external nares become defined in the usual manner as two crescentic apertures the margins of which are completed at Stage Q (fig. 103). At Stage R a very remarkable feature makes its appearance, the nostrils being completely plugged up by a dense cellular mass derived from proliferation of their epiblastic lining. The external nares now appear from the outside as two small round white spots (Fig. 104, *Na.*), being filled up to the level of the surrounding epidermis with the plug of cells just mentioned. This remarkable plugging up of the nostrils takes place about the commencement of the long winter rest which the embryo passes through at Stage R, and probably bears some relation to this process of hibernation. I have no material sufficiently well preserved to enable me to state when

the plug of cells is again removed, but this probably takes place at Stage S, shortly before hatching.

We may, perhaps, compare the plugging up of the nostrils in the Tuatara with the remarkable solidification of the œsophagus which takes place at a certain stage in the development of the tadpole and other Vertebrates (Marshall [7]).[1]

The Alimentary Canal.—The development of the alimentary canal takes place in the usual way by gradual infolding of the splanchnopleure. The front part is enclosed first (figs. 9, 10, 23, 24, 35), and for some time remains filled with yolk particles (figs. 52, 59). The stomodæum is established in the ordinary way by a shallow invagination of the superficial epiblast at Stages J and K (figs. 57, 63, 78). The proctodæal invagination is formed considerably later, commencing at about Stage M.

Of the outgrowths to which the alimentary canal gives rise I have investigated only the allantois (which has been already dealt with), the visceral clefts, and the commencement of the pituitary body and of the thyroid gland.

The visceral clefts are four in number, and appear to develop very much in the usual way as outgrowths of the hypoblastic lining of the front part of the alimentary canal (cf. figs. 63, 78, 91). The first becomes visible externally about Stage K (figs. 74, 75). At Stage L three are thus visible (figs. 83, 84). At Stage N all four can be seen from the outside (fig. 94), and at Stage O they begin to close up again (figs. 96, 98), though the hyomandibular remains visible externally till Stage P (fig. 101).

The pituitary body was first observed at Stage L as a thickening of the lining epithelium (presumably derived from the stomodæum) beneath the anterior extremity of the notochord (fig. 90). At Stage N it forms a deep pit with a very thick wall composed of columnar cells (fig. 95).

The thyroid gland is visible at Stage K as a median ventral

[1] Cf. de Menron, 'Compt. Rend.,' tom. cii, p. 1401 ; and 'Rec. Zool. Suisse,' tom. iii.

groove in the floor of the alimentary canal (fig. 79, *Th.*) just behind the mouth, lined by columnar cells.

The Notochord.—The notochord appears at Stage E, being formed by the separation of an axial rod of cells from the thick sheet of mesoblast which grows forwards from the primitive streak (figs. 26—33, 35, *Not.*). Thus the notochord in Sphenodon appears to be undoubtedly of mesoblastic origin, an origin which, it will be remembered, is also claimed for it by some writers (e. g. Duval [11]) in the chick. It extends forwards to within a short distance of the infundibulum, and in the later stages of its development its anterior end becomes curiously twisted in an irregular spiral (fig. 95, Stage N), which is recognisable even after the cartilage has developed around it (Stage R).

The Cœlomic Cavities.—The first indication of cœlomic cavities appears extremely early, at Stage D, in the form of a wide space in the mesoblast of the area pellucida in front of and entirely outside the embryo itself (fig. 8, *P. C.*). This space appears to be the rudiment of the pericardial cavity which is so conspicuous in later stages, but which is not for a long time shut off from the general body-cavity of the adult. As the head-fold deepens the cœlomic space in question is carried back, and comes to lie just in front of the opening into the anterior enclosed part of the alimentary canal (fig. 50, *P. C.*). It is possible that it does not always appear so early as Stage D, for in embryo 61 (Stage F, fig. 35) I observed no trace of it, and at Stage G (fig. 50) it appears as a space between epiblast and hypoblast, with no mesoblast around it. Possibly it normally appears very early, and is then more or less completely obliterated, perhaps by stretching in the formation of the pro-amnion, to open out again later on. Already at Stage H, however, it forms a conspicuous quadrangular sac containing the heart, lying beneath the anterior enclosed part of the alimentary canal (fig. 52).

Sections of embryos of Stage J, in which the pericardial cavity is even more conspicuous (fig. 59), clearly show that it is in free communication with the general body-cavity above,

on either side of the alimentary canal (fig. 66). The cœlom within the embryo develops in the ordinary manner as a split between the somatopleuric and splanchnopleuric layers of mesoblast (cf. figs. 66—69); this is directly continuous with the large pleuro-peritoneal space outside the embryo, which develops chiefly as a split between the serous envelope and the yolk-sac (cf. figs. 68—72).

The head cavities appear at Stage J as two small spaces in the mesoblast just outside the aortic dilatations on either side of the anterior extremity of the alimentary canal (fig. 62). At Stage K they are visible from the outside as dark patches behind the paired eyes (figs. 74, 75), and each has begun to divide into anterior and posterior portions (fig. 77). At Stage L they have become very large (fig. 91), and those of the anterior pair are connected across the middle line by a short transverse canal (fig. 90, *H. C. C.*), while each of the posterior pair gives off a conspicuous branch into the mandibular arch (fig. 91, *H. C. M.*). The head cavities are lined by short columnar cells.

The Mesoblastic Somites.—The vertebral and lateral plates of mesoblast appear to be derived, at any rate mainly, from the great sheet of mesoblast which grows forwards from the primitive streak (compare Stage D, figs. 11 and 12; Stage E, figs. 29—34; Stage J, figs. 68—71). Transverse sections of embryos of Stage E already, perhaps, show indications of the division of the mesoblast into vertebral and lateral plates (figs. 29—31 and 33), but it is not until Stage H that the vertebral plate begins to be segmented into mesoblastic somites or protovertebræ. The mesoblastic somites do not appear with the same regularity as in the chick, so that they are of little use for the purpose of classifying the embryos in stages. Generally speaking, they may be said to develop from before backwards, though a number of them seem to appear almost or quite simultaneously at first. The cœlomic split between somatopleure and splanchnopleure at first extends into them (fig. 69), but they soon separate completely from the lateral plates, forming squarish blocks composed of radially elongated columnar cells (fig. 68).

The Wolffian Ducts and Tubules.—These organs first appear at Stage J. Their development has not been followed in any detail, but appears to take place very much as described by Weldon (6) in Lacerta (vide figs. 68, 69, 80).

The Vascular System.—The heart was first recognised at Stage H (fig. 52) as a somewhat pear-shaped sac, lying in the pericardium, receiving the two vitelline veins behind, and giving off the short bulbus arteriosus in front. It probably originates as a split in the splanchnopleuric mesoblast. At Stage J, when it was first observed in sections, it looks like a ventral diverticulum of the splanchnopleuric mesoblast, here composed of short columnar cells, containing two thin-walled epithelioid tubules (fig. 66), continuous with the vitelline veins behind, and uniting in front to form the bulbus arteriosus, while the whole heart has begun to be bent into the form of an S (fig. 59). A full description of the circulation at Stage J has already been given, and need not be repeated; it shows no features of special interest, except perhaps the enormous dilatation of the primitive aortæ on either side of the anterior extremity of the alimentary canal (fig. 62). Beyond Stage J the development of the vascular system within the body has not been worked out. The development of the vitelline vessels has already been referred to in dealing with the yolk-sac.

The Teeth.—The only feature of special interest observed in connection with the teeth concerns the development of the two large cutting teeth which are so conspicuous in the front part of each jaw of the adult. At Stage S, shortly before hatching, each of these is represented by three distinct, pointed, conical teeth. No vomerine teeth were observed.

(*d*) The Embryonic Colour Markings.

One of the most remarkable features of the development is the appearance at Stage R of a well-defined embryonic pattern on the integument totally different from that of the adult. This pattern consists mainly of two distinct series of markings: (1) a series of narrow, discontinuous, longitudinal stripes of

white on a grey ground; and (2) a series of less well-defined, much broader transverse bands of white (figs. 104, 105). At Stage S, shortly before hatching, the longitudinal striping has disappeared except under the throat and chin, but the transverse banding is still clearly recognisable on the back and tail. The small whitish or yellowish spots, so characteristic of some adults, have not yet appeared, but the whole body is dirty white, irregularly mottled, and banded with grey. Thus the order in which the markings appear seems to be (1) longitudinal stripes, (2) transverse bands, and (3) spots. The stage at which longitudinal stripes are present without the transverse bands was not actually observed, but as most of the longitudinal stripes disappear before the transverse ones, and are much better defined than the latter at Stage R, we may assume that they also appear before them. These observations are to a large extent in agreement with the conclusions of Eimer (19) and others as to the colour-markings of animals in general, and especially of mammals; but in the latter group, according to Eimer, the spots arise before the cross-stripes. Eimer also observes that the old features linger longest on the fore-parts, as is also the case with the longitudinal striping of the Tuatara, indications of which remain visible on the under surface of the head even in the adult animal sometimes, if not always.

Addendum.

Since this manuscript was forwarded to England I have found that a precisely similar plugging-up of the nostrils has been described by Parker (20, pp. 61, 64, 65, and 111) in the embryo of Apteryx. This fact seems to show that the plugging has no connection with the hibernation of the embryo. It is a most singular coincidence that this strange condition should have been observed in two animals so widely separated zoologically, and yet both occurring in New Zealand.

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6. DESCRIPTION OF PLATES 1—10.

Illustrating Mr. Arthur Dendy's paper on "Outlines of the Development of the Tuatara, Sphenodon (Hatteria) punctatus."

List of Reference Letters.

Al. C. Alimentary canal. *All.* Allantois. *Am.* Amnion. *Am. C.* Amniotic cavity. *Am. O.* Amniotic opening. *Am. S.* Slit along which amnion is not yet closed. *A. O.* Area opaca. *A. P.* Area pellucida. *Au.* Auditory pit (and vesicle). *B. Ar.* Bulbus arteriosus. *B. I.* Blood island. *B. P.* Blastopore. *B. V.* Blood-vessel. *Cb.* Cerebellum. *C. H.* Cerebral hemispheres. *D. A.* Dorsal aorta. *Elb.* Elbow. *Ep.* Epiblast. *Ep. T.* Epithelioid tubule in heart. *Eye.* Ordinary paired eye. *F. B.* Fore-brain. *F. L.* Fore-limb. *F. Sp.* Fold where the hypoblast turns round to run forwards, marking the posterior limit of the anterior enclosure of the alimentary canal. *G. W.* Germinal wall. *H. B.* Hind brain. *H. C.* Head cavity. *H. C. C.* Connection between the two anterior head cavities. *H. C. M.* Branch of head cavity in mandibular arch. *H. F.* Head-fold. *H. L.* Hind limb. *H. M.* and *H. M. C.* Hyomandibular cleft. *Ht.* Heart. *Hyp.* Hypoblast. *I. C. M.* Intermediate cell mass. *Inf.* Infundibulum. *L. A. S.* Line along which amnion and serous envelope are united. *Le.* Lens of paired eye. *L. F. B.* Left half of roof of fore-brain overlapping right half. *Liv.* Liver. *L. Ju.* Line of junction of the two uprising amniotic folds above the medullary groove. *L. L.* Lower-layer cells of blastoderm. *L. L. M.* Mesoblast derived directly from lower-layer cells of blastoderm. *L. L. Y.* Lower-layer cells with yolk. *L. U.* Line along which the two halves of the roof of the fore-brain have united. *L. W.* Lateral wing or sheet of mesoblast growing out from the primitive streak. *Mand.* Mandibular arch. *M. B.* Mid-brain. *M. C.* Medullary canal. *Mes.* Mesoblast. *Mes. E.* Epithelioid mesoblast on under surface of serous envelope. *M. G.* Medullary groove. *Mo.* Mouth. *M. P.* Medullary plate. *M. S.* Mesoblastic somite. *Na.* Nasal pit. *N. En.* Neurenteric canal. *Not.* Notochord. *O. Al.* Ventral opening of unenclosed part of alimentary canal. *O. L.* Optic lobe. *O. P. A.* Opening into posterior enclosed part of alimentary canal. *Op. C.* Optic cup of paired eye. *Op. S.* Optic stalk of paired eye. *O. V.* Optic vesicle of paired eye. *P. A. C.* Posterior amniotic canal. *Pa. E.* Parietal eye. *P. Ao.* Primitive aorta. *P. C.* Pericardium (or cœlomic space which will give rise to pericardium). *P. G.* Primitive groove. *Ph.* Pharynx. *Pit.* Pituitary body. *P. P. S.* Pleuro-peritoneal space. *Pro. Am.* Pro-amnion. *Pr. S.* Primitive streak. *P. S. M.* Mesoblast derived from primitive streak. *P. V.* Primary parietal vesicle. *R. C.* Radial corrugations in the

yolk attached to the under surface of the yolk-sac. *R. M.* Roof of mid-brain. *S. B.* Shell-breaker (patch of cornified epidermis on snout). *S. C.* Segmentation cavity (subgerminal cavity). *S. E. M.* Sub-embryonal membrane. *S. En.* Serous envelope. *Si.* "Sickle," = mass of mesoblast growing out from the primitive streak behind. *S. M.* Superior maxillary process. *Som. P.* Somatopleure. *Sp. C.* Spinal cord. *Spl. P.* Splanchnopleure. *S. T.* Sinus terminalis. *Sta.* Stalk with vitelline vessels attaching embryo to yolk-sac. *Stom.* Stomodæum. *S. V.* Segmental vesicle. *T.* Tail. *Th.* Thyroid gland. *Thal.* Thalamencephalon. *Tr. L.* Transverse line along which the anterior end of the embryo is attached to the overlying blastoderm, and where the yolk-sac turns forwards to form the outer layer of the pro-amnion. *V. A.* Vitelline artery. 1 *V. A.* First visceral arch. 2 *V. A.* Second visceral arch. 3 *V. A.* Third visceral arch. 4 *V. A.* Fourth visceral arch. 5 *V. A.* Fifth visceral arch. 1 *V. C.* First visceral cleft (hyomandibular). 2 *V. C.* Second visceral cleft. 3 *V. C.* Third visceral cleft. 4 *V. C.* Fourth visceral cleft. *V. V.* Vitelline veins. *V.* 4. Fourth ventricle, *W. D.* Wolffian duct. *W. R.* Wolffian ridge. *W. T.* Wolffian tubule. *X.* (Fig. 47). Junction of clear and yolk-laden areas of yolk-sac. *X.* (Fig. 83). Spot where serous envelope is still united with true amnion. *Y.* Yolk. *Y. S.* Yolk-sac.

Note.—The microscopic drawings have in almost all cases been made with the aid of Abbé's camera lucida.

FIGS. 1—5. Stage C.

FIG. 1.—Embryo 9, upper surface, with portion of surrounding blastoderm; drawn from spirit specimen as an opaque object. × 10.

FIG. 2.—Embryo 9, lower surface, with the sub-embryonal membrane partly torn away; drawn as before. × 10.

FIG. 3.—Embryo 9, seen from above as a transparent object after staining with borax carmine and clearing in oil of cloves. Zeiss A (with the bottom lens removed), ocular 1.

FIG. 4.—Embryo 9, longitudinal vertical section, passing through the head-fold in front and the blastopore behind. The dotted line indicates the position of the sub-embryonal membrane. Zeiss A, ocular 1.

FIG. 5.—Embryo 5 (slightly younger than 9, before the formation of the blastopore), seen from above as a transparent object in Canada balsam, after staining with borax carmine. Zeiss A (with the bottom lens removed), ocular 1.

FIGS. 6—14. Stage D.

FIG. 6.—Embryo 58, upper surface, with portion of surrounding blastoderm; drawn from spirit specimen as an opaque object. × 10.

FIG. 7.—Embryo 58, seen from above as a transparent object after staining with borax carmine and clearing in oil of cloves. Zeiss A (with the bottom lens removed), ocular 1.

Figs. 8—14.—Transverse sections of embryo 58, arranged in order; drawn under Zeiss A, ocular 1.

Fig. 8.—Through the blastoderm in front of the embryo, showing the large cœlomic space in the mesoblast (*P. C.*).

Fig. 9.—Through the anterior portion of the embryo lying freely above the blastoderm.

Fig. 10.—Through about the middle of the embryo, showing the alimentary canal still widely open below.

Fig. 11.—Through the medullary plate and primitive groove.

Fig. 12.—Through the primitive streak just in front of the blastopore.

Fig. 13.—Through the blastopore.

Fig. 14.—Through the primitive streak just behind the blastopore. (This figure includes part of the area opaca on each side, which is omitted from the others).

Figs. 15—34. Stage E.

Fig. 15.—Embryo 56, upper surface, with portion of surrounding blastoderm; drawn from spirit specimen as an opaque object. × 10. (The anterior portion of the embryo now lies beneath the blastoderm of the area pellucida, through which it is seen.)

Fig. 16.—Embryo 56, seen from below after removal of nearly the whole of the sub-embryonal membrane, showing the anterior half of the embryo projecting freely beneath the blastoderm of the area pellucida; drawn as before. × 10.

Fig. 17.—Embryo 56, seen from above as a transparent object, the front half showing through the blastoderm of the area pellucida; after staining with borax carmine and clearing in oil of cloves. Zeiss A (with the bottom lens removed), ocular 1.

Fig. 18.—Embryo 64, seen from above, with portion of surrounding blastoderm; drawn from spirit specimen as an opaque object. × 10.

Fig. 19.—Embryo 64, seen from below after removal of the sub-embryonal membrane from beneath the anterior end of the embryo, while the posterior part is concealed by this membrane and the adherent yolk; drawn as before. × 10.

Fig. 20.—Embryo 64, seen from above as a transparent object, after staining with borax carmine and clearing in oil of cloves. The front part of the embryo is seen through the overlying blastoderm. Zeiss A (with the bottom lens removed), ocular 1.

Fig. 21.—Embryo 64, anterior end seen from below, as before.

Figs. 22—32.—Transverse sections of embryo 56 arranged in order; drawn under Zeiss A, ocular 1. (In Figs. 23—26 the overlying blastoderm is omitted.)

Fig. 22.—Through the head in front of the alimentary canal. The overlying blastoderm (*A. P.*) is shown above.

Fig. 23.—Through the anterior enclosed part of the alimentary canal, in front of the cœlomic space, which will give rise to the pericardium.

Fig. 24.—Through the cœlomic space (*P.C.*), which will give rise to the pericardium.

Fig. 25.—Just behind the opening into the anterior enclosed part of the alimentary canal.

Fig. 26.—Through the anterior end of the notochord.

Fig. 27.—Just behind the spot where the front end of the embryo, enclosed in the pro-amnion, becomes free from the underlying blastoderm (compare Fig. 35, *Tr. L.*).

Fig. 28.—Through the trunk region.

Fig. 29.—Through the trunk further back, showing the very small amniotic cavity overlying the medullary groove.

Fig. 30.—Through the posterior amniotic opening, showing the two uprising folds of the amnion not yet united.

Fig. 31.—Just in front of the primitive streak.

Fig. 32.—Through the neurenteric canal (*N. En.*).

Fig. 33.—Embryo 64. Transverse section just in front of the posterior amniotic opening. Zeiss C, ocular 1.

Fig. 34.—Embryo 64. Transverse section through the posterior amniotic opening and primitive streak, showing the primitive groove (*P. G.*). Zeiss C, ocular 1.

Figs. 35—46. Stage F.

Fig. 35.—Embryo 61. Median longitudinal vertical section, slightly diagrammatic.

Fig. 36.—Embryo 61. Similar section a little to one side of the middle line.

Fig. 37.—Embryo 72, seen from above, with portion of surrounding blastoderm; drawn from spirit specimen as an opaque object, the front part of the embryo being seen through the overlying blastoderm of the area pellucida. × 10.

Fig. 38.—Embryo 72, seen from below after removal of the sub-embryonal membrane and adherent yolk; drawn as before. × 10.

Fig. 39.—Embryo 72, seen from above as a transparent object, after staining with borax carmine and clearing in oil of cloves. The front part of the embryo is seen through the overlying blastoderm. Zeiss A (with bottom lens removed), ocular 1.

Fig. 40.—Embryo 61, seen from above, with portion of the surrounding blastoderm; drawn from spirit specimen as an opaque object; the front part of the embryo seen through the overlying blastoderm of the area pellucida. × 10.

Fig. 41.—Embryo 61, seen from above as a transparent object, after staining

with borax carmine and clearing in oil of cloves; the front part of the embryo seen through the overlying blastoderm of the area pellucida. Zeiss A (with bottom lens removed), ocular 1.

Figs. 42—46.—Transverse sections of embryo 72, arranged in order.

Fig. 42.—Through the mid-brain. Zeiss A, ocular 1.

Fig. 43.—Through the hind brain, overlapped by the roof of the mid-brain. Zeiss A, ocular 1.

Fig. 44.—Through the hind brain further back. Zeiss A, ocular 1.

Fig. 45.—Through the posterior amniotic canal (*P. A. C.*). Zeiss C, ocular 1.

Fig. 46.—Through the opening of the posterior amniotic canal on the surface of the blastoderm. Zeiss C, ocular 1.

Figs. 47—50. Stage G.

Fig. 47.—Embryo 59, seen from below as an opaque object in spirit. The yolk-sac (*Y. S.*) is partly torn away from beneath the serous envelope (*S. En.*). × 10.

Fig. 48.—Embryo 59, seen from above as a transparent object, after staining with borax carmine and clearing in oil of cloves. (The broad dark line across the embryo at *Tr. L.* is due to tearing and crumpling of the yolk-sac.) Zeiss A (with bottom lens removed), ocular 1.

Fig. 49.—Embryo 59, seen from below, as before.

Fig. 50.—Embryo 59. Median longitudinal vertical section, slightly diagrammatic. (The posterior amniotic canal lies to one side of the middle line in this specimen, and is therefore not shown. The separation of the serous envelope and yolk-sac in front is exaggerated for this stage.)

Figs. 51, 52. Stage H.

Fig. 51.—Embryo 78, seen from below with portion of the surrounding blastoderm as an opaque object in spirit. At the bottom right-hand corner the thick, blanket-like yolk-sac (*Y. S.*) is turned up to show the overlying serous envelope (*S. En.*). × 10.

Fig. 52.—Embryo 78, anterior half, seen from below as a transparent object, after staining with borax carmine and mounting in Canada balsam. Zeiss A, ocular 1.

Figs. 53—73. Stage J.

Fig. 53.—Embryo 60, seen from above as an opaque object in spirit, with portion of the surrounding blastoderm; the body of the embryo is seen dimly through the 8-shaped clear area and serous envelope. × 10.

Fig. 54.—Embryo 46, seen from above as an opaque object in spirit, with portion of the surrounding blastoderm as before, but the serous envelope (*S. En.*) is removed from the bottom left-hand corner, showing the underlying yolk-sac (*Y. S.*). × 10. (This embryo is exceptional in having the amnion

incompletely formed dorsally, leaving a slit-like opening (*Am.S.*) into the amniotic cavity.)

Fig. 55.—Embryo 46. Transverse section through the dorsal amniotic opening, a little in front of the neurenteric canal. Zeiss A, ocular 1.

Fig. 56.—Embryo 44, seen from above as an opaque object in spirit, with portion of surrounding blastoderm. The embryo itself is seen dimly through the fœtal membranes. × 10.

Fig. 57.—Embryo 44, drawn from below, as before. × 10.

Fig. 58.—Embryo 44, seen from above through the fœtal membranes as a transparent object, after staining with borax carmine and clearing in oil of cloves. Zeiss A (with bottom lens removed), ocular 1.

Fig. 59.—Embryo 44, anterior half, drawn from below as before. Zeiss A, ocular 1.

Figs. 60—72.—Transverse sections of embryo 44, arranged in order; drawn under Zeiss A, ocular 1.

Fig. 60.—Through the fore-brain, where the left half of its roof overlaps the right.

Fig. 61.—Through the optic vesicles of the paired eyes.

Fig. 62.—Through the anterior enclosed part of the alimentary canal in front of the stomodæum.

Fig. 63.—Through the stomodæum and hind brain.

Fig. 64.—Through the hind brain and auditory pits.

Fig. 65.—Through the first pair of mesoblastic somites, heart and pericardium.

Fig. 66.—Through the heart further back.

Fig. 67.—Through the fourth pair of mesoblastic somites, showing the alimentary canal widely open below and the vitelline veins on each side.

Fig. 68.—Through the sixth pair of mesoblastic somites.

Fig. 69.—Through the twelfth pair of mesoblastic somites.

Fig. 70.—Through the ventral opening of the neurenteric canal.

Fig. 71.—Through the primitive streak.

Fig. 72.—Through the pleuro-peritoneal cavity behind the embryo, to show the posterior amniotic canal (*P. A. C.*) lying in the thickness of the serous envelope.

Fig. 73.—Part of the last section more highly magnified, to show the posterior amniotic canal lying in the serous envelope.

Figs. 74—82. Stage K.

Fig. 74.—Embryo 39, seen from above as an opaque object in spirit, with the fœtal membranes torn away from the front half. × 10.

Fig. 75.—Embryo 39, seen from below, as before. × 10.

Figs. 76—82.—Transverse sections of embryo 39, arranged in order; drawn under Zeiss A, ocular 1.

Fig. 76.—Through the mid-brain and fore-brain, showing the primary parietal vesicle (*P. V.*) on what is really the left side of the middle line.

Fig. 77.—Through the hind brain and fore-brain, showing the development of the ordinary paired eyes.

Fig. 78.—Through the hind brain and stomodæum.

Fig. 79.—Through the hind brain and auditory pits.

Fig. 80.—Through the trunk, showing the Wolffian ducts and tubules.

Fig. 81.—Through the trunk further back, showing the thickening of the epiblast on the flanks of the embryo.

Fig. 82.—Through the neurenteric canal, placing the hinder part of the medullary canal in communication with the posterior enclosed part of the alimentary canal.

Figs. 83—91. Stage L.

Fig. 83.—Embryo 47, seen from above as an opaque object in spirit. The yolk-sac and serous envelope have been removed from above the head and on the right. × 10.

Fig. 84.—Embryo 47, from below, as before. × 10.

Fig. 85.—Embryo 50, hinder end, seen from above as a transparent object, after staining with borax carmine and clearing in oil of cloves. To show the origin of the allantois.

Fig. 86.—Embryo 50, hinder end, seen from below, as before.

Figs. 87—89.—Transverse sections of embryo 50, selected and arranged in order to show the origin of the allantois; Fig. 87 being the most posterior, and Fig. 89 the most anterior. Zeiss A, ocular 1.

Fig. 90.—Embryo 50. Longitudinal section through the head, nearly in the median plane, but not quite truly vertical; showing the notochord and pituitary body. Zeiss A, ocular 1. (The section passes a little on one side of he communication between mid- and hind brains.)

Fig. 91.—Embryo 50. Similar section on one side of the median plane, showing visceral clefts and head cavities. Zeiss A, ocular 1.

Figs. 92, 93. Stage M.

Fig. 92.—Embryo 51, seen from below as an opaque object in spirit. (The Wolffian ridge and hind limb were not really seen until after the removal of the yolk-sac.) × 10.

Fig. 93.—Embryo 51, sketched from above while alive to show the vitelline circulation, seen through the serous envelope. × 10.

Figs. 94, 95. Stage N.

Fig. 94.—Embryo 96, seen from below as an opaque object in spirit. The yolk-sac and serous envelope have been removed on the left side of the figure. × 10.

Fig. 95.—Embryo 96. Part of a median longitudinal vertical section through the head, showing the notochord and pituitary body. Zeiss C, ocular 1.

FIGS. 96—100. Stage O.

FIG. 96.—Embryo 103, seen from below, with the fœtal membranes intact, as an opaque object in spirit. × 5.

FIG. 97.—Embryo 92, seen from below as an opaque object in spirit, showing the front end partially withdrawn from the invaginated yolk-sac. × 5.

FIG. 98.—Embryo 92, from the left side, after removal of the fœtal membranes, seen as an opaque object in spirit. × 5.

FIG. 99.—Embryo 92. Ventral view of head, seen as an opaque object in spirit. × 10.

FIG. 100.—Embryo 92. Sketch of living embryo from above, to show allantois, allantoic and vitelline circulations. (The arrows show the direction in which the blood was flowing; only the veins were conspicuous: the transparent serous envelope is not indicated.) × 5.

FIG. 101. Stage P.

FIG. 101.—Embryo 87, drawn from above (right side of embryo) as an opaque object in spirit, to show the complete extrication of the front part of the embryo from the yolk-sac. The amnion and serous envelope have been partially removed, and the allantois has shrivelled. × 5.

FIGS. 102, 103. Stage Q.

FIG. 102.—Embryo 1, drawn from the right side as an opaque object in spirit, after removal of the fœtal membranes. × 5.

FIG. 103.—Embryo 1, drawn as before, but from the left side. × 5.

FIGS. 104—107. Stage R.

FIG. 104.—Embryo 2, drawn from the right side as an opaque object in spirit, after removal of the fœtal membranes. × 5.

FIG. 105.—Embryo 2, drawn from above, as before. × 5.

FIG. 106.—Embryo 3. Portion of yolk-sac, with yolk particles adhering to the absorbent vessels so as to give rise to a radially columnar structure; seen as an opaque object in spirit on a dark background. × 5.

FIG. 107.—Embryo 3. Yolk-spheres and crystalloids; drawn from a preparation mounted in glycerine, after treatment with Kleinenberg's picric acid and alcohol. Zeiss D, ocular 3.

Abstract and Review of the Memoir by G. Hieronymus "On Chlamydomyxa labyrinthuloides, Archer."

By

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I. Previous Work.

a. Archer.—Chlamydomyxa labyrinthuloides was originally discovered and named by Archer (1) in 1875. He supposed it to be related to Labyrinthula (4), and described it as a protoplasmic body, rounded or irregularly lobed, invested by a cellulose cyst, colourless, or straw-yellow, and generally of several layers, and living on water-plants, or parasitically in the cells of Sphagnum or Eriophorum leaves, or in the air-spaces of roots of Eriocaulon. He further saw it streaming out through a rupture in the cyst as a sort of labyrinth-like meshwork, the nodes of which were amœboid, ingested Algæ, and finally encysted themselves anew.

In the protoplasmic body were (1) a hyaline, vacuolated ground substance; (2) red granules of different sizes, varying in number, and not always present; (3) yellowish-green, round or irregular granules closely resembling the chloroplastids of certain Algæ except in colour; they were smaller but more numerous than the red, which Archer supposed to be derived from them; (4) there were numerous small, pale blue homogeneous bodies, rounded in the resting stage, fusiform during the streaming, capable of division, and of gliding on or in the threads of the meshwork with a movement, according to Archer, of their own; and (5) in the streaming condition there was a "diffuse chlorophyll."

Reproduction was effected in two ways: (1) by the separation of the nodes of the meshwork; and (2) by the division, inside the cysts, of the protoplasmic body into several portions, each of which became surrounded with a proper cyst of its own.

b. Lankester (8) suggested that the spindles of Chlamydomyxa, as also those of Labyrinthula, were to be regarded as nuclei.

c. Geddes (6) described the resting stages. He did not observe the streaming out of the protoplasm, although he repeatedly saw hernia-like protrusions of the contents of the cyst, which re-encysted themselves outside the Sphagnum cell. He also observed division of the contents of a cyst and the formation of proper cell-walls round these, as well as the formation of a septum between separate portions of a cyst.

He describes, in addition, two other kinds of cysts, one of which he calls the Protococcus form, and believes to have arisen from separated portions of the labyrinthine meshwork; the other sort being due, he supposed, to individuals which had migrated out of their cysts, spent some time as naked amœbæ, and then re-encysted.

He mentions, without figures or further description, cell-nuclei; and, besides these, masses of "protoplasm," usually of a red colour, and occasionally nucleus-like bodies containing a nucleolus, to be regarded as secondary cysts exceptionally formed inside the primary ones. A yellowish pigment, pro-

bably xanthophyll, is present in conjunction with the chlorophyll; the distribution of the latter is irregular, but occasionally definite corpuscles, the primitive form of the chloroplastids of the higher plants, occur. The red colouring matter he believes to be derived from the green; it is found sometimes in minute drops between the consecutive layers of the cellulose cyst.

As processes of reproduction he mentions fission, budding, free cell formation, and rejuvenescence. Lastly, he believes the organism to occupy the same position relatively to the lower Algæ as do the Myxomycetes to the lower Fungi.

d. Askenasy (2) has criticised these statements, suggesting that the organism described by Geddes was not Chlamydomyxa at all, the latter being, he supposes, really related to the Rhizopoda.

e. Bütschli (3) places Labyrinthula among the colonial Rhizopoda (Mikrogromia, etc.), but considers it doubtful whether Chlamydomyxa is really related to it, the "spindles" of the two not being homologous.

f. It is highly probable that Janisch (7) really observed Chlamydomyxa in 1859, mistaking it for Pleurostaurum or Cocconema.

[*g.* Hieronymus has apparently not seen Lankester's (9) account of Chlamydomyxa montana. This was seen in the streaming condition, and later on encysted. No ingestion of food particles was observed. The figure given l. c. is quite like that of Hieronymus copied here. Lankester expresses the opinion that the threads are pre-formed and comparable with the axis of the Heliozoan pseudopodium; he believes they are covered by a layer of invisible hyaloplasm, the streaming of which was the cause of the motion of the "oat-shaped corpuscles" ("spindles").

In the encysted condition crimson oil-drops were observed, and the division of the protoplasmic body inside the cyst into several portions. He observed no nuclei.—J. W. J.]

II. Preliminary Account.

The present author has found Chlamydomyxa in the Riesengebirge. It occurs in cells and intercellular spaces of Sphagnum and dead Cyperaceæ and Gramineæ leaves. The extrusion of the

Fig. A.—An amœba dividing.

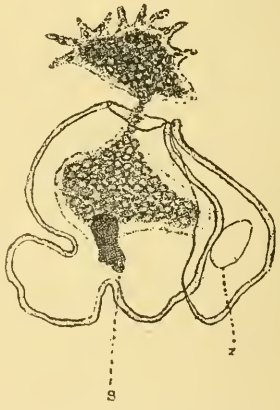

Fig. B.—A cyst out of which the amœba is emerging, and at the same time dividing; one half will remain behind. *s*, an egested oil-drop; *z*, a cell-wall thickening.

cell contents, the ingestion of food by these, the re-entrance into Sphagnum cells, and the re-encystment have all been observed.

Other organisms are found in the Sphagnum cells in company with the Chlamydomyxa. One of these is Chlorochytrium Archerianum (mentioned by Archer). Another

Alga, Urococcus Hookerianus, also occurs. It is mentioned by Geddes, but supposed by him to be a stage in the development of Chlamydomyxa; he figures it in his figs. 3, 4, 5 *a*, and 5 *b*. There is, however, no genetic connection between the

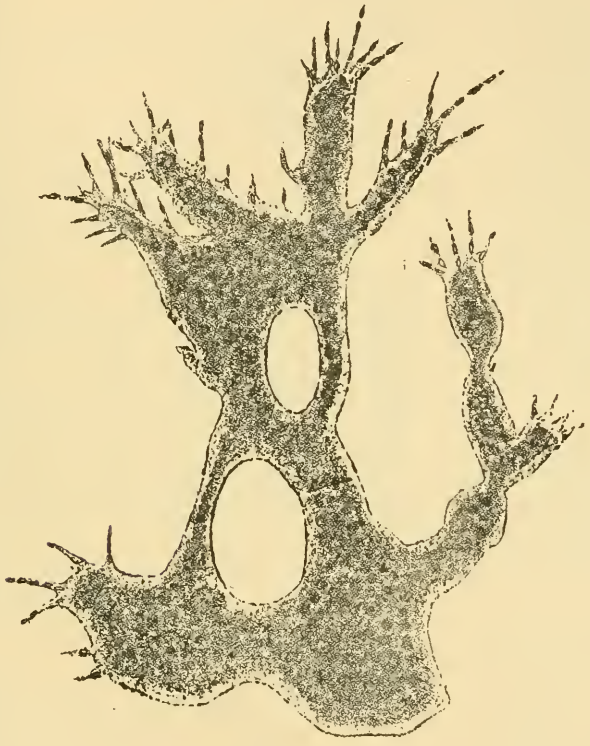

Fig. C.—A labyrinthine amœba dividing into several parts simultaneously.

two forms. His fig. 2 is a true Protococcacean, also often found with the other two.

In Chlamydomyxa the nuclei are of a different shape, smaller size, and much greater number than they are in Urococcus; the latter is also much more easy to cultivate.

With regard to Chlamydomyxa itself, we have to attempt an answer to the following questions:

1. Do nuclei exist?
2. What are the "spindles"?
3. Are the yellow-green bodies chromatophores or symbiotic Algæ?
4. What are the red bodies?
5. Is "diffuse chlorophyll" present? and—
6. What is the systematic position of the organism?

The following is an enumeration of the cell-contents according to Hieronymus:

1. The Nuclei.—Of these there is only one in the quite young cysts, in larger and older cysts two or more, and in the largest several; and conversely in those amœbæ which have just left the cyst there are many nuclei, in the final products of division only one.

2. The Chromatophores.—These contain a green pigment, probably identical with chlorophyll, and a yellow-brown pigment. During the period of ingestion they fade.

3. Oil Bodies.—These are of a red colour, sometimes olive-green or blackish brown. They are formed from aggregations of dead chromatophores, and can be artificially produced in sunlight. They are egested by the amœbæ, and often left behind in the cyst.

4. Rod-shaped crystalline bodies of calcium oxalate; they are formed in the hyaloplasma or in the cell-sap, and exhibit Brownian movements in the vacuoles.

5. A vacuolated hyaloplasma, containing—

6. Granular or drop-shaped bluish, highly refractile corpuscles, deeply staining in the living cell, and identical with Archer's spindles and Crato's physodes.

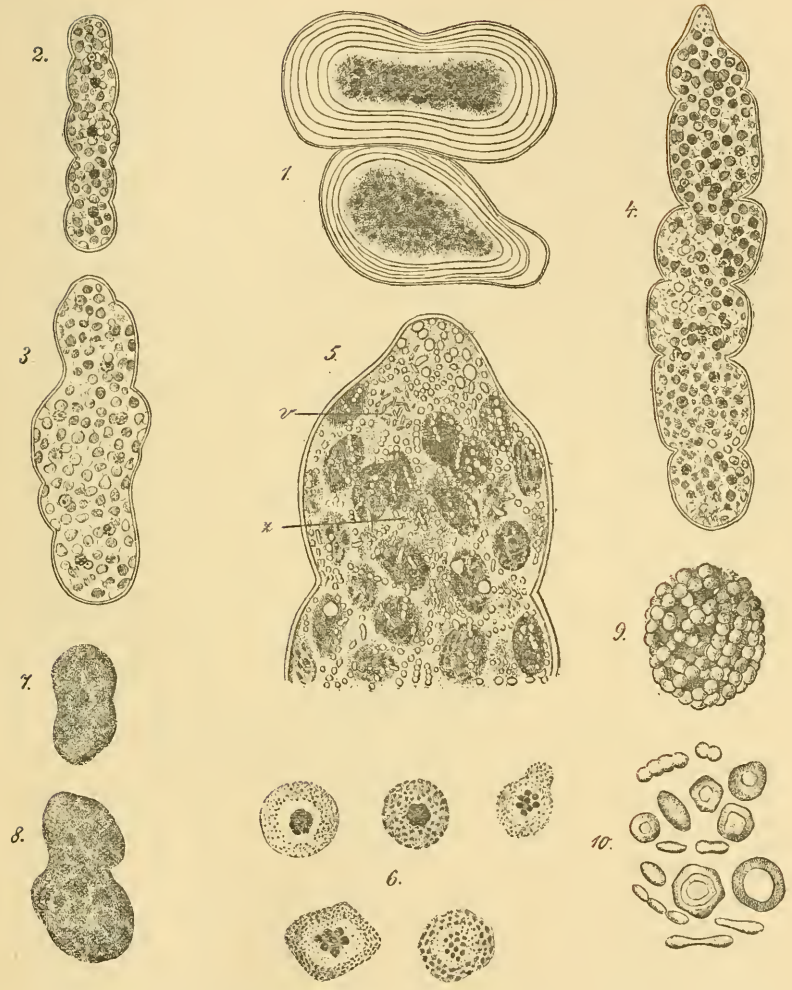

FIG. D.—1. Two thick-walled many-layered cysts. × 620. 2—4. Cysts showing the distribution of the nuclei. Chrom-aceto-osmic and hæmatein-ammonia preparations. × 620. 5. The end of a living cyst treated with methylene blue and pure water. × 5000. 6. Nuclei. Chrom-aceto-osmic and hæmatein-ammonia preparation. × 10,000. 7 and 8. Chromatophores, living. × 10,000. 9. Chromatophore. Chrom-aceto-osmic and fuchsin preparation. × 10,000. 10. Living physodes. × 10,000.

III. The Life History.

a. The Amœba Stage.—The amœbæ commonly arise by the extrusion from the cyst of the whole of the multinucleate contents, which then divide into two with the appearance of pseudopodia. The process is repeated until the whole is divided into uninucleate amœbæ, which then encyst (Fig. A).

There are often, however, modifications of this process. For instance, the first division may take place during extrusion from the cyst, in which single products of division may remain behind and re-encyst (Fig. B); or the original amœba may divide into more than one at a time, or the second division may begin before the first is completed.

Less frequently the whole body breaks up simultaneously into several pieces (Fig. C), assuming then the labyrinthine appearance observed by Archer, the thickened nodes of the meshwork being the separate energids of the protoplasmic body.

The present author, however, only observed the streaming of the protoplasm out of such cysts as that depicted in Fig. B, not out of the Urococcus-like many-layered cysts, as seen by Archer.

It is probable that this streaming and simultaneous division are due to favourable conditions of temperature, and so forth, supervening on a prolonged period of unfavourable circumstances, during which the forces concerned have become much intensified. At different stages of division, or even before the first division has taken place, the amœbæ ingest food particles, mostly diatoms, and encyst themselves with these; the uninucleate amœbæ are only able, however, to ingest bacteria, or very small green or blue-green Algæ. More rarely they ingest small pieces of Alga filaments, such as Œdogonium; starch grains they devour readily, and they will also take up grains of sand or bits of decaying plants, though they soon egest these again.

After encystment there is, in all cases, a rapid multiplication of the nuclei. In this condition they are generally found on

the outside of Sphagnum, grass leaves, etc., but sometimes inside the Sphagnum cells with diatoms ingested, apparently after their entrance into the cells. The ingested Algæ are not entirely digested, there being always left over the membrane, and some grey or black granules, which are either egested when next the creature creeps out as an amœba, or if, as frequently, a new cyst is formed inside the old one, are deposited between the two. Once the contents of the cyst were observed to divide, one half creeping out, the other re-encysting itself with the previously ingested diatom; and on another occasion, after the cyst contents had divided, each half re-encysted, the chromatophores of that half which contained the diatom becoming quite pale (Fig. E).

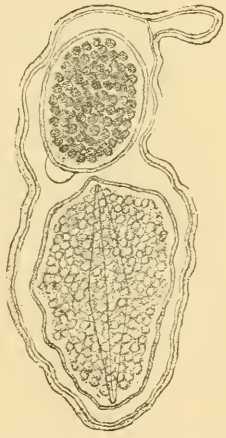

Fig. E.—A cyst containing two daughter-cysts: one of these, which has pale chromatophores, contains a large diatom; the other has dark brown chromatophores.

It very frequently happens that the amœbæ re-encyst without having ingested any food at all. The cause of this is probably to be sought in differences of temperature and moisture; if, for instance, certain conditions were to induce rapid multiplication of nuclei without a corresponding development of chromatophores, the creature might give up almost entirely a

holophytic mode of nutrition, and become for the time holozoic. The paleness of the chromatophores at the period of ingestion is probably to be explained on this assumption, that they then become superfluous and go through a period of rest, during which they are comparable to the leucoplastids of the higher plants.

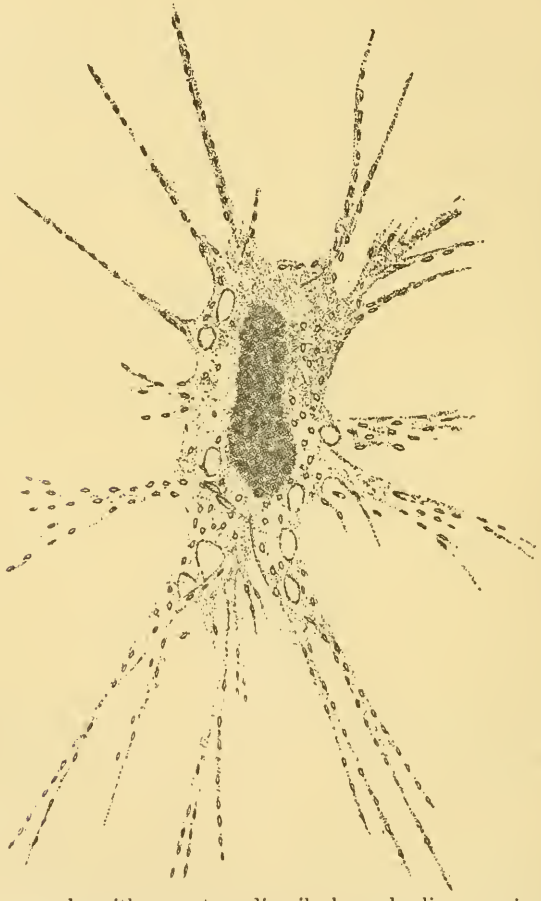

Fig. F.—An amœba with an extraordinarily large hyaline margin (ectosarc). Physodes are moving along the pseudopodia.

The amœbæ generally creep on the surface of the Sphagnum and other plants (Figs. A, C, and F); very rarely they are seen

swimming (compare Lankester's figure [9]). In this condition they never divide, and are always circular or elliptical. The dark central mass which contains the nuclei and chromatophores is surrounded by a margin of clear protoplasm, from which emanates a halo of hyaline pseudopodia, which continually shorten and lengthen again, or may be altogether withdrawn and then again protruded. These pseudopodia are similar to, although much larger than, those of the creeping amœbæ, which latter are only developed on the forwardly directed end (Fig. C). Along them move numerous round or spindle-shaped physodes, carried hither and thither by the streaming of the protoplasm. The emergence of the amœbæ from their cysts, and their subsequent division, only take place during the summer months, and then only during the warm hours of the day, and in the presence of light of sufficient intensity.

In the amœbæ the olive-green or red oil bodies are never seen, nor the crystals of calcium oxalate; but both are frequently found in deserted cysts, being sometimes egested at the moment of emergence.

b. The Cysts.—The form of the cyst, when on the surface of leaves, etc., is generally spherical or ovoid, occasionally elongated or irregularly lobed, the last when large Algæ have been ingested. In the case of those cysts, however, which are inside the cells of the plants on which they live, the form is adapted to that of the cell; but if this is too small for the enclosed cyst, the latter may protrude (cf. Geddes, fig. 1) as a hernia-like swelling into which a portion, or in some cases the whole, of the protoplasmic contents migrate. A new spherical cyst forms round the protruded protoplasm, while the old cyst splits, thus allowing the newly formed one to escape without becoming amœboid.

Again, it often happens that the protoplasmic contents divide into two or more parts, each forming a proper cell-wall of its own. The large many-layered cyst described by Archer, enclosing several small cysts, is probably a sort of formation of resting spores. The repeated formation of a new membrane round, and the subsequent division of the

whole cell contents, would produce a form quite similar to that of Urococcus, except that it would be multinucleate and possess no starch grains.

Two or more cysts are often found in the same Sphagnum cell. These by their growth exert a pressure upon one another, which may result in the death of one of them, or in the bursting of the Sphagnum cell, and consequent protrusion of the cyst. This mutual pressure also occurs in any cysts which happen to lie closely together, producing thickenings of their walls where they rest on one another. This latter also occurs in solitary cells. The completely grown cysts contain at least eight nuclei, sometimes as many as thirty-two.

The number of chromatophores, their size and distribution, are extremely variable. The quite young cysts generally contain few, but those large; the full-grown cysts much smaller ones, but in very much greater numbers. In the ripe cysts they can only be distinguished from one another by squeezing out the contents; but if the cyst be unripe there are numerous large vacuoles in the protoplasm, which make it quite possible to see the separate chromatophores as well as the minute crystals of calcium oxalate with which the vacuoles are crowded.

The over-production of this calcium oxalate seems to be in some way deleterious to the organism; if the cell contents migrate the crystals are egested, but the amœba stage may be altogether prevented by their presence. If the organism be kept in lime water calcium oxalate is produced in excessive quantities, and many cysts are killed; the chromatophores are the first to die, being transformed directly into brown or blackish masses, and after them the nuclei become disorganised.

The vacuoles mentioned above as occurring in the unripe cysts may run into one another. The protoplasm is then divided up into numerous rounded or angular portions, each containing a nucleus, and termed originally by Sachs (**10**) an "energid." They are connected with one another by threads of hyaline streaming protoplasm, along which are carried the glistening physodes (Archer's spindles), and sometimes chromatophores as well, but never nuclei. The separation of the

cyst contents into energids can be artificially effected by exposure to strong sunlight, when the protoplasm and chromatophores are found to have grouped themselves round the individual nuclei, apparently to screen them from excessive light; and if this treatment be prolonged, many of the chromatophores become transformed into first olive-green and then red oil-drops, which always occupy a position outside the energids, where they can shield the nuclei and the remaining chromatophores from the sun. By still further prolonging this treatment all the chromatophores can be so transformed, but this is followed by the death of the individual.

These oil-drops are also always most plentifully found in material taken from dry or sunny places, and they are often to be seen, as Geddes observed, between the layers of the cyst, in which case it seems probable that they are rejected by the organism when no longer of any use to it. The red colour of the oil-drops finally changes to a deep brown or black, similar in appearance to the granules of the digested Algæ, whence it seems probable that they may be used as food.

In favourable places the whole cycle of the life history may be repeated several times in a summer. In winter, and in dry or otherwise unfavourable places, the cyst becomes very thick and stratified (Fig. D, 1).

IV. The Cell Contents.

a. The Nuclei.—These can only be satisfactorily demonstrated by appropriate fixing and staining reagents, although in the living cysts the clear masses of protoplasm in which the nuclei lie can sometimes be seen if the chromatophores and other cell contents are not too numerous. The calcium oxalate crystals are also a great hindrance to accurate observation, and must, before staining, be got rid of by treatment with hydrochloric acid.

The fixing reagents used were absolute alcohol, aqueous picric acid, chromic acid, and various mixtures of these two with acetic and osmic acids. Finally, and most generally,

iodine added to the water in which the creature was living. The iodine material was gradually hardened in alcohol, while that preserved in picric and chromic acid was transferred to and kept in water containing numerous crystals of naphthalin.

The stains used were hæmatein-ammonia and Mayer's hæmalum, the former of which gave the better results. The objects were deeply over-stained, and then decoloured in alum until only the granular portions of the nuclei retained the stain; they were finally mounted in Canada balsam in the usual way. Only those amœbæ were preserved which happened to be migrating into Sphagnum cells; but their nuclei, and therefore presumably the nuclei of all other amœbæ, exhibited the same appearance as those of the cysts.

In the uninucleate amœbæ or cysts the nucleus always occupies a nearly central position, never being situated against the cyst wall, or in the hyaline border of the amœbæ. In the multinucleate stages the nuclei are evenly distributed. They may lie in a row if the cyst is compressed by the narrowness of the Sphagnum cell, or, if the cyst is broader, they may alternate with one another in two rows, or in large cysts may lie equally in all directions (Fig. D, 2—4), and this uniformity is quite uninterrupted by local thickenings of the cell-wall, or by the protrusions of the cyst. The size of the resting nuclei varies, the diameter being from $1\frac{1}{2}\,\mu$ to $3\,\mu$. They are generally round, almost spherical, more seldom lens-shaped, ovoid, or faintly lobed. They lie embedded in a more or less thick layer of hyaloplasm, which passes indistinguishably into the nuclear membrane. The structure of the nucleus is reticulate, though the threads of the reticulum are so excessively minute that they can only be recognised by the rows of chromatin granules that lie upon them.

In properly stained preparations the nuclei appear to be divided into layers. In the centre space—about one third of the diameter of the whole nucleus—are generally several, twelve or more, but sometimes only a few, or even one, large, deeply staining granules (Fig. D, 6). These are probably nucleoli; they are not protein crystals, which do not stain with hæmatoxylin,

nor chromatin granules, for the small peripheral granules (see below) go blue when stained with iodine-green fuchsin, or methylene-blue acid fuchsin, while the central granules colour red. Next there is a zone free from granules, and traversed only by achromatic threads, between which is the so-called nuclear fluid; this zone varies in breadth, but may occupy as much as one fifth of the whole diameter. Around this clear zone, again, is the outermost of all, which may be in breadth as much as two thirds of the whole diameter, but which varies with the width of the clear zone. It contains numerous small deeply staining granules of chromatin, occasionally equalling the nucleoli in size. The division of the nuclei is apparently amitotic.

b. The Chromatophores.—No "diffuse chlorophyll" can be detected either in the amœbæ or in the cysts. The pigment, yellowish or brownish green, is always contained in definite though very minute corpuscles. These are either discoid or lens-shaped, appearing fusiform in profile, or may be nearly spherical, sometimes angular or lobed. In the largest chromatophores the thickness varies from $1\frac{1}{2}\,\mu$ to $2\,\mu$, the diameter from $4\frac{1}{2}\,\mu$ to $5\frac{1}{2}\,\mu$, but the size seems to vary inversely with the number. In the amœbæ, as also in the mature and in the uninuclear cysts, they are generally very small indeed, about $\frac{1}{2}\,\mu$ in diameter, which may account for the diffuse chlorophyll of other authors. They attain their maximum size in the adult but still immature cysts.

In the uninjured cell very little structure can be detected in the chromatophores, except now and then darker spots on a lighter ground substance, due, perhaps, to thicker accumulation of the oil which lies in the chromatophoric reticulum (Fig. D, 7, 8). But when fixed, and with the oil and pigment removed by alcohol, a skein-like structure of threads twisted over one another exhibits itself. These threads are composed of rounded segments, Mayer's granules, and can be readily stained. In the smaller chromatophores there is only one twisted thread, but in the larger several. This fibrillar structure can be readily seen and the threads isolated by crushing the chromatophores under the cover-glass, or by prolonged

treatment with strong salt solution. The granules are then seen to be the vehicles of the pigment which lies in their peripheral portion, the centre being colourless (Fig. D, 9). The yellow-green or brown-green pigment seems to be a combination of two distinct colouring matters. If the chromatophores are treated with dilute alcohol they become of a grass-green before they eventually fade; there is thus a brown or yellow pigment, more soluble in alcohol or in water after the death of the organism than the green. In absolute alcohol, however, the yellow-brown pigment is the less soluble.

By the action of concentrated hydrochloric or sulphuric acid the chromatophores are coloured bluish green or blue, and by continued treatment with the former hypochlorin masses are differentiated as dark green drops or crystals. These reactions prove that the green colouring matter is identical with, or very nearly related to, the chlorophyll of the higher plants. The yellowish-brown pigment, on the other hand, is probably itself a mixture of two: one of these may be identical, as Geddes supposed, with carotin or xanthophyll, or else with diatomin (phycoxanthin); the other may be either the phycophæin of the Phæophyceæ, or the phycopyrrhin of the Peridineæ.

These chromatophores are almost certainly not symbiotic Algæ, neither a cell-wall nor a nucleus being detectable in them.

c. The Oil-drops.—These, as above stated, arise by the degeneration of the chromatophores, for when they are formed the number of the chromatophores decreases, and if they be decoloured in alcohol they exhibit traces of the chromatophoric structure. They are at first olive-green, then red, and finally brown or blackish. They are formed under the influence of direct sunlight, and can be artificially produced by insolation; if the organism is then removed from the direct light, the shade, which it appears to be the function of these oil-drops to afford, is no longer necessary, and they become disorganised, changing to the brown or black colour. They may, as Geddes observed, be egested and deposited between successive layers of the cysts; he figures them red, but the present author only

found them there in the brown condition. They may also become enclosed inside the cyst by a proper cell-wall of their own.

The red colouring matter is probably one of the fatty pigments (lipochromes). By treatment with sulphuric acid the masses are coloured a bright blue, but the formation of crystals of lipocyanin was not observed. The change of the red to a brown colour possibly points to there being here also two pigments in combination ; the red colour, also, is not always the same, being sometimes rose or carmine, sometimes a brick-red.

d. The Physodes and the Hyaloplasma.—The physodes are glistening, highly refractile bodies, with sometimes, when of considerable size, a blue sheen. In the cyst they are spherical and arranged in rows, less frequently spindle-shaped, angular, or lobed. They are Archer's "spindles," taken by later writers for nuclei. Archer observed in them a change of form, which, however, has not been seen by the present author. This change of form is probably to be explained, as Archer believed, by supposing that the bodies become viscid under stress of the varying forces exercised upon them from time to time by the protoplasm.

The smallest of them exhibit no structure of any kind, although the larger show a sort of stratification, there being a central more refractile portion.

Crato (5) has recently figured similar cell contents as occurring in many Fucaceæ, and also in Chlorophyceæ. To these he has given the name physodes, and has asserted that in many cases they contain phloroglucin. Now by treating Chlamydomyxa with vanillin-hydrochloric acid a red coloration, intensified by the addition of sulphuric acid, is found in these bodies, a characteristic test for phloroglucin. A similar reaction can be obtained by treatment with alcoholic piperonal solution and sulphuric acid. With iron chloride they stain a pale greenish blue, and finally, in the living cell they take up very strongly such stains as iodine green, methylene blue, methyl violet, and methyl green, and retain these after the cell has been killed in water or alcohol.

All these facts point to their being related to Crato's physodes. They are also partially soluble in absolute alcohol and ether, though they do not entirely disappear in these liquids as Crato's physodes do. If killed in 30 per cent. alcohol they only contract slightly, at the same time losing much of their staining capacity, and refusing to show the characteristic reaction with vanillin, so that the phloroglucin is apparently dissolved out. By staining the fixed material with iodine-green fuchsin or methylene-blue acid fuchsin, the ground substance of the physodes appears kyanophilous ; it also takes up hæmatein-ammonia as readily as the chromatin and nucleoli, but can be destained with alum more readily than these.

In the living cell the physodes often lie in rows, sometimes very closely together (Fig. D, 7), which rows have apparently an oscillating movement. Along the protoplasmic threads, also, which connect the separate energids of the cell, the single physodes, as well as the rows, are seen to move. This may be readily observed by intra vitam staining (Fig. D, 5) with a very dilute aqueous solution of methylene blue ; if too strong a solution be used the protoplasm becomes vacuolated, many of the deeply stained physodes being ejected into the vacuoles, where, together with the crystals of calcium oxalate, they are seen to execute the so-called Brownian movements, evidence that in the cyst, at any rate, they are of a more or less permanent consistence. The cell sap in the vacuoles becomes at the same time faintly reddish, showing an acid reaction. If the organism be placed in a dilute methylene-blue solution for a very short time, so that a few only of the physodes —those situated peripherally—are coloured, and be then left for some time in pure water, then the more energetic streaming of the protoplasm which ensues causes the physodes, stained and unstained, to become closely packed together, and finally deposited in one or more vacuoles which lie near the cell-wall. These would probably, like the oil-bodies, become covered in time by a layer of cellulose. The arrangement in rows of the physodes is probably due to frequent division, and is seen best after the organism has been kept a short time only

in culture. They lie embedded in the threads (visible after treatment with any reagent, and probably present though undetectable in the living state) of the hyaloplasma, which themselves are coiled in a scarcely staining mass (Kupfer's paraplasma), the kyanophilous physodes being immediately surrounded by an erythrophilous layer. This can be readily made out with iodine, which stains this layer deeply, the physode itself not at all.

The hyaloplasmic threads are closely packed round the nuclei, less so round the chromatophores; aggregations of them, with their contained physodes, are especially common in places where the cell membrane is undergoing a marked growth, which would suggest that the physodes are reserve food material, the nature of which cannot, however, be at present more precisely affirmed. The hyaloplasmic threads never branch or anastomose.

e. The Crystals.—These are found, in "Brownian" movement, in the vacuoles. They are generally egested by the organism when it assumes the amœboid stage, and are sometimes left behind in the cyst. That they are crystalline is proved by polarisation, and that they are of calcium oxalate is proved by their insolubility in acetic acid, whereas they readily dissolve, even in weak solutions of hydrochloric, sulphuric, and nitric acids, without the evolution of bubbles. If strong sulphuric acid is used crystals of calcium sulphate are formed, and finally, if they be heated, the calcium oxalate is converted into what is probably calcium oxide, which is then soluble in acetic acid.

The form of the single crystal is rod-shaped; when cut it is to be supposed that they are mono-symmetric; double and triple crystals also occur, as well as aggregations, which stick together and may completely fill up a vacuole. These crystals are never found in the hyaloplasmic threads, and therefore probably arise in the paraplasma, or may possibly be sometimes formed in the vacuoles themselves.

f. The Cell-wall.—This gives the characteristic cellulose reaction of blue or dark violet with iodine and sulphuric acid,

and with chlor-zinc-iodine. In the case of the many-layered resting cysts the outer layers were violet; the inner, as also the single membranes of the quite young cysts, blue. Thickenings in the cell-wall stain more deeply and are less easily decolourised than other parts with reagents like iodine green, Congo red, safranin, etc.

V. The Systematic Position of Chlamydomyxa.

The views of Janisch, Archer, Geddes, Askenasy, and Bütschli have already been stated. Lankester (9), while refraining from asserting its exact relation to the other Gymnomyxa, believes, with Archer, its nearest ally to be Labyrinthula. It is held by Hieronymus that this organism is on the border-land between plants and animals, although nearer, by reason of its chromatophores and cellulose membrane, to the former; on the other hand it is, at times, certainly holozooic. This form of nutrition is, however, known in Chromulina and Ochromonas (12), and in Peridineæ, even in chromatophore-containing forms (11).[1]

Had Chlamydomyxa no chromatophores it could have been related to Vampyrella; also the paramylum grains, which are present in this latter, have not been detected in Chlamydomyxa.

Assuming, with Bütschli, one single stem for the origin of all organisms, one might (says Hieronymus) regard Chlamydomyxa as a specialised derivative of Vampyrella; on the other hand, if we assume with Nägeli a polyphyletic origin for organisms, there being between members of the different lines of descent apparent relationships due to convergence, then Chlamydomyxa must be placed in a separate family, which will occupy the lowest grade among the yellow-brown Algæ, leading up to the Dinoflagellata and diatoms on the one hand, to the

[1] Which is not surprising if we do not (as do a certain section of botanists) go out of our way to make the indefensible assumption that the Flagellata and Dinoflagellata belong to the vegetable series!—E. R. L.

Chromomonadinaceæ and Phæophyceæ on the other. It will also have relations, in a wider and different sense, with the Vampyrellaceæ, Labyrinthuleæ, Myxomycetes, and Fungi on one side and on the other with the Heliozoa, Rhizopoda, and Radiolaria.

A parasite, probably Pseudospora maligna, sometimes occurs in the cysts.

VI. Remarks.

[Hieronymus, of course, approaches his account of this organism entirely from the botanical standpoint.[1] He adduces chiefly the presence of chromatophores and of a cellulose cyst as reasons for regarding the organism as a plant; but these occur in several well-known instances in animals. It would have been more to the point, perhaps, had he relied on the presence of phloroglucin and calcium oxalate, and the alleged periodic holophytism. Phloroglucin is unknown among animals. Calcium oxalate occurs only, I believe, in the Myxomycetes. Hieronymus may, of course, be mistaken in his identification of the substance found in the spindles with phloroglucin; he certainly mentions a fair number of tests, but these seem all merely to rest on our ignorance of any other substances which would give the same reaction. His figures of the spindles (physodes) differ a good deal from those given by Archer and Lankester.

It is also, perhaps, a curious coincidence that these physodes should have so marked an affinity for the basic aniline stains; it is perfectly true, on the other hand, that they exhibit no structure in the least comparable to that of a nucleus.

Hieronymus's account of the nuclei seems sufficiently to

[1] The notions indulged in by Hieronymus as to the relationship of Chlamydomyxa to the yellow-brown Algæ, and of every yellow-brown organism with every other, are devoid of any serious basis in fact. Whilst his paper contains some observations of importance, *e.g.* as to the nuclei, and some the accuracy of which seems to need further inquiry, *e.g.* as to the chromatophores, the general views which dominate the author's speculations appear to be those of a botanical specialist whose knowledge of Protozoa is defective.—E. R. L.

explain why his figures show so little of the typical reticular structure. He has, of course, omitted to emphasise reasons which might induce a zoologist to claim this organism for his own province, such as the ingestion of solid food, and the existence of pseudopodia covered with streaming protoplasm. —J. W. J.]

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On the Development of the Parietal Eye and Adjacent Organs in Sphenodon (Hatteria).

By

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With Plates 11—13.

Table of Contents.

1. Introduction.

My attention was first directed to this investigation by observing a stage in the development of the parietal eye in an Australian skink, in which the portion of the optic vesicle behind the lens appears to consist of two very distinct layers,

thus forming an optic cup structurally resembling that of the ordinary paired eye. The conclusion that this cup had been formed in the same way as in the paired eye was irresistible, but fortunately I refrained from publishing until further evidence was forthcoming, and in the meantime made arrangements for securing a supply of Tuatara eggs, with a special view to investigating this point. Thanks to the generous assistance of Mr. P. Henaghan, I have obtained a considerable number of eggs, from which I have extracted a very good series of embryos. I have recently published a general account of the development of Sphenodon.[1] This will be followed by more detailed accounts of the development of special organs, by myself and other zoologists, and towards these the present communication may be considered as a first instalment.

My material was hardened in Kleinenberg's picric acid, followed by different grades of alcohol, and stained with borax carmine in the usual way. The sections were cut by the ordinary paraffin method.

Owing to the work having been carried out in Christchurch, New Zealand, where, unfortunately, there is no proper scientific library, the difficulties of the investigation have been greatly increased. So much valuable work has been published of late years on the "epiphysis" and parietal eye of various types, that it was thought undesirable to publish my own observations before having an opportunity of consulting the more important recent papers on the subject by other writers. In order to attain this end, great delay has been caused by the necessity for communicating with England and America, but it is hoped that this delay may be more than compensated for by the increased interest attaching to the work when compared with that of other investigators. As a result of this comparison, I find that most of my conclusions have been anticipated by one or other of the numerous writers on the subject. Especially is this true in the case of the admirable work of Béraneck (**5, 6**) on lizards, and Hill (**16, 17**) on fishes.

[1] This vol., p. 1.

Sphenodon is generally recognised as being the most ancient type of reptile still existing, and it might therefore well have been expected that the parietal eye in this animal would be better preserved than in any other type. Thanks to the classical works of Baldwin Spencer (33) and de Graaf (15), this has long been known to be the case in the adult, and I hope in the present memoir to be able to show that its development has also undergone less modification than in other reptiles, a fact which enables us to make a remarkably close comparison with the development observed by Hill in fishes, and presents us with an important clue to the phylogenetic history of this remarkable organ.

As on so many previous occasions, I have to tender my most sincere thanks to Professor G. B. Howes, LL.D., F.R.S., for his kindness in revising the proof sheets of these pages in my absence from England, as well as for much valuable advice and assistance in the matter of literature. I am also greatly indebted to Dr. Gaskell, to Professor J. E. Reighard, and to Professor W. B. Benham for sending me works which I was unable to obtain in Christchurch.

The letters employed to designate the different stages are the same as those of which I have already made use in my general account of the development.

2. Systematic Account of Observations on Sphenodon punctatus.

Stage K.

The first indication of the parietal eye appears at the stage in the development which I have called K, comparable with a chick of about two days. The fore-brain is already bent downwards and backwards, so that the mid-brain lies at the anterior extremity of the body. The optic vesicles[1] of the

[1] In the summary of my results recently sent to England I have, by an unfortunate slip, stated (10) that the parietal eye first appears shortly after the development of the optic lobes. I should have said optic vesicles.

ordinary paired eyes have begun to invaginate to form the optic cups, while the lens already appears as a thickening of the superficial epiblast.

A short way behind the ordinary paired eyes (actually in front, owing to the cerebral flexure) a small round vesicle appears as a bud on the roof of the fore-brain, slightly to the left of the middle line. Owing to the cerebral flexure it appears in sections on the ventral surface, as shown in fig. 2. This vesicle I propose to term the primary parietal vesicle. In the section figured, the roof of the fore-brain, with the primary parietal vesicle, is cut almost tangentially, in a direction not favourable for studying its histological characters, and I therefore reserve the description of these until later.

The position of the primary parietal vesicle to the left of the middle line is very remarkable, and appears to be constant (see also fig. 5, which is reversed as compared with fig. 2), but the discussion of this problem must be postponed till the sequel.

Stage L.

At Stage L, comparable with a chick of about three days, the primary parietal vesicle has become conspicuous, when the head of the embryo is examined in profile, as a small protuberance on the roof of the fore-brain between the paired eyes. Longitudinal vertical sections of the head show it lying a short way in front of the mid-brain (fig. 3). It still communicates by a wide aperture with the brain-cavity, but the vesicle itself is somewhat flattened dorso-ventrally and extends on both sides of this aperture, rather more forwards than backwards (fig. 4), so that it appears to be attached to the brain by a very short, hollow stem. Its wall is composed of a layer of columnar cells similar to those forming the underlying wall of the brain, with large, oval, finely granular nuclei and indistinct cell boundaries. The outer wall of the vesicle lies immediately beneath the epidermis, and the mesoblast cells are only just beginning to spread in between the two. The cavity of the vesicle is filled with a finely reticulate

coagulum, in which at least one small nucleus is present (fig. 4). A similar coagulum, with occasional nuclei, is visible in the cavity of the fore-brain itself.

Stage M.

At Stage M, comparable with a chick at about the middle of the fourth day, with the limbs just beginning to bud out, the primary parietal vesicle still remains as a simple outgrowth opening into the cavity of the fore-brain, slightly to the left of the middle line (fig. 5).

Stage N.

The Parietal Eye and Stalk.—At Stage N, comparable with a chick at about the commencement of the fifth day, a great advance has taken place in the development of the parts under discussion. Longitudinal vertical sections of the fore-brain at this stage (figs. 7, 8) show the parietal eye as a hollow oval vesicle lying on the roof of the fore-brain, in front and slightly to the left of its so-called "stalk." The latter, which I shall refer to in future as the "parietal stalk," is a glove-finger-shaped diverticulum of the roof of the fore-brain, curving forwards and lying nearly or quite in the middle line. The parietal eye appears to be nearly if not entirely separated from the stalk. It lies wedged in between the superficial epiblast and the roof of the fore-brain, and is somewhat compressed dorso-ventrally; its front or dorsal wall is already thickening to form the lens (fig. 8). The cavity of the stalk communicates freely with the cavity of the fore-brain. In histological structure its wall closely resembles that of the parietal eye, being composed of columnar cells similar to those of the wall of the brain of which it is an outgrowth. Both stalk and eye contain the usual coagulum.

It appears to me that there are two possible ways in which the condition of the parietal eye and stalk at this stage may have been derived from that which I have described as existing at preceding stages.

(1) The primary parietal vesicle may have divided into two

parts, an anterior one forming the parietal eye and a posterior one forming the stalk (or the eye may have budded out from the stalk, which comes to much the same thing). This appears to be the view generally adopted.

(2) It appears to me more likely that the parietal eye is formed from the primary parietal vesicle, and that the parietal stalk arises as a second, distinct evagination of the roof of the fore-brain after the primary parietal vesicle has become almost if not quite constricted off. This second evagination would appear to take place slightly to the right of the primary parietal vesicle, which seems to be pushed forward in the process. This view of the case is strongly supported by the following considerations :—(*a*) The primary parietal vesicle is already almost constricted off from the brain, and has acquired the characteristic flattened oval form of the young parietal eye before the parietal stalk makes its appearance at all. At this stage (fig. 4) it projects backwards as well as forwards from its attachment to the brain, while the parietal stalk when it appears projects forwards only. (*b*) The parietal eye, like the primary parietal vesicle, at first lies to the left of the middle line, while the parietal stalk is median, or nearly so.

The constancy of the left-sided position of the young parietal eye will be discussed later on ; meanwhile I may state that I have observed it in a sufficient number of cases to justify me in attaching considerable importance to it, and I believe it affords almost conclusive evidence in favour of my view that the parietal stalk is a second and independent outgrowth of the roof of the fore-brain. If its cavity ever communicates with that of the parietal eye, which is doubtful, it is probably because the second evagination involves the point of attachment of the first to the brain before the opening of the first has closed up.

The Paraphysis.—This structure does not appear until some time after the appearance of the primary parietal vesicle. It is certainly not present at Stage L, and I have not been able to satisfy myself as to whether it is present or not at Stage M.

At Stage N, however, when the parietal eye and stalk are both already clearly differentiated, the paraphysis appears as a small, broadly rounded diverticulum of the roof of the brain, close to the junction of the prosencephalon with the thalamencephalon. Owing partly to a not very well marked depression[1] of the roof of the brain just behind it, this diverticulum already points backwards, as shown in fig. 6, towards the parietal eye, from which it is separated by almost the entire length of the roof of the thalamencephalon.

The cavity of the paraphysis on its first appearance communicates by a wide aperture with that of the fore-brain, and its wall is composed of a layer of columnar cells similar to those forming the wall of the brain in its immediate neighbourhood. It is interesting to note that already a distinct blood-vessel is conspicuous in sections just behind the paraphysis, lying on the roof of the thalamencephalon (fig. 6).

Stage O.

The Parietal Eye and Stalk.—At Stage O, comparable with a chick of about five and a half days, the parietal eye and its stalk are conspicuous externally (as, indeed, they were already at Stage N) when the head is examined as an opaque object without staining. It will be seen, from an inspection of fig. 12, that there is on top of the head a dark-looking, elongated, quadrangular area,[2] tapering behind to an acute angle which lies just in front of and between the optic lobes, while anteriorly it ends in a less acute angle just behind and between the cerebral hemispheres.

In the posterior angle of this area, just behind the paired eyes and in front of the optic lobes, are visible two round spots of an opaque white appearance. One of these lies just in front of and slightly above the other, and also slightly to the left.

[1] This depression, or slight fold of the brain-roof, appears to mark the junction between prosencephalon and thalamencephalon, so that the paraphysis is to be regarded as an outgrowth of the hinder part of the prosencephalon.

[2] This area belongs chiefly to the thalamencephalon, but its most anterior portion, containing the paraphysis, belongs to the prosencephalon.

This is the parietal eye. It is further distinguished by the presence of a distinct double circular outline, the inner circle indicating the margin of the lens. The hinder of the two spots, lying in the middle line or nearly so, represents the parietal stalk, and, except that it sometimes appears dark in the centre owing to its central cavity, it exhibits no further features of interest.

It is important now to notice the position of the parietal eye and stalk with regard to the paraphysis, shown also in fig. 12. It will be seen that whereas the former occupy the posterior angle of the roof of the thalamencephalon, the latter appears as a round white spot between the cerebral hemispheres, being separated from the parietal eye by almost the entire length of the thalamencephalon. The same relations are shown in fig. 9, which represents somewhat diagrammatically a longitudinal vertical section of the head at this stage, the parietal eye being shown as though it lay in the middle line instead of slightly to the left.

The vesicle of the parietal eye is now somewhat deeper than before, and the lens thicker (fig. 10). The ventral wall of the vesicle is flattened against the roof of the thalamencephalon, and its posterior wall against the anterior wall of the parietal stalk. A few mesoblast cells have made their way in between the lens and the superficial epiblast (epidermis), which appears in the section figured to be slightly depressed just above the parietal eye (probably owing to shrinkage in the course of preparation).

The cavity of the parietal stalk still opens into that of the fore-brain, but the section passes a little to the left of the opening. Its front wall is much flattened against the posterior wall of the parietal eye, so that it appears to project vertically upwards from the roof of the brain.

Except for the thickening of the lens the wall of the parietal eye at this stage has undergone no marked alteration. The lens (fig. 19) is made up of more or less elongated columnar cells with correspondingly elongated nuclei. These cells are longest in the middle and much shorter towards the periphery

of the lens. Here and there a clear-looking space appears between the elongated cells, containing what seems to be a much smaller nucleus.

In the cavities of both stalk and eye the usual coagulum is present with an occasional small nucleus. These nuclei present a curiously lobed or agglomerated appearance, and appear to me to be thrown off from the surrounding walls (figs. 19, 20, *Nu.*). In fig. 19 one appears to be just leaving the inner surface of the lens. They are possibly identical with the smaller nuclei noticed above in the interior of the lens, and which also occur in the ventral wall of the eye, and perhaps in the wall of the stalk, but I have not sufficient evidence to decide this point. Karyokinesis is going on both in the wall of the stalk and of the eye.

Just behind the parietal stalk the roof of the brain has already become differentiated into two layers (fig. 10), the outer finely punctate in longitudinal section and almost without nuclei, the inner with numerous large oval nuclei.

The Paraphysis.—At this stage the paraphysis has elongated backwards, and its wall has begun to be irregularly folded (fig. 11). Its cavity still communicates freely with that of the brain, and the blood-vessel is still conspicuous just behind its apex.

I have already pointed out in dealing with the parietal eye and stalk the relative positions of these organs and of the paraphysis, which are readily seen on examining the upper surface of the head of embryos at this stage (fig. 12).

Stage P.

Of Stage P I have only a single specimen, and it is so similar in external characters to Stage O that I have not thought it necessary to cut sections at present, especially as its histological condition is doubtful.

Stage Q.

Of Stage Q I have also only a single specimen, which was dead when it reached me, and useless for histological purposes.

Stage R.

Of Stage R, however, I have numerous examples, as the embryo passes a long period in this condition. According to my classification of the embryos, this is the last stage but one before hatching. The embryo is very advanced. The limbs and digits are well formed, and pigment is present in the integument, forming a characteristic embryonic pattern of transverse bands and longitudinal stripes.

The upper surface of the head of an embryo at about the commencement of Stage R is represented in fig. 13. When this figure is compared with fig. 12 it will be seen that owing to the straightening out of the cerebral flexure the optic lobes and cerebral hemispheres have become closely approximated, and the length of the thalamencephalon thereby greatly reduced, its roof appearing as a dark-looking, diamond-shaped area between the two.[1] In the centre of this area the parietal eye appears as a round white spot with an intensely black border, the white centre representing the lens, and the black border the now deeply pigmented margin of the retina. As far as one can judge, the parietal eye has now become median in position.

Topography of the Brain in the Neighbourhood of the Third Ventricle.—As the brain has progressed greatly in development since the last stage described, it will be desirable to briefly describe the topographical relations of the parts in the neighbourhood of the third ventricle before proceeding any further, and this may best be done with the aid of a series of transverse sections.

Fig. 22 represents a transverse section taken a short way in front of the foramina of Monro. It shows the anterior commissure (*Com. Ant.*) passing across above the anterior end of the third ventricle. Above the anterior commissure is a crescentic band of fibres (*Com. Man.*) uniting the two inner

[1] This dark patch, however, does not exactly mark the limits of the roof of the thalamencephalon, for it will be shown later on that the parietal eye lies over the hinder part of the roof rather than over the centre as it appears externally.

mantle walls of the cerebral hemispheres. I identify this with the mantle commissure or corpus callosum mentioned by Hoffmann,[1] and I think a comparison of his fig. 4, pl. clxiii, representing a corresponding section of an embryo of Lacerta, leaves no doubt as to the correctness of this identification. Beneath the third ventricle is seen the optic chiasma (*Op. Ch.*).

The section represented in fig. 23 passes through the foramina of Monro, and is drawn to show especially the choroid plexus of the lateral ventricle on either side springing from a common origin between the inner mantle walls of the cerebral hemispheres.

Fig. 24 represents a similar section immediately behind the foramina of Monro, and the only feature about it which calls for special comment in this place is the presence of a conspicuous transverse commissure (*Com. F.*) running across above the third ventricle from the inner mantle wall of one cerebral hemisphere to that of the other. This commissure, here very strongly developed, appears to be identical with the rudiment of the fornix discovered in 1881 by Rabl-Rückhard (27) in Psammosaurus.[2] I shall refer to it in future as the Commissura fornicis.

Fig. 25 represents a transverse section across the third ventricle in the plane of the parietal eye.[3] The ventricle itself (fig. 3) is partially divided into two parts, upper and lower. The lower is a deep, slit-like space, bounded on either side by the thick optic thalamus. The upper is a much wider cavity, having its roof formed by the thin choroid plexus, and its floor by the two ganglia habenulæ (*Gang. Hab. R. and L.*), which lie upon the right and left optic thalami respectively, on either side of the slit-like opening into the lower division of the ventricle. There is no noticeable difference as regards size between the two ganglia habenulæ. On either side of the

[1] (18), pp. 1977, 1979, et seq.

[2] Vide also Hoffmann (18), pp. 1975 et seq.

[3] The slightly asymmetrical position of the eye itself is evidently due to an artificial crease in the integument.

thalamencephalon one of the cerebral hemispheres (*C. H.*), with its large lateral ventricle (*L. V.*), is cut through.

Fig. 26 represents a section through the hinder part of the third ventricle, the upper and lower divisions of which are seen to be completely separated from one another in this region by the development of a strong band of commissural fibres (*Com. Sup.*) which runs across from side to side, between and beneath the hinder ends of the two ganglia habenulæ. This commissure I identify with the "superior commissure" described by Burckhardt (9) in Lacerta, and by Hill (17) in various bony fishes. Hill further identifies it with the superior commissure found by Osborn [1] in Amphibia, and with a commissure found by Balfour [2] in Elasmobranchs. Its position corresponds very closely with what Béraneck (5) calls the "parietal centre," and one can hardly help identifying it with this structure as represented in his fig. 5.

Owing to its very strong development in Sphenodon, I at first mistook the superior commissure for the posterior commissure, which at this stage is very feebly developed.[3]

The upper division of the third ventricle is bounded beneath by the ganglia habenulæ, and arched over by the here much folded choroid plexus (*Ch. P.*). The lower division is bounded as before by the two optic thalami, and beneath it lies the infundibulum (*Inf.*).

Fig. 27 represents a transverse section through the entrance to the iter, and shows the very feebly developed posterior commissure (*Com. Post.*) running across just above the latter. The extension of the upper division of the third ventricle (*V.* 3), which runs back above the superior commissure, is cut through between the anterior extremities of the two optic lobes (*O. L.*).

[1] 'Journal of Morphology,' vol. ii, pp. 51—96 (quoted by Hill).

[2] Vide Balfour's 'Comparative Embryology,' vol. ii, p. 356, fig. 254.

[3] This mistake occurs in my summary of results (10), in which the inferior commissure is referred to as the posterior. I shall show later on, however, that there is some reason for believing that the inferior commissure may unite with the posterior commissure in the adult.

With the foregoing description of a series of transverse sections should be compared the sagittal section represented in fig. 15, especially with regard to the position of the commissures. This figure shows very clearly the strong upward arching of the thin roof of the third ventricle, which is doubtless due to the compression of the thalamencephalon in the straightening out of the cerebral flexure. The thin-walled upward extension of the third ventricle thus caused is bounded anteriorly by the commissura fornicis (*Com. F.*), and posteriorly by the superior commissure (*Com. Sup.*).

The Parietal Stalk.—The parietal stalk (figs. 14, 15, 16, 27, *Pa. S.*) has now become considerably elongated. It is attached to the roof of the brain, immediately behind the superior commissure, and in front of the posterior commissure (fig. 15). From its point of attachment it first runs upwards and slightly backwards; it then curves forwards over the bay formed by the roof of the third ventricle, to which its anterior wall is closely applied. Thus, in a transverse section, such as is represented in fig. 27, the proximal part of the stalk is cut longitudinally and tangentially below the thin-walled upper division of the third ventricle, while the distal portion is cut transversely above it.

At the commencement of Stage R the blind distal extremity of the parietal stalk ends in a not very strongly marked and somewhat flattened expansion close beneath the parietal eye (fig. 14). In somewhat older embryos of the same stage it ends in a glove-finger-shaped extremity separated by a considerable interval from the parietal eye (figs. 15, 16), this separation being due apparently to a forward shifting of the parietal eye itself.

At its proximal end the parietal stalk becomes greatly narrowed, as if by compression from the strong development of the superior commissure in front of it; and its lumen, elsewhere wide, is here completely obliterated, though its walls can still be traced into direct continuity with the epithelial lining of the brain. Except at its proximal extremity the wall of the parietal stalk is thick and densely packed with large

oval nuclei, between which, on the inner aspect, the boundaries of columnar cells are faintly discernible. In one late embryo of this stage I have observed the wall of the parietal stalk to be clearly differentiated into two layers (fig. 21), an outer thinner one composed of a single layer of short columnar cells, and an inner very much thicker one, so densely crowded with large oval nuclei arranged in many but irregular tiers, that it is impossible to make out the cell boundaries satisfactorily.

Towards the end of Stage R a patch of black pigment is deposited in the antero-ventral wall of the parietal stalk, near its distal extremity (figs. 15, 16, 21). This pigment resembles that of the parietal eye, having the form of small black granules lying between the innermost cells of the wall, next to the lumen. It appears to be constant, for I have observed it both in transverse and longitudinal sections of embryos at this stage. De Klinckowström (19) has observed pigment in the same situation in Iguana at a certain stage of development, but apparently disappearing later. In Sphenodon also it would seem to disappear in the adult, for none is shown in Spencer's figure (33). Ritter also (29) in Phrynosoma coronata has observed pigment in what he terms the "epiphysial vesicle," which appears to be homologous with the distal extremity of the parietal stalk in Sphenodon.

The Parietal Eye.—At the commencement of Stage R we see the parietal eye in sagittal section lying over the hinder part of the thalamencephalon, and above the flattened distal extremity of the parietal stalk (fig. 14). The end of the stalk lies wedged in between the eye and the brain, and between the stalk and the eye there is a very thin layer of connective-tissue cells. Above the eye, between it and the superficial epidermis, is a rather thick layer of connective tissue, forming part of the dermis, which, however, is here thinner but denser than elsewhere. The upper surface of the lens is pressed close against the under surface of the dermis.

In the eye itself important changes have taken place. The

lens is very sharply marked off from the rest of the wall of the vesicle. The latter, in fact, forms a thick-walled cup with a sharp inturned edge, which appears to grasp the margin of the lens just where the less convex outer surface of the latter joins the more convex inner surface.

Histologically the lens shows little or no change. The rest of the wall of the vesicle, however, forming what we may fairly call the optic cup, has undergone marked alterations. In the first place it has become distinctly differentiated into two layers, an inner (*In. W.*), next to the cavity of the vesicle, and an outer (*Out. W.*, fig. 14). The inner layer is very much thicker than the outer, and the two show a strong tendency to separate from one another, except round the margin of the cup, where no such separation takes place. The outer layer is a single layer of rather short columnar cells with large elongated oval nuclei; it contains no pigment. The inner layer shows a series of columnar cells next to the cavity of the vesicle, with very faint cell boundaries and large oval nuclei, while its deeper part is densely packed with large oval nuclei in several tiers, between which no cell boundaries can be made out. The deepest nuclei, nearest to the outer wall of the cup, are smaller than the others, but except for this no differences can be made out between them. The nuclei are not arranged in distinctly recognisable layers.

Small granules of black pigment, arranged in radiating lines, are now conspicuous between the cells of the inner layer of the wall of the optic cup, but almost confined to the inner half of its thickness, and most abundant in the deeper part of that half, i.e. about the middle of the inner layer. Round the margin of the cup, however, the pigment is especially dense, and lies more on the inner surface next to the cavity of the vesicle.

Between the outer and inner layers of the wall of the optic cup, in the space formed by their slight separation, a small quantity of finely granular, non-staining material is present. This appears to correspond to Spencer's "molecular layer," which in the adult parietal eye seems still to divide the retina

into an outer thinner and an inner thicker layer, as shown in Spencer's figures (33).

Towards the end of Stage R, as already noted, an important change has taken place in the relative positions of the parietal eye and stalk. The eye no longer lies over the end of the stalk, but appears to have shifted forwards, so that it lies slightly in front of the middle of the thalamencephalon and over the tubules of the paraphysis, being separated from the end of the parietal stalk by a wide interval. The relative positions of the parts under discussion at this period are shown in fig. 15, representing a longitudinal vertical section through the thalamencephalon in the median plane. With the exception of the forward shifting of the eye the relations are much the same as in the earlier part of Stage R.

As regards the parietal eye itself, the changes are not very great as compared with younger embryos of this stage. The optic cup, however, is deeper than before, and its axis is inclined backwards and downwards (fig. 16). The retina is still clearly differentiated into two layers, as shown in figs. 16 and 17, and as described in the younger embryo. The black pigment (*Pig.*) is more abundant, but is still almost confined to the inner half of the inner layer of the wall.

In both the earlier and later periods of Stage R a considerable amount of coagulated humour is present in the cavity of the parietal eye, chiefly adhering to the inner surface of the retina, as shown in fig. 17 (*Coag.*), with occasional nuclei, as shown in fig. 18. It is seen also in much smaller quantity adhering to the inner surface of the lens. In figs. 14, 15, and 16 it is omitted for the sake of clearness.

The Nerve of the Parietal Eye.—By far the most important change which marks the later part of Stage R is the appearance of the nerve of the parietal eye. This is seen in longitudinal vertical sections as a faintly staining, delicately fibrillated band attached somewhat posteriorly to the outer layer of the retina, as shown in fig. 16 (*Pa. N.*). From its point of attachment it runs backwards and downwards between the distal part of the parietal stalk and the tubules of

the paraphysis. I have not been able to trace it to a connection with the brain, but it certainly does not appear to be connected with the apex of the parietal stalk. It is probably not connected with the parietal stalk at all, though it lies close under the antero-ventral surface of the latter. It appears to contain elongated nuclei, but I am not quite certain that these may not belong to surrounding connective-tissue cells.

I suspect that the nerve of the parietal eye grows downwards and backwards from the outer layer of the retina, and ultimately becomes connected with the roof of the thalamencephalon. If this should prove to be the case we shall have here another noteworthy resemblance between the parietal eye and the ordinary paired eyes.

The Paraphysis.—The relation of the paraphysis to the other parts of the brain towards the end of Stage R is best shown in fig. 15. It will be seen that the paraphysis arises from the roof of the brain just in front of the commissura fornicis. It curves upwards and backwards over the now strongly arched and more or less folded roof of the third ventricle. Its walls, which consist of short columnar cells arranged in a single layer, are greatly folded and produced into numerous blind diverticula, which form a mass of somewhat convoluted tubules lying in front of the parietal stalk and beneath the parietal eye. Intermingled with these tubules are numerous blood-vessels, easily recognisable by the corpuscles which they contain, and the whole is bound together by loose connective tissue. Histological details are shown in fig. 16. It is worthy of note that the blood-corpuscles in this region contain numerous small black pigment granules similar to those which occur in the retina of the parietal eye and in the parietal stalk. These pigment granules occur also in a blood-vessel lying above the end of the parietal stalk, as shown in fig. 16 (compare Bernard [7]).

The paraphysis at this stage may also be advantageously studied in transverse sections. Thus fig. 23 shows that it originates from the same point as the choroid plexuses of the

lateral ventricles, between which its opening (*O. Par.*) is situated. Fig. 24 shows the paraphysis (*Par.*) cut through above the commissura fornicis. Fig. 25 shows it cut through above the roof of the third ventricle, and below the parietal eye, and fig. 26 shows it cut through just in front of the parietal stalk.

Accessory Vesicle.—At the commencement of Stage R the tubules of the paraphysis do not extend so far backwards as is represented in fig. 15, and between them and the parietal stalk there lies a wide space of irregular shape (fig. 14, *Ac. V.*). This space I propose to term simply an accessory vesicle; its possible homologies will be discussed later on. It is at once distinguished from the paraphysial tubules by the histological characters of its walls, which are composed of much thinner epithelium, though not so thin as those of the blood-vessels. It is further distinguished from the blood-vessels by the absence of corpuscles from its cavity. It gives off a few irregular diverticula or sacculations, but I have not been able to detect any connection between its lumen and the cavity of the third ventricle, nor does it appear to communicate with either the paraphysial tubules or the blood-vessels. Although so conspicuous at the commencement of Stage R, it has completely disappeared in later embryos of this stage, its place being taken by paraphysial tubules (figs. 15, 16).

Stage S.

Of this stage, the last before hatching, according to my classification, I have received only dead specimens, useless for histological purposes.

In the adult Tuatara the parietal eye is no longer recognisable externally, although still very highly organised; but, according to Thomas (38), in the recently hatched Tuatara it still shows as a dark spot through the translucent skin over the parietal foramen.

3. Discussion of Results.

(*a*) Structure of the Parietal Eye.

The Retina.—Certainly the most remarkable feature about the retina of the parietal eye in Sphenodon is its differentiation at Stage R into two very distinct layers, as shown in figs. 14—17. It was this feature, which I first observed in an Australian skink embryo (Hinulia, sp.), which led me to undertake the present investigation. The parietal eye at a certain stage thus acquires an extraordinarily close resemblance to the ordinary paired Vertebrate eye, consisting of a two-layered optic cup grasping a cellular lens within its margin. The developmental history shows, however, that this result is arrived at in a totally different way in the two cases, for in the parietal eye there can be no doubt that the lens is formed simply by thickening of the front wall of an originally single-layered vesicle. It will also be observed that the situation of the pigment is different in the two cases, being in the inner layer, next to the cavity of the cup, in the parietal eye, and in the outer layer, away from the cavity, in the paired eye. Baldwin Spencer (33) has also shown that the rods lie next to the cavity in the parietal eye, instead of being formed from the outermost part of the inner layer as in the paired eye.

It appears to me, from what Baldwin Spencer says of the structure of the retina, that even in the adult parietal eye of Sphenodon we can still trace the two embryonic layers.

This author describes no less than six series of histological elements in the retina of the adult eye. His "molecular layer, consisting of fine punctated material, which takes the stain (hæmatoxylin) with difficulty," evidently marks the embryonic separation of the wall of the optic cup into two primary layers, as I have noticed previously. In the adult the inner of the two primary layers is still much thicker, and appears to have become differentiated into (1) a layer of rod-like bodies enveloped in deep pigment, evidently formed from the innermost layer of columnar cells which I have described in the embryo, with the pigment granules between them;

(2) a double or triple row of spherical nucleated elements, apparently formed from the deeper cells of the inner embryonic layer.

The outer of the two primary layers, consisting in the embryo of a single layer of short columnar cells, appears to have given rise to spherical elements, cone-shaped bodies and spindle-shaped elements according to Spencer, but Hoffmann (18) only recognises one layer in place of these three.

The Lens.—In the adult Sphenodon the lens of the parietal eye has become much more strongly convex on its inner aspect (by elongation of the columnar cells occupying its centre) than it is in the latest embryo examined. In advanced embryos the lens (figs. 14—16) is much more sharply marked off from the retina than is indicated in Spencer's figure, and I have noticed the same fact in the development of the parietal eye in an Australian Hinulia. Beard (4) has also called special attention to the sharp delimitation of the lens from the retina in Anguis, in support of de Graaf's original description (15).

Supposed Arthropod Characters.—The "humour," so frequently noticed as forming a coagulum in the cavity of the parietal eye vesicle, apparently persists in the adult, as indicated in Spencer's fig. 3. As a result of his observations on Ammocœtes, Gaskell (12) has put forward what I cannot help regarding as a mistaken view as to the nature of this humour, at any rate so far as the types examined by me are concerned. He says, "Everything seems to me to point to the conclusion that the appearance of a large central cavity [in the parietal eye] is brought about by the partial degeneration of elements which originally filled it, and that their remains have given rise to the impression held by Spencer and Beard, that a large cavity exists which is filled by a coagulated albuminous fluid." Gaskell accordingly claims an Arthropod structure for the parietal eye of vertebrates.[1]

It appears to me, however, that the observations of Beard

[1] I learn from a paper by Prenant (26) that Leydig has also compared the parietal organs with Arthropod eyes.

(4), Spencer (34), Studnička (35), and others, upon the structure and development of this organ in Cyclostomes, as well as those of the numerous observers who have studied it in Lacertilia, afford conclusive evidence against Gaskell's views; and the weight of this is now only increased by my own observations on Sphenodon, in which animal the parietal eye certainly exhibits no trace of an Arthropod character.

(*b*) The Nerve of the Parietal Eye.

The real nerve of the parietal eye appears, so far as I can learn, to have been first discovered by Béraneck. I regret that I have been unable to consult his earlier work on the subject,[1] but fortunately he has given an excellent account of his observations, together with a discussion of the question, in a later paper (5), which I have been able to obtain. From this I learn that Francotte, Strahl, and Martin have also observed this nerve in Lacertilia. It appears, according to Béraneck, that in Anguis fragilis the nerve is connected distally with the retina of the parietal eye, and proximally with the roof of the brain at a point which corresponds very closely with the superior commissure as described by Hill (17) in fishes, by Burckhardt (9) in Lacerta, and by myself in Sphenodon, but which Béraneck terms the "centre ou noyau pariétal." De Klinckowström (19) has also described the nerve of the parietal eye in Iguana, and states that the "parietal centre" from which it originates is situated asymmetrically on the right side of the origin of the "epiphysis" (= parietal stalk). In this connection it is worthy of note that Gaskell (12) describes the nerve of the parietal eye in Ammocœtes as being connected proximally with the right ganglion habenulæ.

Baldwin Spencer (33), as is well known, believed the nerve of the parietal eye in Sphenodon to be formed from the distal end of the "epiphysis" (parietal stalk). This conclusion, strongly supported by Hoffmann (18), has, I understand, been already challenged by Leydig, and my own

[1] "Ueber das Parietalauge der Reptilien," 'Jenaische Zeitschrift,' 1887.

observations certainly seem to indicate that such an interpretation is incorrect. As already stated, I found at Stage R a nerve which appears to correspond exactly so far as observed with that described by Béraneck. It passes in front of and beneath the distal extremity of the parietal stalk, and becomes connected with the outer layer of the retina (figs. 15 and 16). Unfortunately I have been unable to trace it to its point of origin from the roof of the brain, but I have little doubt that the connection takes place at or near a point immediately in front of the origin of the parietal stalk, corresponding to what Béraneck terms the parietal centre, and lying between the posterior ends of the two ganglia habenulæ, above the fibres of the superior commissure (fig. 26). It seems probable that the nerve is actually connected with one or other of the ganglia habenulæ, as in Ammocœtes, but I have been unable to detect any such difference in size between the two ganglia as occurs in the lamprey. The fact that the parietal eye itself in Sphenodon originates on the left side of the middle line seems to make it probable that the nerve is connected with the left ganglion habenulæ. The observations of de Klinckowström, however, point to the right ganglion. Possibly the nerve crosses over from right to left, but this is a question which cannot be decided in the present state of our knowledge, and one which is well worthy of further investigation. We may safely take it as an established fact, however, that the parietal eye has a special nerve which lies in front of and is not derived from the parietal stalk ("epiphysis").

(*c*) Relations of the Parietal Eye to the Parietal Stalk.

Embryologists who have studied the question appear to be divided into two schools on this subject. The first and older school maintain that the parietal eye is either the distal extremity of the stalk ("epiphysis") separated from the proximal, or that it is an outgrowth or diverticulum of the stalk. The second and more modern school hold that the

parietal eye and the stalk originate as separate outgrowths from the brain.

As one of the earliest of the first-named school I may cite McKay (**25**), whose valuable work appears to have been overlooked by many of the more recent writers. The figures given by McKay of the early stages in the development of the parietal eye in Grammatophora agree very closely on the whole with what I have observed in Sphenodon, but he interprets the appearances as follows:—"The epiphysis cerebri or pineal gland arises, as is seen, as an outgrowth of the thalamencephalon. At this stage the outgrowth is composed of a single layer of columnar cells with well-marked nuclei (fig. 1). Second stage.—In the next stage the evagination or vesicle undergoes the following changes. The anterior wall begins to grow forward, and this soon leads to the formation of a second evagination in the wall of the primary one (fig. 2, *Pn.*). Thus we have two vesicles formed, an anterior larger (*Pn.*), destined to become the pineal eye, and a posterior smaller one (*Ep.*). Since the anterior vesicle grows faster than the posterior it bends forwards, and its inferior wall rests on the superior surface of the columnar cells of the thalamencephalon (fig. 2). The walls of both vesicles are composed of a single layer of columnar cells with oval nuclei." Of his third stage he observes, "The anterior of the two vesicles becomes constricted off to form the pineal eye (fig. 4, *Pn.*); while the posterior remains as the end of the epiphysis (fig. 4, *Ep.*)."

Unfortunately I have been unable to consult Hoffmann's original memoir[1] on the development of Lacerta, but this author has also described and illustrated his results in his memoir on the Reptilia in Bronn's 'Klassen und Ordnungen des Thier-reichs' (**18**). From this description it appears that in Lacerta also the early development of the parietal eye and stalk is very similar to what takes place in Sphenodon and Grammatophora. A hollow outgrowth is budded off from

[1] 'Weitere Untersuch. zur Entwicklungsgesch. der Reptilien. Morphol.,' Jahrb. xi, 176 (quoted by McKay).

the hinder part of the roof of the thalamencephalon. This vesicle, according to Hoffmann, becomes divided by a constriction into two parts, anterior and posterior, of which the anterior develops into the parietal eye and the posterior into the "eigentlichen Epiphyse" (= parietal stalk). Later on the parietal eye vesicle no longer lies in the same plane as the "epiphysis," but somewhat obliquely in front and to the right of it.

As a representative of the second school I may mention Béraneck, who in his later papers (5 and 6) has contended very vigorously for what he terms the individuality of the parietal eye. He maintains that the two vesicles, observed by all who have investigated the subject, originate independently of one another from the brain, and that therefore the parietal eye is not formed from the "epiphysis." In support of this view he lays special stress upon the fact that the nerve of the parietal eye is developed independently of the so-called epiphysis or parietal stalk, and not at the expense of the latter as was formerly supposed.

In spite of the arguments advanced against Béraneck by de Klinckowström (19), who has investigated the development of the parietal eye and "epiphysis" in Iguana, I must declare myself strongly in favour of the "individuality" of the parietal eye. Béraneck (6) has thoroughly argued the question in his reply to de Klinckowström, and I think he has hit upon the true explanation of the diversity of opinion in the following passage:—"Ces divergences assez importantes s'interprètent facilement avec l'hypothèse que l'œil pariétal et l'épiphyse sont des évaginations distinctes du thalamencéphale. En effet, ces deux organes peuvent avoir un développement simultané a successif, suivant certaines conditions embryogéniques, sans que leurs caractères morphologiques en soient pour cela altérés."

I have already given my arguments for believing that in Sphenodon the parietal stalk and eye originate independently. Béraneck (6) believes that the primitive evagination of the brain described by Francotte in Anguis corresponds

not to the "pineal gland," but to the parietal eye only. This is exactly my view with regard to this organ in Sphenodon, in which it appears that the "stalk" or "pineal gland" or "epiphysis" (so called) develops a little later than the parietal eye itself. The eye, it will be remembered, first appears as a small round vesicle, which I have termed the "primary parietal vesicle," budded out on the left side of the middle line. This vesicle, according to my interpretation, soon becomes completely closed, and a second vesicle appears as an outgrowth of the roof of the fore-brain, beneath and slightly to the right of the primary vesicle, pushing the primary vesicle forwards. This second vesicle forms the "parietal stalk." For a long time its cavity retains free communication with the cavity of the fore-brain, but at Stage R it has become completely shut off from the latter, a fact which will be seen to have considerable importance when we come to consider the relation which the parietal stalk bears to the "epiphysis" of the adult.

(*d*) Evidence of the Paired Origin of the Parietal Eye and Stalk.

The evidence in favour of the originally paired character of the parietal eye in Sphenodon is derived principally from the fact that the eye itself arises on the left-hand side of the middle line, while the "parietal stalk" appears almost or quite in the middle line, and therefore a little to the right of the parietal eye. These relations appear to be very constant. Thus I have observed the first origin of the primary parietal vesicle (= parietal eye) to the left of the middle line in the only two embryos of which preparations suitable for demonstrating this point have been made (vide figs. 2, 5); and later on, when the parts in question have become conspicuous externally, I have observed the young parietal eye lying to the left of the stalk (fig. 12) in nearly every embryo examined, though by the time Stage R is reached, as already noticed, it appears to have become median (fig. 13).

As this is a point of some importance it may be well to enumerate all the cases observed belonging to Stages N, O, and P.

Stage N	Embryo 93, „ 95, „ 96	Parietal eye lying to left of stalk.
	„ 94	Parietal eye lying directly in front of stalk.
	„ 97	Parietal eye lying pretty well in front of stalk, but both to left of middle line.
Stage O	„ 89, „ 90, „ 92, „ 103	Parietal eye lying to left of stalk.
Stage P	„ 87	Parietal eye lying well to left of middle line, stalk indistinct (externally).

At very early stages in the development, immediately antecedent to the first appearance of the primary parietal vesicle, the roof of the fore-brain is in a markedly asymmetrical condition, the left half overlapping the right for some distance. This overlap is shown in fig. 1, in the region between the optic vesicles of the ordinary eyes; it also extends to the region in which the primary parietal vesicle will shortly appear. It will be found more fully illustrated in my memoir on the general development of Sphenodon. Granting that the right parietal eye was once well developed, it seems not unnatural to associate its suppression with this remarkable overlapping of the left side of the roof of the fore-brain, which at first seemed to me to be due to restraint exercised by the very early developed pro-amnion upon the growing brain; but the probability of this explanation is greatly diminished by the fact that a very similar asymmetry of the developing parietal organs occurs in fishes, as described by Hill (**16**, **17**). The part of the brain roof from which the right parietal eye should arise is covered over at first by the overlap, which may perhaps be supposed to retard its development.

If the right parietal eye ever develops at all it must be represented by the parietal stalk, and there is certainly good ground for considering this to be the case. In the first place the parietal stalk originates, if I am right in my interpretation

of the development, in precisely the same manner as the left parietal eye, of which it is quite independent. In the second place it has for some time a very similar structure, though it never acquires the same degree of perfection.

The similarity in structure of the parietal eye and the parietal stalk is a point on which I am inclined to lay considerable stress as an argument in favour of their paired origin. Both have the form of hollow vesicles, which become completely shut off from the brain cavity. Except for the fact that no lens thickening is formed in the case of the "stalk," the histological characters of the two are, up to a certain stage at any rate, identical. Fig. 21 represents a transverse section through the "stalk" at Stage R, taken near its distal extremity, just at the place where pigment is deposited in its ventral wall as already mentioned. It will be seen that the wall is divided into two layers, as in the left parietal eye,—an outer thin one composed of a single tier of short columnar cells, and an inner very much thicker one containing many large oval nuclei not regularly arranged in tiers, and with a layer of columnar cells next to the central cavity. The pigment is deposited between the cells of the inner half of the inner layer of the ventral wall, exactly as in the left parietal eye. On comparing this figure with the sections of the left parietal eye represented in figs. 14, 16, and 17, and bearing in mind its developmental history, the conclusion that the so-called "parietal stalk" or "epiphysis" in Sphenodon represents a right parietal eye appears to me inevitable.

The probability of this conclusion is greatly strengthened when we come to compare certain observations of other writers dealing with other types, by far the most important of which are those of Hill (16, 17) on certain Teleostean fishes, and on Amia.

In 1891 Hill described (16) in Coregonus albus two "epiphysial outgrowths" from the roof of the primary forebrain. He says, "On the roof of the brain—in the median line, and in a plane passing through the middle of the optic

vesicles—is seen the posterior epiphysial outgrowth. It is a small spherical body, having its lateral walls thickened so that the cavity within it is laterally compressed. This cavity is narrowest at the middle, on account of the greatest thickness of the lateral wall of the vesicle falling at the middle of its antero-posterior axis; consequently, in a dorsal view the cavity has the form of a dumb-bell. Just in front of this vesicle, and a little to the left of it, is a second similar outgrowth. This anterior evagination is smaller than the posterior one, and appears to be solid. It lies close against the wall of the posterior vesicle, and is partly hidden by it."

At earlier stages Hill found that each vesicle arises as a separate outgrowth from the roof of the brain, and each contains a cavity which opens separately into the cavity of the brain. "The anterior vesicle shows an increase in size for about twenty days, and after that a decrease, while the posterior vesicle shows from the beginning a gradual increase." The author further compares these two "epiphysial outgrowths" with those described by Leydig[1] in Lacertilia, and points out "that while the early stages of these two epiphysial outgrowths of Coregonus agree in many details with the corresponding early stages of the two outgrowths in Lacertilia, as described by Leydig, yet the ultimate fate of these two outgrowths in the two forms is widely different. In Coregonus the anterior outgrowth, which is the smaller, gradually disappears, while in Lacertilia, according to Leydig, it develops into the adult parietal organ." He further thinks it probable that the two outgrowths will be shown to be homologous with the primary and secondary parietal vesicles described in adult Petromyzon by Ahlborn (1), and in Lacertilia by Ritter (29).

In a later paper (17), published in 1894, Hill continues his excellent researches on this subject, and arrives at closely similar results in the case of various fishes. He shows that there are two epiphysial outgrowths from the roof of the

[1] "Das Parietalorgan der Amphibien und Reptilien," 'Anatomische-histologische Untersuchung,' Senckenberg. Naturf. Ges., Band xvi, p. 441. I regret that I have been unable personally to consult this important memoir.

primary fore-brain of Salmo, Catostomus, Stizostedion, Lepomis, and Amia. The anterior of these two is rudimentary, and appears to lie constantly to the left of the posterior. He concludes that the anterior epiphysial vesicle is homologous with the parietal eye of Lacertilia, while the posterior, or epiphysis itself, is homologous with the epiphysis of Lacertilia; and he thinks it probable that in their primitive position the two vesicles were side by side.

I need hardly point out that my own observations on Sphenodon in the main strongly support the important results arrived at by Hill. Indeed, except that in Coregonus the posterior vesicle appears to arise first, the agreement between the early stages of the types investigated by us is most remarkable; and I think there can be little doubt that the parietal eye was not originally an unpaired sense-organ, as usually supposed, but one of a pair, of which, in most cases at any rate, the left at the present day has the eye-like structure most fully developed. In bony fishes the right parietal eye is evidently represented by the "epiphysis," as described by Hill. In Lacertilia it is represented by what I still prefer to term the "parietal stalk," which is therefore homologous with the "epiphysis" of bony fishes; but how far this latter is homologous with the "epiphysis" of Sauropsida and Mammalia as that organ is ordinarily understood is another question.

The fact that, both in the various types of fishes investigated by Hill and Sphenodon, it is the left vesicle which alone or first separates from the brain, and either degenerates or gives rise to a parietal eye, appears to me very remarkable. It is not, however, by any means certain that it is always the left parietal eye which is best developed. Thus in Lacerta, according to Hoffmann's description (18), the parietal eye would appear to be formed from the right vesicle, and the stalk from the left one.

Gaskell also (12), who lays great stress upon the paired origin of the parietal eye in the lamprey, believes it to be the right parietal eye which is most perfectly developed, and which

overlies the much smaller left vesicle, a conclusion which appears to be supported by the observations of Studnička (35) on the development of Petromyzon planeri. The latter author, however, does not appear to realise the paired character of the two vesicles, which he terms "pineal" and "parapineal" respectively; the former, which he supposes to be of greater antiquity, apparently corresponding to the right, and the latter, which he supposes to be of more recent origin, to the left parietal eye. Why these two organs should so persistently tend to alter their positions from the primitive transverse to the sagittal plane is a mystery which cannot at present be explained.

Before leaving the question of the paired origin of the parietal eye, I should recall the very interesting and significant fact that Locy (21) considers the epiphysis of Elasmobranchs to be formed from a united pair of accessory optic vesicles; and that he has recorded (23) the discovery of accessory optic vesicles in the chick, and put forward the view that the Vertebrate eyes are segmental.

(*e*) The Relations between the Parietal Stalk, the Epiphysis, and the Brain.

The organ which I term in this paper the "parietal stalk," as I have already pointed out, is commonly, if not invariably, identified by writers on the subject as a portion, at any rate, of the epiphysis cerebri or pineal gland. By those who hold that the parietal eye is formed at the expense of the stalk, the former is commonly regarded as the distal, and the latter as the proximal part of the epiphysis.

In my summary of results I announced my belief that the parietal stalk does not represent the epiphysis, basing this conclusion upon a more or less mistaken identification of the early and strongly developed superior commissure which arises just in front of the stalk as the posterior commissure.[1] Although my conclusion was thus originally founded upon a mistake, and

[1] I still believe that the superior commissure may form the anterior part of the posterior commissure of the adult.

therefore requires considerable modification, I still believe that there is very grave doubt as to the homology of the so-called "epiphysis" throughout the Vertebrate series. I am convinced that in Sphenodon, at any rate, the parietal stalk does not give rise by itself to what is usually regarded as the epiphysis of the adult, as described, for example, by Hoffmann (18), who begins his description of the epiphysis of Sphenodon thus: "Die epiphyse selbst bildet eine ziemlich weite, schlauchförmige Fortsetzung der dritten Hirnhöhle."

Perhaps one of the most important facts established by my investigation of the development of Sphenodon is the complete closure of the proximal end of the parietal stalk at Stage R, so that its cavity is completely shut off from that of the brain. This closure seems to be effected by compression between the large superior commissure immediately in front of the base of the stalk, and the posterior commissure immediately behind it (fig. 15). It appears to me highly probable that these two commissures ultimately unite at the point where the opening of the parietal stalk was placed. If this be so, the so-called posterior commissure of reptiles and higher vertebrates must be regarded as a composite structure, the front part of which is homologous with the superior commissure of fishes.

In any case it is clear that in Sphenodon the parietal stalk cannot give rise to the main part of the epiphysis of the adult as that organ is described by Hoffmann, though it may give rise to a portion thereof.

This conclusion is strongly supported by the examination of other types. Ritter's observations on Phrynosoma are especially instructive in this respect, and are capable of a very simple interpretation in accordance with my views. Before discussing these observations it may be well to quote his following paragraph (29):

"In previous discussions of the nature and function of the parietal organ, I believe sufficient attention has not been given to the structure and development of the epiphysis and its relation to the parietal vesicle, and especially its relation to the so-called choroid plexus. I have designated the entire

structure found in connection with the roof of the thalamencephalon as the epiphysis; but, as already said, I have considerable doubt as to the wisdom of so doing. For the sake of precision it would seem best that the term epiphysis should be limited to the structure which arises as an evagination from this portion of the brain. Certain it is that the large blood sinus which I have described as a part of the epiphysis in Phrynosoma cannot be regarded as forming an essential portion of the structure, and I think it quite possible that what I have called the epiphysial vesicle is not a portion of the epiphysis, should the term be limited as I have suggested that it ought to be. The distinctness of the epiphysial vesicle from the proximal portion of the epiphysis in the adult Phrynosoma is without exception, so far as my observations have gone; and if it is regarded as having been derived from the epiphysis, then we have two vesicles instead of one that have arisen in this way, and the difficulty of explaining the nature and function of the whole structure is correspondingly increased."

Now if we limit the term epiphysis as I understand Ritter to suggest, we shall limit it to the comparatively late evagination of the thin roof of the third ventricle between the parietal stalk and the paraphysis. There appears to me to be a good deal in favour of this definition of the epiphysis; but if we accept it, then it is quite clear that neither the parietal stalk nor the parietal eye has anything whatever to do with the epiphysis, but both develop much earlier, and also behind it. It is also obvious that the epiphysis in Ritter's sense is not homologous with the epiphysis of fishes as described by Hill.

The parietal stalk in Phrynosoma is undoubtedly represented by what Ritter terms the "epiphysial vesicle," whose cavity, as in Sphenodon, is completely shut off from that of the brain.

In comparing Ritter's fig. 9, representing a sagittal section through the thalamencephalon and adjacent structures in Phrynosoma, with my fig. 15, representing a corresponding section of an advanced embryo of Sphenodon, it is necessary

to notice one point very carefully. The commissure which Ritter identifies as the superior commissure evidently does not correspond with that structure as described by Hill in fishes and myself in Sphenodon. It corresponds rather to what I have identified as the commissura fornicis in Sphenodon. Where, then, are we to look for the superior commissure in Phrynosoma? I believe that it has united with the posterior commissure in the manner already indicated. This interpretation renders Ritter's figure directly comparable with mine.

Further evidence in favour of my views on this subject is afforded by my fig. 28, representing, in a slightly diagrammatic manner, a sagittal section through the third ventricle and neighbouring organs in Hinulia.

(*f*) The Paraphysis.

In the interpretation of the so-called "epiphysis" or "pineal gland" of the higher vertebrates, the paraphysis, only discovered within recent years, must in future play a very important part. An excellent summary of the history of our knowledge of this organ has been given by Studnička (**37**). From this it appears that Selenka, whose original paper[1] I have been unable to see, gave the name "paraphysis" to an unpaired outgrowth of the membranous roof of the prosencephalon in embryos of reptiles and Selachians. He interpreted this structure as a rudimentary sense-organ, and homologised it with the auditory organ of Ascidians, as Spencer and de Graaf had previously homologised the epiphysis with the eye.

The organ itself had been found previously by Hoffmann in Tropidonotus and Lacerta, and by Francotte[3] in Anguis, the latter interpreting it as the choroid plexus. It has since been found in representatives of almost all vertebrate

[1] "Das Stirnorgan der Wirbelthiere," 'Biol. Centralblatt,' x.

[2] "Weitere Untersuchungen zur Entwicklung der Reptilien," 'Morph. Jahrb.,' xi.

[3] "Recherches sur la Développement d l'Epiphyse," 'Archiv. de Biologie,' viii.

groups. McKay figured it in Hinulia (25, figs. 7, 8) as far back as 1888, but described it only as an evagination in front of the pineal eye, "very similar to the eye itself."

Leydig—as I learn from a short paper by Eycleshymer (11)—finds instead of a single vesicle a group of five, which later came into such close relation with the "epiphysis" that Spencer figured both as one structure. Eycleshymer himself describes the paraphysis in Amblystoma as a median outgrowth of the posterior portion of the roof of the prosencephalon. Lateral diverticula appear at its distal end, but its cavity becomes obliterated proximally in a manner analogous to that which occurs in the "epiphysis." The two structures in Urodela never come into close relation, as in Reptilia, but remain widely separate.

The development of the paraphysis in Sphenodon has already been described in these pages, and appears to agree closely with what takes place in Lacertilia. It gives rise to a mass of convoluted tubules, lined by short columnar cells and intermingled with blood-vessels, which lies beneath the parietal eye and in front of the parietal stalk (figs. 15, 16). These tubules undoubtedly form a part of what is usually recognised as the "pineal gland."

It thus appears that my observations with regard to the paraphysis support the conclusions of Leydig already noticed. Hoffmann also, according to Ritter (29), considers that the paraphysis or "ependyma" in the grown animal "comes to take a not inconsiderable part in the formation of the epiphysis." There appears to be no reason whatever for regarding it as a sense-organ, while its structure certainly suggests a glandular function. In origin it is intimately connected with the choroid plexus of the lateral ventricles, as I have already shown.

(*g*) The Epiphysis of Sphenodon and Lacertilia.

It thus appears that the "epiphysis" of Sphenodon and Lacertilia, as ordinarily understood, is a composite structure, made up of the following constituent parts:

(1) An upward arching of the thin roof of the third ventricle between the superior commissure and the commissura fornicis. This outgrowth arises comparatively late in development, and is probably the result, at any rate to a large extent, of the compression of the thalamencephalon between the prosencephalon and the mid-brain in the straightening out of the cerebral flexure. It is evidently homologous with the Zirbelpolster described, for example, by Burckhardt (9) in Lacerta. It is to this part that Ritter (29) proposes to restrict the term "epiphysis."

(2) An outgrowth from the membranous roof of the prosencephalon arising in close relation with the choroid plexus of the lateral ventricles, and known as the paraphysis. This outgrowth grows upwards and backwards over the commissura fornicis, and gives rise to a number of blind diverticula or tubules, which lie beneath the parietal eye.

(3) A number of blood-vessels, which make their way in amongst the diverticula or tubules of the paraphysis.

(4) The parietal stalk, which arises from the roof of the thalamencephalon a short way in front of the optic lobes, and passes upwards and forwards over the superior commissura until it meets the backward-growing paraphysis. The parietal stalk, however, does not appear to form any very important part of the epiphysis of the adult, but is a vestigial structure representing (in Sphenodon) the right parietal eye. It undoubtedly gives rise to the "epiphysial vesicle" of Ritter, and to a part, at any rate, of what Spencer (33) calls the "portion of the epiphysis equivalent to the pineal stalk." It is clearly shown, for example, in Spencer's fig. 41, representing a sagittal section through the brain of Varanus bengalensis, where it is labelled Ep.[1]; and in his fig. 25, representing a corresponding section of Anguis fragilis.

This analysis of the so-called "epiphysis" into its constituent

parts is very obvious in the case of an advanced embryo of Hinulia, as seen in sagittal section and represented in my own fig. 28. It is also made very clear by Burckhardt's admirable figure of the brain of the embryo of Lacerta (9), in which, however, he does not represent any commissura fornicis.

(*h*) The Accessory Vesicle.

The accessory vesicle (fig. 14, *Ac. V.*), which appears between the tubules of the paraphysis and the end of the parietal stalk in the early part of Stage R of Sphenodon, is extremely difficult to account for. The histological character of its walls and the absence of any communication of its cavity with those of the surrounding organs seem to indicate an independent origin. It is conceivable that it may be formed from the outgrowth which I have identified as the commencing paraphysis at Stages N and O (figs. 6, 9, 11, *Par.*), and that the paraphysis itself originates later, between Stages O and R; but this view appears to me to be highly improbable.

The absence of blood-corpuscles, the large size of the cavity, and, to a less extent, the nature of the lining epithelium, prevent us from regarding it as a blood-vessel. It may perhaps be a large lymphatic sac, but this again does not seem very probable. It may be homologous with the accessory vesicle or "parapineal organ" described by Ritter (30) in Phrynosoma, but it is very doubtful if it is homologous with the "parapineal organ" of Studnicka in the lamprey.

Burckhardt (9) figures a small vesicle in a similar position in Lacerta vivipara, and, following Leydig, terms it the "Nebenscheitel-Organ." Unfortunately I have not seen Leydig's work, and therefore am unable directly to compare the organ as described by him with the transitory accessory vesicle of Sphenodon. Judging from the observations of Prenant (26), however, it seems to me hardly likely that the accessory vesicle of Sphenodon is homologous with the accessory parietal organs of Anguis fragilis, which are supposed to be budded off from the "epiphysis" (parietal stalk), and, like the latter, have thick walls.

4. General Conclusions.

As a result of my study of the writings of other embryologists, as well as of my personal observations, I have been led to formulate the following general conclusions as to the homologies and phylogenetic history of the "epiphysis" and associated organs.

(1) The "epiphysis" of Selachians is formed by a pair of equally well-developed optic vesicles, originating as outgrowths of the thalamencephalon, and uniting together in the middle line (as shown by Locy).

(2) In Teleosts and Amia there is also a pair of epiphysial outgrowths arising in a similar way (with more or less displacement), but the right vesicle alone gives rise to the "epiphysis" of the adult, while the left one separates completely from the brain and degenerates (as shown by Hill).

(3) In Cyclostomes there is also a similar pair of epiphysial outgrowths, which suffer displacement in such a manner that the right vesicle comes to overlie the left. The right vesicle forms a parietal eye, and the left one the "parapineal organ." These two, together with the nerve of the parietal eye, constitute the "epiphysis" (compare Ahlborn, Beard, Gaskell, Studnička).

(4) In Sphenodon and Lacertilia the "epiphysis" is a composite structure in which the paraphysis and "Zirbelpolster" take a very large share, while the parts which correspond to the paired epiphysial outgrowths of fishes take a very small one. These outgrowths originate, however, very much as in fishes, and are subject to more or less displacement, and one or other of them may give rise to a parietal eye. In Sphenodon it is the left parietal eye which is thus developed.

(5) The right parietal eye is represented in Sphenodon by the "parietal stalk." In Lacertilia the parietal stalk represents either the right or left parietal eye.

(6) The parietal eye has no real connection with the parietal stalk beyond that of fellowship, and is supplied with a special nerve of its own not derived from the parietal stalk.

(7) The ancestors of existing vertebrates possessed a pair of parietal eyes, which may have been serially homologous with the ordinary vertebrate eyes.

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EXPLANATION OF PLATES 11—13,

Illustrating Mr. Arthur Dendy's paper on the "Development of the Parietal Eye and Adjacent Organs in Sphenodon."

Explanation of Lettering.

Antr. Anterior. *Au.* Auditory vesicle. *B. V.* Blood-vessel. *Cart.* Cartilage. *C. H.* Cerebral hemisphere. *Ch. P.* Choroid plexus. *Ch. P. L. V.* Choroid plexus of lateral ventricle. *Coag.* Coagulated humour in cavities of parietal eye and stalk. *Com. Ant.* Anterior commissure. *Com. F.* Commissura fornicis. *Com. Man.* Mantle commissure. *Com. Sup.* Superior commissure. *Com. Post.* Posterior Commissure. *Corp. Stri.* Corpus striatum. *Derm.*

Dermis. *Epd.* Epidermis. *Eye.* Ordinary paired eye. *F. B.* Fore-brain. *F. M.* Foramen of Monro. *F. N. P.* Fronto-nasal process. *Gang. Hab. R. and L.* Right and left ganglia habenulæ. *H. B.* Hind brain. *H. C.* Head cavity. *H. M. C.* Hyomandibular cleft. *Inf.* Infundibulum. *Int.* Integument. *In. W.* Inner layer of wall of parietal optic cup and of parietal stalk. *Iter.* Iter a tertio ad quartum ventriculum. *L.* Lens of parietal eye. *L. P.* Lens of paired eye. *L. V.* Lateral ventricle. *Mand.* Mandibular arch. *M. B.* Mid-brain. *Mes.* Mesoblast. *Mo.* Mouth. *Mol.* Molecular layer in retina of parietal eye. *Not.* Notochord. *Nu.* Nucleus. *O. L.* Optic lobes. *O. Par.* Origin of paraphysis between the choroid plexuses of the lateral ventricles. *Op. ch.* Optic chiasma. *Out. W.* Outer layer of wall of parietal optic cup and of parietal stalk. *O. V.* Optic vesicle of paired eye. *Pa. E.* Parietal eye. *Pall.* Pallium of cerebral hemisphere. *Pa. N.* Nerve of parietal eye. *Par.* Paraphysis. *Par. T.* Tubules of paraphysis. *Pa. S.* Parietal stalk. *P. B.* Pituitary body. *p. m.* Pia mater. *P. V.* Primary parietal vesicle. *Pig.* Pigment. *R. T.* Roof of thalamencephalon. *Thal.* Thalamencephalon. *Thal. Op.* Optic thalamus. *V. 3.* Third ventricle. *X.* (Figs. 16 and 17.) Space due to separation of outer and inner layers of wall of parietal optic cup. *X.* (Fig. 18.) Inner surface of retina of parietal eye. *Z.* Evagination of the roof of the third ventricle forming the proximal part of the "epiphysis" of the adult, and sometimes known as the "Zirbelpolster."

FIGS. 1—27. Sphenodon punctatus.

FIG. 1.—Stage J. Embryo 44. Transverse section of head, showing overlap of the left side of the roof of the fore-brain on the apparent ventral aspect. (Reversed as compared with Fig. 5.) Zeiss A, oc. 1, camera outline.

FIG. 2.—Stage K. Embryo 39. Transverse section of head, showing first appearance of the primary parietal vesicle to the left of the middle line on the apparent ventral aspect. (Reversed as compared with Fig. 5.) Zeiss A, oc. 1, camera outline.

FIG. 3.—Stage L. Embryo 50. Longitudinal vertical section of head (nearly median), showing origin of primary parietal vesicle as an evagination from the roof of the fore-brain. Zeiss A, oc. 1, camera outline.

FIG. 4.—Stage L. Embryo 50. Portion of the same section, more enlarged, to show the histological structure of the primary parietal vesicle. Zeiss D, oc. 1, camera outline.

FIG. 5.—Stage M. Embryo 81. Transverse section of head, showing origin of primary parietal vesicle on the left side of the middle line, and apparently ventral owing to cerebral flexure. Zeiss A, oc. 1, camera outline.

FIG. 6.—Stage N. Embryo 96. Median longitudinal vertical section through the paraphysis at its first appearance, showing its origin as a backward-pointing outgrowth of the roof of the fore-brain. Zeiss D, oc. 1, camera outline.

Fig. 7.—Stage N. Embryo 96. Longitudinal vertical section through parietal eye and stalk. The eye lies to the left of the stalk, and is cut on one side of its median plane. Zeiss D, oc. 1, camera outline.

Fig. 8.—Stage N. Embryo 96. Similar section a little further to the left, cutting through the middle of the parietal eye and escaping the stalk. Zeiss D, oc. 1, camera outline.

Fig. 9.—Stage O. Embryo 92. Diagrammatic sagittal section through the head, to show the relative positions of the parietal eye and stalk, paraphysis, and infundibulum. (The parietal eye really lies to the left of the middle line.)

Fig. 10.—Stage O. Embryo 92. Sagittal section a little to the left of the middle line, through the parietal stalk and eye, passing a little to the left side of the opening of the cavity of the parietal stalk into the brain cavity. Zeiss D, oc. 1, camera outline.

Fig. 11.—Stage O. Embryo 92. Sagittal section through the paraphysis, showing its commencing convolution. Zeiss D, oc. 1, camera outline.

Fig. 12.—Stage O. Embryo 89. Upper surface of head, to show relative positions of parietal eye and stalk, paraphysis, cerebral hemispheres, and optic lobes. × 10.

Fig. 13.—Stage R (early). Embryo 143. Upper surface of head, to show positions of parietal eye, cerebral hemispheres, and optic lobes. × 5.

Fig. 14.—Stage R (early). Embryo 143. Part of sagittal section through parietal eye and stalk, paraphysis, and accessory vesicle. Zeiss C, oc. 1, camera outline.

Fig. 15.—Stage R (late). Embryo 2. Sagittal section through the thalamencephalon and neighbouring parts, combined from several of the same series. Zeiss A, oc. 1 (assisted by camera).

Fig. 16.—Stage R (late). Embryo 2. Sagittal section through parietal eye, end of parietal stalk, nerve of parietal eye, and paraphysial tubules. Zeiss D, oc. 1, camera outline.

Fig. 17.—Stage R (late). Embryo 2. Part of retina of parietal eye in vertical section, to show its histological structure. Zeiss F, oc. 1, camera outline.

Fig. 18.—Stage R (late). Embryo 2. Part of vertical section of parietal eye, to show coagulated humour,[1] containing a group of nuclei and adhering to the inner surface of the retina (*X*). The sharp bend in the surface of the retina marks the bottom of the optic cup. Zeiss F, oc. 1.

Fig. 19.—Stage O. Embryo 92. Vertical section of lens (sagittal) with

[1] In many of the drawings of the sections the coagulated humour has been omitted for the sake of clearness.

adherent humour containing a nucleus, and with another nucleus apparently escaping from the under surface of the lens. Zeiss F, oc. 1, camera outline.

Fig. 20.—Stage O. Embryo 92. Part of wall of parietal stalk from same section as last, with adhering humour and nucleus. Zeiss F, oc. 1, camera outline.

Fig. 21.—Stage R (late). Embryo 3. Transverse section through the parietal stalk near its distal extremity. Zeiss D, oc. 1, camera outline.

Figs. 22—27.—Stage R (late). Embryo 3. Transverse sections through the third ventricle and adjacent parts, arranged in order from before backwards. × 16½. Camera outlines.

Fig. 28.—Hinulia, sp. Part of a sagittal section through the third ventricle and adjacent parts of an advanced embryo, to show the relations of the "epiphysis," parietal eye, and associated parts. Zeiss A, oc. 1. Slightly diagrammatic.

The Molluscs of the Great African Lakes.

III. Tanganyikia rufofilosa, and the Genus Spekia.

By

J. E. S. Moore.

With Plates 14—19.

On the coast line formed by the red sandstone and conglomerate precipices which flank the picturesque shores of Lake Tanganyika there are to be found a number of rock molluscs, which dwell upon the submerged stones, much in the same manner as the periwinkles do upon the half-tide rock faces of the ocean coasts. Of these small Gastropods, what have appeared to be at least three distinct species have been known, but hitherto only by the characters of their empty shells, and in the absence of all other information they have been regarded by the conchologists as approaching the genus Lithoglyphus.[1] In the literature, one of these forms, L. neritinoides, still bears that generic name, but the species rufofilosa was eventually placed by Cross in what he supposed to be an allied but separate genus, under the name of Tanganyikia, while the original L. zonatus was placed by Bourguignat in the genus Spekia.

No shells exactly similar to these have been found, hitherto, outside the confines of the great lake in which they were originally observed by Speke; and from this very remarkable fact we might suspect, without further investigation, that they

[1] That is as members of the family Hydrobiidæ.

belong to the singular marine fauna for which the lake is famed.

In the present paper it is my purpose, in the first place, to exhibit the wide morphological difference which exists between these types by a description of the anatomy of Tanganyikia rufofilosa as compared with that of Spekia zonata, both forms having been collected during my expedition to the lake in the summer of 1896. In the second, I wish to show that the morphological characters of both these forms, like those of all the halolimnic species, suggest that they are the constituents of a fauna that some ancient sea has left behind. And lastly, I wish to lay emphasis upon the fact that they possess anatomical peculiarities, as a concomitant of their vast antiquity, which are worthy of the closest study. For, quite apart from the fact that these molluscs are zoological curiosities in the sense that they are relics of a departed biological era, it would be difficult to instance any forms possessing a higher scientific value as a means of clearing up the obscure inter-relationship of numerous molluscan types.

Tanganyikia rufofilosa.

The shell of this form is represented in Pl. 14, fig. 1, and was first described by Smith,[1] from the empty shells obtained by Captain Speke and Mr. Hore. The animals, which I observed alive, have a white, semi-transparent foot, with a very broad, wrinkled, and pigmented snout. The tentacles are not long, and the eyes are situated on the posterior base of each. The proboscis is retractile but non-protrusible, like that of Typhobia. The Littorinoid characters of the operculum are apparent in fig. 1. The buccal mass in this species is extremely small, and the whole radular apparatus is so very little developed, and the radula itself proportionately reduced to such a flimsy structure, that it is by no means easy to find. It actually exists, however, in the bed of a very slight dila-

[1] 'Proc. Zool. Soc.,' 1881, p. 288, and 'Ann. and Mag. N. H.,' 1880, vi, p. 426.

tation of the gullet (Pl. 14, fig. 3, *rad.*), and this dilatation must be taken as the only indication of the almost entirely undeveloped buccal mass. The radular dentition is interesting and somewhat peculiar; a single row of the delicate teeth is represented in fig. 5. In many ways the dentition suggests physiological adaptation. Both the rows of lateral teeth are very much alike, and there does not appear to be any difference in the relative size of the serrations on the head of either the outer or the inner of these teeth. In this feature the lateral teeth resemble those of Modulus lenticularis, as figured by Troschel, and to a certain extent the whole row of teeth approximates to this type; but on closer examination the admedian, no less than the median tooth itself, differs widely from any of the more normal melanioid forms. The predominant denticle on the reflexure of the admedian teeth, which is such a constant feature of so many radulas (see the radula of Spekia zonata (Pl. 18, fig. 3, *pred.*), is here quite wanting, but it is clearly visible in Troschel's figure of Modulus to which I have referred. Neither is there any posterior denticle on the median tooth of T. rufofilosa which would either correspond to that of Modulus, or that on the median teeth of the forms truly belonging to the genus Lithoglyphus. The admedians, laterals, and to some extent the medians of this radula approximate to those of Tympanotomus fuscatus given by Troschel, and in a more general way to those of Planaxis sulcatus (Lam.); and from these characters of the radula we might conclude with certainty that the genus Spekia has little or no connection with the Hydrobiidæ, to which family it has been hitherto thought to belong.

In all the specimens of T. rufofilosa which I examined, the mouth, œsophagus, stomach, and rectum were crammed with sharp rock fragments, about the size of a pin's head; and it is a curious fact that these animals should thus exhibit a propensity to fill themselves with stones, while at the same time the radula, or normal rasping apparatus, is almost entirely lost. The salivary glands are simple and somewhat long.

The œsophagus is narrow, and opens posteriorly in the

hinder chamber of a small stomach (Pl. 14, fig. 4, *œ.*). In the anterior chamber of this organ (fig. 4, *ant. ch.*) there is lodged a well-developed crystalline style, represented in fig. 4, *c. st.* The intestine passes out of the left side of the posterior stomachic chamber much in the same manner as in Typhobia. It takes the course represented in fig. 4, *int.* Towards the rectal extremity of the intestine there is a large, round, glandular lump (figs. 4 and 9, *R. g.*), very similar at first sight to that connected with the oviducts in the female Littorina.

The liver is large, and opens by two very small hepatic ducts on to the base of the posterior stomachic chamber (fig. 6, *h. d.*).

The kidney surrounds the heart, and extends posteriorly to that organ. It opens by a minute aperture, represented in Pl. 14, fig. 4, *R.*, at the extreme upper end of the mantle cavity.

The heart has the normal tænioglossate characters. It lies in a large pericardial cavity (Pl. 14, fig. 4), and consists of—

1. A thin-walled auricle in direct connection with the pulmonary vein.

2. A thick-walled ventricle which opens by a valvular aperture into—

3. An aortic trunk, from which the anterior and posterior aortæ proceed forward and backwards (fig. 4).

The gill is large and somewhat peculiar (Pl. 14, figs. 2, 14). It occupies an elongated oval space upon the inner mantle wall, and consists of numerous strong, broad, triangular gill leaves, the projecting apices of which occupy the median line of the structure. The apex of each leaf is prolonged into a filiform process, which gives the curious appearance to the gill represented in figs. 2, 14.

These filaments do not project quite straight, but are more or less inclined to the rectal (left) side of the gill. On this same side, each gill leaf below the median process is slightly pigmented, the whole left side of the ctenidium appearing consequently dark in comparison with the other.

The osphradium is long, and lies at the bottom of a groove

below the gill. It is a simple ropy structure at its base, but it becomes very distinctly pectinated at its distal extremity (Pl. 14, figs. 2, 14, *os.*).

The nervous system of T. rufofilosa (Pl. 14, fig. 6) consists of two large cerebral ganglia, closely united together as in the genus Melania (Pl. 15, fig. 58).

The cerebral ganglia are indistinguishably fused with the pleural ganglia below, but from their respective loci there spring the super- and sub-intestinal cords (Pl. 15, fig. 6), which pass to the super- and sub-intestinal ganglia. From the locus of the left pleural ganglion a nerve passes out towards the mantle, and this nerve apparently anastomoses with a branch from the subintestinal ganglion (Pl. 15, fig. 6). The nervous system is therefore dialyneurous on the left.

In like manner there springs a nerve from the right pleural ganglion, which also unites with a branch from the super-intestinal. The nervous system is therefore entirely dialyneurous on both sides, and when viewed from above it is practically indistinguishable from the similar view of the nervous system of Melania (?) which I have given in Pl. 15, fig. 5. On the under side of the cerebral ganglion, however, there is a curious and, so far as I am aware, unique median protuberance (Pl. 14, fig. 8, *m.p.c.g.*), but which is in itself quite sufficient to distinguish the nervous system of T. rufofilosa from that of any known Melania. The cerebro- and pleuro-pedal commissures are of median length, and unite at the upper extremity of the well-developed pedal ganglia (Pl. 14, fig. 8, *p. g.*). On the upper posterior faces of the pedal ganglia there are slight and peculiar projections (Pl. 14, fig. 8).

The otocysts are fairly large; they lie behind and slightly above the pedal ganglia, the otocyst nerve passing diagonally across the outer faces of the cerebro- and pleuro-pedal connectives to the cerebral ganglia on each side (Pl. 14, fig. 8, *o t.*). The otoliths are rectangular, small and numerous; there are a small osphradial and two visceral ganglia.

Reproductive Apparatus.

In the female Tanganyikia rufofilosa the whole arrangement of the genital apparatus is most interesting, and there is little doubt that in both sexes of this species we are dealing with one of the most archaic types of reproductive apparatus that any of the Gastropods possess. The genital gland is situated on the upper surface of the apical body-whorls, and in the male (Pl. 14, fig. 4) it is united by a number of small ducts to a rather long but not greatly coiled vas deferens, which passes beneath the intestine, and opens by a fine aperture immediately below the rectum (fig. 4, *v. d.*). Beyond this aperture, in some male specimens, I was able to trace a fine groove running forward along the lateral wall of the body, and dying out beneath the eye (fig. 2, *g. v.*); while in others this groove, which is well known in the males of several Gastropods, was not to be found. Such grooves are known to exist in the males of various representatives of the Struthiolaridæ, and many other forms. In the Strombidæ and Littorinidæ they are to be traced in both the male and female.

In the female T. rufofilosa the ovary lies in the same position as the testis in the male (Pl. 14, fig. 10), and the ova are collected by a number of small channels into a common long non-convoluted oviduct (Pl. 14, fig. 10, *g. d.*).

The aperture of the oviduct is somewhat further forward in the female than the corresponding opening in the male; and I was surprised to find that the aperture of the oviduct in T. rufofilosa is always related to a very strongly marked furrow which runs forward in the same position as the spermatic groove in the male (Pl. 14, fig. 14, *g. v.*), but it here terminates in a little pit beneath the eye (fig. 14, *x.*). On the other side of the head, that is on the left, there appeared in all the females that were killed during the breeding season a relatively great protuberance, which had the appearance of a pathological swelling, behind and below the left tentacle (Pl. 14, fig. 14, *b.p.*). On being opened this swelling was seen to be full of small round bodies, which I at first took to be parasites, but which when more

closely examined were found to be embryo rufofilosi in all stages of their late development. By pushing a bristle into the pit beneath the eye, on the right where the groove leading from the aperture of the oviduct terminated, it was easy to ascertain that this singular brood-pouch on the left was connected with the base of the groove and the pit on the right by a small tube (Pl. 14, fig. 10, *c.*), which passed completely through the foot beneath the buccal mass. Thus the external groove passing forward from the opening of the oviduct, and which unquestionably corresponds with the ciliated spermatic groove in the male, is connected in the female with an invaginated tube and brood-pouch, and we must consequently regard all these structures as the correlated parts of an accessory reproductive apparatus, which in the complete form just described is not present, so far as I can ascertain, in any of those Prosobranchs the anatomy of which is known. In searching for the meaning of this singular apparatus I discovered by accident among some of the so-called Melanias, which the authorities of the British Museum had courteously placed at my disposal for comparison, an obviously analogous but not quite similar condition of the reproductive apparatus in several of the viviparous females belonging to these forms.

In the so-called Melania episcopalis (Lea) which I received through Mr. Smith we found, on removing the body-wall, really in order to expose the nerves, that not a little to our surprise we had cut into a sac unconnected with the œsophagus, and which was filled with embryos in all their late stages of development (Pl. 14, fig. 13, *B. P.*). On examining the position of the external opening of the oviduct, it was soon seen that this was put into relation with a subocular pit (Pl. 14, fig. 13, *x.*) by a most prominent groove (fig. 13, *g. v.*), and it was easy to pass a bristle (*B.*) from the exterior opening of the pit into the brood-pouch which had already been opened up (Pl. 14, fig. 13, *B. P.*).

From this dissection it became at once evident that in M. episcopalis there existed the same relations between the oviduct in the female and an external groove connected with

an internal brood-pouch which had been observed in Tanganyikia rufofilosa. But in the M. episcopalis the pouch is directly above the œsophagus, and median instead of being below and to the left side as in the former case. (Compare Pl. 16, figs. 1 and 2.)

I do not think, however, that this relative change observed in the position of the brood-pouch between T. rufofilosa and M. episcopalis is of much morphological importance, for the whole series of structures is far too similar in both cases to admit of the slightest doubt that they must be regarded as morphologically the same.

In two small Melanias (species?) which had been collected by Mr. Cummings from the fresh waters of the Philippine Islands similar grooves were present in the females, and in like manner these were connected with median dorsal brood-pouches.

In a male Faunus, also lent to me by the authorities of the British Museum, there was a most pronounced groove (Pl. 15, fig. 11, *g. v.*); but, as I have had no opportunity of examining the female, I am naturally ignorant whether the groove is here connected with a brood-pouch or not.

From all this it is evident that in some Prosobranchs, T. rufofilosa, M. episcopalis, and several other forms, there exists in the female a complex accessory reproductive apparatus, which consists of an external ovigenetic groove in direct relation with an internal brood-pouch, which is either median and dorsal, or lateral and on the left side. In the males of these forms these structures are represented by spermatic grooves exactly similar to those of the Struthiolaridæ, the genera Strombus, Pteroceras, and the like. But so far as at present known the complete female apparatus is only represented in Spekia and the so-called Melanias which I have named. In various female Prosobranchs, however, more or less of the groove at any rate appears. In the female Strombus gigas there is a very pronounced groove leading downwards across the foot, which is figured by Haller.[1] Also in the female Littorina I

[1] 'Morph. Jahrb.,' Bd. xix, 1893, Taf. xix, fig. 17.

have traced a similar groove, faintly representing the spermatic structure relating to the penis in the male (Pl. 15, fig. 15, *g. v.*).

In none of these cases, however, do these grooves open into an internal chamber; and since in the female they can serve no conceivable purpose, unless it be that of oviposition, we must conclude that the groove, where it exists in those Prosobranchs which do not possess a pouch, represents a vanishing structure, and that the pouch, although it was probably possessed by the ancestors of these forms, has been completely lost. In some forms, moreover, such as Typhobia, all trace of this complex accessory reproductive apparatus has entirely disappeared, and there is no trace of either groove or pouch in the female or the male.

In Tanganyikia, as in those forms of Melania which possess the complete female apparatus I have described, there is no penis, and only a slight spermatic groove represents the apparatus in the male. But in Strombus (Pl. 16, fig. 3, *g. v.*) and Pteroceras the penis exists, and the groove is not only pronounced in these genera, but, as is well known, it is prolonged along the posterior surface of the organ to the tip. Modifications of the male intromittent apparatus can, however, go even further than this. In some forms, such as Buccinum (Pl. 16, fig. 4), there is no spermatic groove, nor indeed does the vas deferens open in the mantle cavity at all. In these forms the grooves have become enclosed, and as a simple tubular continuation of the vas deferens pass through the whole internal length of the penis, and open at the tip. (Compare figs., diagram I, Pl. 17.)

Various modes of modification of the parts of the complex apparatus existing in Tanganyikia and Melania episcopalis are thus seen to be widely distributed among entirely different prosobranchiate forms; and although, so far as is at present known, the entire apparatus exists fully developed only in the four species I have named, it must be concluded, from the fact that traces of these structures exist in most widely separated types, that they are representatives of an archaic condition of the reproductive apparatus, and we may almost certainly

conclude that the fully developed apparatus was present in the remote ancestors of all these forms. Hitherto little or no attention has been paid to the spermatic grooves which have long been known to exist in the males of many forms, but directly their relation to the fully developed female apparatus which I have described becomes apparent their morphological importance becomes immense, for it will doubtless have been seen how closely the fully developed apparatus in these female Prosobranchs corresponds to the spermatic grooves and invaginated penes of the Opisthobranchs; and indeed, when we look further into the matter, the comparison becomes so striking and so complete that there can be little doubt of the existence in this feature of an actual bond of morphological unity between the two. I may, however, preface the remarks which I have to offer on this subject by stating that it has already been shown, as a result of Professor Pelseneer's elaborate and painstaking investigations[1] concerning the morphology of the Opisthobranchs, that the characteristic hermaphroditism of these organisms appears to have been secondarily acquired, and to have arisen in the female by the evolution of a functional male gland.

If we bear in mind this result of a profound research while comparing the genital ducts in the Opisthobranchs with those of the Prosobranchs which I have just described, much of the initial prejudice that would be likely to exist against such a comparison will disappear, and it will be more readily seen that in their simplest forms the external genitalia in both orders are exceedingly similar.

In Aplysia, Pelta (Pl. 16, fig. 5), and several other forms the hermaphrodite genital aperture, as is well known, leads into a forwardly extended groove (Pl. 16, fig. 5, *g. v.*), which terminates anteriorly in a pit beneath the eye, and this pit communicates with the cavity of an internal sac, the walls of which can function as an introvertible penis when required. Except in the addition of the muscles which introvert this sac,

[1] 'Arch. de Biol.,' t. xiv, 1895. See also 'Quart. Journ. Micr. Sci.,' vol. 37, p. 19, 1895.

there is absolutely no difference in the relation of the curious external genital apparatus in Pelta or Aplysia, and those similar structures which T. rufofilosa and several Melanias are now known to possess. (Compare figs., diagram I, Pl. 17.) It is true that the genital opening in the Opisthobranchs is that of an hermaphrodite duct, but we have seen that there is every reason to regard this character as secondarily acquired; and just as would be expected from Professor Pelseneer's view, that the female first acquired the hermaphrodite character in these forms, we find the sac, which in its original ancestral prosobranchiate condition was a brood-pouch, here converted with very little modification into an introvertible penis. There seems, therefore, to me to be no admissible objection to the conclusion that the parts of the external genital apparatus which are present in T. rufofilosa and Melania episcopalis are structurally homologous with the similar parts of the external genital apparatus in the simpler Opisthobranchs. Clearly, therefore, in the existence of these curious structures which I found in T. rufofilosa and M. episcopalis, we have still more evidence which will help to bridge the rapidly diminishing gap between the two molluscan orders in which they exist.

The ordinary external genital apparatus of the Opisthobranch undergoes similar modifications to those occurring in the Prosobranchs, but it exhibits these modifications in a more pronounced degree. Thus the simple open grooves of Pelta and Aplysia pass through modifications such as that appearing in Auricula myosotis (Pl. 16, fig. 6), where an external groove still exists, but the vas deferens has become invaginated, so as to form a distinct internal tube. In Lobiga and Actæon (Pl. 16, fig. 7) the groove has apparently disappeared, and the vas deferens, as in Buccinum among the Prosobranchs, appears as an enclosed tube running to the extremity of a permanent external penis (Pl. 16, fig. 7).

Lastly, in Helix (see diagram, Pl. 17), both male and female conduits may become enclosed as separate tubes, their original connection with one another only appearing in early

ontogenetic life, and the line of their invagination remaining as a faint streak in the adult.

Whether we deal with the Prosobranchs or the Opisthobranchs in this matter of the modifications which the original external genital apparatus may undergo, it will thus be seen that in both orders the modification is along the same lines, and results in the conversion of an open groove into one or two closed conduits as the case may be. In both orders the original brood-pouch of the Prosobranchs and the introvertible penis of the Opisthobranchs tend to become lost, and to be replaced by permanent external penes, supplied with secondarily acquired internal tubes. Very obviously, therefore, we are here dealing with one of those numerous examples of parallel modifications of an originally similar structure present in two types which have become distinct. The whole apparatus in its complete ancestral prosobranchiate form is too complex and too peculiar in the arrangement of the different parts to have been evolved twice from different things. We know further that the Melanias to which I have referred, as well as the T. rufofilosa of Tanganyika, are indubitably extremely ancient forms, and that the complete structures they possess were once widely distributed among their prosobranchial ancestry, for parts of these structures as we have seen are present in widely different prosobranchiate forms. It would appear, therefore, that we are fully justified in concluding that in all these cases we are dealing with an incompletely represented condition of the reproductive apparatus that was once common to the Prosobranchs and the Opisthobranchs alike.

Lastly, it will have been seen that this view, drawn from the study of Prosobranch morphology alone, and Professor Pelseneer's view respecting the origin and nature of the Opisthobranchiata, mutually confirm each other; for, as I have already pointed out, Pelseneer has shown from an entirely different line of inquiry—

1. That the Opisthobranchs arose from the Prosobranchs.

2. That their characteristic hermaphroditism was secondarily acquired by the females of this group.

How exactly this view accords with the results of the anatomical comparison just given will be at once apparent. And in conclusion I may add that, when I arrived at the views above stated I was ignorant of Pelseneer's work, and of the conclusion at which he had arrived.

Spekia zonata.

The shell of this remarkable mollusc is represented in Pl. 18, fig. 1, and it is certainly most curious that no attention has been drawn by any of the conchologists to the extremely naticoid character which it presents, for the shell of this species is so completely similar to that of numerous fossil naticoid forms that, had it appeared fossilised instead of having been found living in a great fresh-water lake, there is not the slightest doubt that it would have been placed in one of the numerous fossil genera which are supposed to group themselves about the living Naticas. The oblique aperture, and the tendency of the outer wall of the mouth to be continued as a cup-shaped ring round the bases of the older shells, are exactly what is observed in many fossil Naticas; while the presence of a very pronounced umbilical opening, which is more or less filled up with a deposit of callous substance, are features which are generally regarded as almost diagnostic of naticoid shells.

The external appearance of this form is superficially similar to that of T. rufofilosa. The foot is rather less broad, and the snout is not so much pigmented; but, apart from the naticoid appearance of the shell, it is only in the internal anatomy that we begin to appreciate the wide morphological differences which exist between these forms.

In S. zonata the buccal mass is well developed, and the radular sac is conspicuous, but not of any considerable length (Pl. 18, fig. 6). There are two very strong muscles attaching the buccal mass to the body-wall, and the salivary glands are long and simple. The radular dentition is characteristic, and very strongly developed. A single row of teeth is represented in Pl. 18, fig. 3. It will be at once seen that the characters of

these teeth are highly suggestive of those of a number of well-known forms. In gross detail the radular dentition is very similar to that occurring in various forms of Anchylotus figured by Troschel. The predominant denticle on the admedian tooth is well developed in zonatus, as indeed it is in a very large number of dissimilar forms; and an exactly analogous and widely prevalent feature is presented in the difference of size and character between the denticles on the heads of the outer and inner lateral teeth. Perhaps, however, the most notable feature which the radula presents is the peculiar structure of the median tooth. The outer surface of this tooth is concave, like the median tooth in Anchylotus, Thiara, Melania brevis (Dorb.), and Melanopsis. But it differs from all these forms in having no predominant median denticle, there being instead two lateral predominant denticles and a median concavity. The only forms which appear to possess this peculiarity of the median tooth are the different species of the genus Sigaretus; and although in other respects the radula differs widely from that of either a Sigaretus or a Natica, I shall show in the concluding part of the paper that this comparison is not nearly so far-fetched as it might appear, since it can be clearly demonstrated that what we may call the melanio-planaxoid form of radula which S. zonata possesses is that of an extremely ancient type, and in all probability, with the exception of the Rhipidoglossa, is antecedent to that of all the other Prosobranchiates with which we are acquainted.

In S. zonata the œsophagus leads into a peculiar stomach, represented in Pl. 18, fig. 2, which contains at its anterior end a body which must be regarded as representing a crystalline style, although it is so small and so little elongated that at first sight it does not seem to have the characters which this structure usually presents.

The intestine takes the course represented in Pl. 18, fig. 6, and communicates with a dilated rectum, opening in the usual way just within the border of the mantle. The heart has the regular tænioglossate characters. The description of this

organ given for T. rufofilosa (p. 158) would stand equally well for that of S. zonata. In S. zonata, however, owing to the naticoid shape of the body, there is a forward displacement of the internal viscera, which results in the pulmonary vein not going directly forward to the base of the stenidium, as in T. rufofilosa and most other Prosobranchs, but in its being bent slightly backwards in a more or less acute curve before it reaches the base of the gill (Pl. 18, fig. 6). This relative displacement of the heart and gill is fully described by Haller[1] in the Naticas Trochita radians, Ergaea plana, Crepidula peruviana (Lam.), Tanacus unguiformis (Lam.), and Crucibulum (sp. ?), and it is curious to find that this displacement has proceeded less in Spekia than in any of the forms which Haller figured.

The gill in S. zonata is very much like that of rufofilosa. The leaves are similarly broad, low, and triangular, and their apices are prolonged into the same littorinoid finger-like processes (Pl. 18, fig. 2).

Such processes are figured by Haller in the gills of Natica lineata: they are present in the gills of Littorina (Pl. 15, fig. 1), but curiously enough they are not represented in Haller's figure of the gill of Sigaretus neritoides (Lam.). Neither are they present in the gills of the Faunus which I examined, and which are represented in Pl. 15, fig. 9.

The osphradium is lodged in a groove beneath the gill; it is simple, and slightly but distinctly tending to become pectinated (Pl. 18, fig. 2, *os.*). At its outer extremity it is curiously bent downwards and back, exactly repeating in this the condition of the same organ in Sigaretus neritoides and Natica lineata as represented by Haller (loc. cit., pl. xiii, figs. 17—20).

The nervous apparatus of S. zonata is the most interesting feature which this form presents, the whole completely simu-

[1] Haller, "Die Morphologie der Prosobranchia," iii, 'Morph. Jahrb.,' xviii, 1892, p. 451, and plates.

lating in every detail that of Lamellaria perspicua, as figured and described by Bouvier[1] and Haller.

The cerebral ganglia are united by a short but distinct commissure, and are almost completely fused with the pleural ganglia (Pl. 18, fig. 5, *b.*). The right pleural ganglion gives rise to a nerve-cord which passes upwards and directly across the axis of the animal's body. It almost immediately expands into a ganglionic mass, which represents the supra-intestinal ganglion (Pl. 18, fig. 5, *Sup. int. g.*).

This ganglion is in turn connected with the left pleural by the commissure (Pl. 18, fig. 5, *a. x.*). The nervous system is therefore completely zygoneurous on the left. From the left pleural ganglion a nerve-cord passes almost parallel to and below the supra-intestinal commissure to a ganglionic enlargement which represents the subintestinal ganglion on the right (Pl. 18, fig. 5 *b*, *Sb. int. g.*). This ganglion is in direct connection with the right pleural ganglion by the connective *x'*. The nervous system is therefore completely zygoneurous on both sides. All these details could be made out by dissection alone, but the animals were so small that for confirmation I was obliged to resort to sections.

In all essential details the nervous system just described corresponds with that of Lamellaria, and as the modifications it presents are most complete and peculiar, there can be no doubt that the nervous system of S. zonata offers a true indication of the naticoid affinities of this form.

The reproductive apparatus very much resembles that of Littorina, the genital gland occupies the upper part of the apical body-whorl, and in the male is related to a simple vas deferens which opens at the extreme upper end of the mantle cavity (Pl. 18, fig. 2). This opening is, however, prolonged as a ditch or groove (Pl. 18, fig. 2, *g. v.*), which is overhung by a flap, which latter structure terminates at the more usual position of the genital opening.

An exactly similar state of affairs is present in Littorina (Pl. 15, fig. 1, *g. v.*), and I found it also very well defined in

[1] 'Ann. des Sciences Nat.,' Series 7, "Zoolog.," tom. iii.

the specimen of Faunus to which I have referred (Pl. 15, fig. 2).

There is little or no trace of the spermatic groove in S. zonata beyond the termination of the flap, but this groove is very plainly indicated in this position in Faunus (Pl. 15, fig. 11, *g. v.*) and in Littorina (Pl. 15, fig. 1, *g. v.*).

In all essential details the female apparatus of zonatus repeats that of the male, but there is neither an external groove nor pouch.

Comparative.

In attempting to ascertain with what known molluscan forms the foregoing species are to be placed, it will be abundantly apparent from the previous anatomical descriptions that each bears little if any relation to the other; and further, that from the broad features of their general anatomy the existing conchological determinations of their affinities with the Hydrobiidæ must be at once dismissed. As regards the so-called S. zonata, the general anatomical features of this form, the gills, the position of the viscera, the nerves, and so forth, all place it unquestionably among the simpler Naticoids; the only features which at first sight might militate against such a determination of its phylogenetic relationships being those presented by the radula and dentition. We have seen that the dentition of S. zonata is in many ways identical with that Melanio-planaxoid type which I have already described, and that it only corresponds with that of any of the Naticoids in the minor features of the median tooth. It is therefore incumbent on us to ascertain whether this difference in the radula is really an important feature, or whether it is not wholly or partly due to the antiquity or the specialisation of the Tanganyika form. But in order to obtain a clear conception of this matter it is necessary to examine the radula question from a more general point of view. I have long been impressed by the possibility that the usually adopted methods of estimating the gross relationships of these structures simply

by the number of teeth which the transverse rows contain may not be sound, for it was clearly shown by Troschel himself that the Rhipidoglossate radula of Trochatella could be regarded as having been naturally evolved from the Tænioglossate radula of such a form as Cistula through the splitting up into numerous segments of the outer lateral teeth.

He says, "Im Gebisswurdesich wohl von den Tænioglossen zunächst ein Uebergang zu den Rhipidoglossen verleiten lassen indem die Cyclostomaceen einerseits die Amphiperasidæ, anderseits durch die tiefen Kammärtigen Einschnitte der Seitenplatten auffallend und die ganz zerschnittenen aüsseren Seitenplatten der Rhipidoglossen mahnen. Ich ziehe es jedoch vor, die Abtheilungen mit kammärtigen Kiemen, wenn gleich das Gebiss, sehr beträchtlich abweicht, zunächst zu behandeln. Anatomische Erforschung aller übrigen Verhältnisse und die Entwickelungsgeschichte gestatten uns vielleicht, künftig hierüber eine sichere Entscheidung."

If we accept this view—and, as I shall immediately show, there is every reason why we should—it consequently follows that in the Rhipidoglossate and Tænioglossate types of

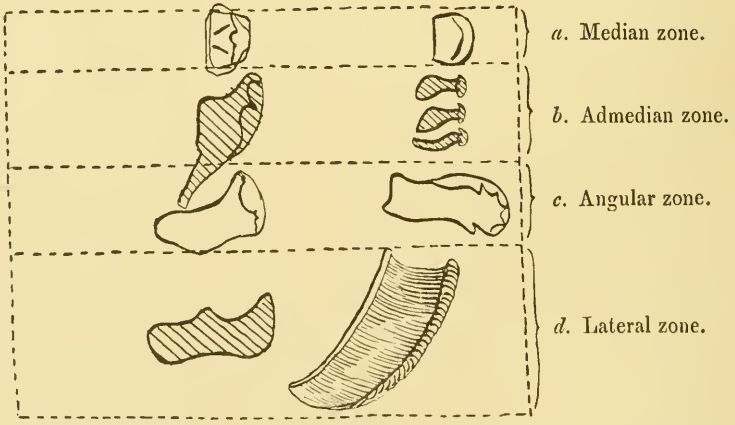

Diagram showing the corresponding zones and denticles in the Rhipidoglossate radula of Cocculina Rathbuni, Dall. (right figures), and in the Tænioglossate radula of Melania Episcopalis, Lea (left figures).

radulas represented in the accompanying diagram the areas lettered *a, b, c, d,* are morphologically the same. But if this is so, the comparison can be pushed much further, since it is apparent that there is a very real morphological distinction between certain areas of the dentition, which is quite independent of the number of denticles which the individual areas may contain.

What is of primary importance, however, is this, that in a very large number of Rhipidoglossa the radula presents a simple division into separate denticular areas, three on each side of a median line, there being precisely the same morphological distinction between the elements in these areas as that which subsists between the molar and premolar teeth of mammals. We know that the Rhipidoglossa stand in the relation of ancestors to the great Tænioglossal group, and the question naturally presents itself whether the 3, 1, 3 radula of the Tænioglossa is not to be interpreted as a condensation into seven single denticles of the more numerous Rhipidoglossate teeth; for in the latter group the denticles are already differentiated into a similar number of morphologically distinct denticular areas. But although Troschel pointed out that the Tænioglossate radula in certain forms approaches that of the Rhipidoglossa, he seems for some reason or other to have missed this fundamental similarity, which becomes apparent throughout the radulæ of both groups if we consider the denticular areas, as we have every right to do, and not the separate teeth. In some Pleurotomaridæ[1] this division into lateral, angular, and median denticular areas is marked, and as this division is present in most Rhipidoglossa we are fully justified in regarding it as a fundamental feature of molluscan tongues. In fact, the presence of such a division in a group like the Rhipidoglossa appears to be as strong, if not stronger evidence for the fundamental character of the arrangement than that

[1] Dall., 'Bull. Mus. Comp. Zool. Havard,' vol. xviii, 1889, Pleurotomaria adansoniana, C. and F., pl. xxxi, fig. 3. It is not so apparent in Bouvier's figures of P. quoyana, 'Arch. Zool. Ex.,' 3e série, vol. vi, pl. xii, fig. 1.

which may and ought to be obtained by the study of the ontological formation of the teeth. Consider also the very marked existence of this division revealed by the study of the existing figures of the Dochoglossa, while in the Cephalopods themselves we frequently encounter the regular Tænioglossate type of tongue.

From these considerations it is clearly incontestable that in a large series of molluscs of very different types there exists a fundamental similarity of plan, or symmetry, upon which the dentition has been formed; and the conception of this unity of groundwork seems to me important, in that it throws light upon the means whereby the radulæ of those groups which have emanated from the Rhipidoglossa, the Tænioglossa, the Rachiglossa, the Ptenoglossa, and the Opisthobranchs have respectively been evolved.

In the light of these general conclusions respecting the unity of nature, and the probable inter-relationships subsisting between the different molluscan dentitions, we may pass now with advantage to certain more particular considerations, which are pertinent to the determination of the true phylogenetic position of the molluscs with which this paper is primarily concerned.

We have seen that the Rhipidoglossate radula may be made to pass, by a series of gradations, through forms like Trochatella into that of the Tænioglossa such as cystula; while in the genus Ovula, the teeth in both the lateral and angular areas tend to be split up. If the above conception of the formation of the Tænioglossate radula from the Rhipidoglossate type be true, we should expect to find some definite approach to the Rhipidoglossate condition in the Archi-tænioglossa as they at present exist. Through the work of Bouvier and Lacase-Duthier there seems much reason to believe that the Cystostomidæ have originated from the ancient Littorinas, a group which, if fossil identifications can be trusted, is as old as any that we know.

Now there is a whole series of gradations between the quasi-Rhipidoglossate radula of the Cyclostomes and that of the Paludinas, another unquestionably Archi-tænioglossate group;

and if we look into the matter a little more closely, we see that the only real difference between the single outer lateral tooth of the Viviperas and that of the Cyclostomes lies in the fact that the former is somewhat more elongated vertically, and a little less numerously serrated on the crown.

From this form of Tænioglossate radula we may pass, by insensible gradations, to what I may call the Cerithio-planaxoid radulas, in which the outer lateral has become still more specialised and perpendicularly lengthened out. Thus the Cerithio-planaxoid radula comes very close to the quasi-Rhipidoglossate radula of the truly Archi-tænioglossate forms, and, as we should expect from the abundant geological evidence respecting the antiquity of the Cerithidæ, what I have termed the Cerithio-planaxoid radula would rank among the more primitive of the existing Tænioglossate types. It is this conclusion which I wish to emphasise, for it has a most direct bearing on the nature and affinities of Spekia zonata, as I shall now proceed to show.

It has been seen in the descriptive part of this paper that S. zonata has the anatomical peculiarities, and especially the nerves, of several Naticoids, but, unlike any Naticoid hitherto examined, it presents us with a well-developed Cerithio-planaxoid tongue. The radula of the more typical Naticoids is very marked in type, and more generally resembles the radulæ of the Xenophoridæ, the Chenopodidæ, and their associates, than it does that of the Cerithio-planaxoid forms. On the other hand, it will at once be admitted by anyone acquainted with such structures, that among these more highly specialised forms there exist numerous individual examples which exhibit more or less distinct traces of the retention of the Cerithio-planaxoid teeth. The radulæ of Vermetus, Crucibulum, and Crepidula all show undoubted stages in the gradual transformation of the Ceritho-planaxoid radula into that common to the more aberrant types to which these genera respectively belong.

Now, unquestionably, S. zonata is extremely old. It lives in association with forms which have every appearance of being identical with several Jurassic molluscan types. Therefore

the co-existence in this species of a Naticoid organisation with a Cerithio-planaxoid tongue is exactly what, judging from the foregoing observations, we should naturally expect; for the complete retention of a Cerithio-planaxoid radula in zonatus indicates, like the rest of this animal's anatomy, that we have in this form what is probably the most primitive Naticoid at present known.

Concerning the Lamellarian nervous system of S. zonata it has already been rendered evident, by the work of Haller,[1] how such a modification has been derived from the more normal Gastropod types. To illustrate this I have diagrammatically represented the nervous system of Sigaretus neritoides, in which it will be seen that the shortening of the subintestinal cord is very pronounced (Pl. 17, fig. I). In Natica lineata (Pl. 17, fig. II), on the contrary, the nervous system remains simply zygoneurous and bilaterally symmetrical, with nothing remarkable about it except the simultaneous shortening of the supra- and sub-intestinal cords. In fig. 4 is represented the nervous system of S. zonata, and it will be seen that the peculiarities which this form presents are primarily due simply to a more complete shortening up of the supra- and sub-intestinal cords. In fig. 5, which represents the nervous system of Crucibulum, another line of modification is introduced, for in this form it is the sub- and not the supra-intestinal cord which is becoming short. A still further progressive shortening of the subintestinal cord is witnessed in Trochita radians (fig. 6), and the most extreme case of this progressive development is represented in Crepidula. These modifications, it will be observed, are the exact inverse of those obtaining in Sigaretus (fig. 1). It is thus apparent that Sigaretus and Crepidula represent the extreme terms of modification in opposite directions of a mean type of nervous system, which is exemplified by that of Natica lineata.

We witness thus the very instructive fact that both these lines of modification have been carried to opposite extremes in genera which are unquestionably close allies. S. zonata (fig. 4)

[1] Loc. cit.

does not incline to either of these extreme types, but, like Lamellaria (fig. 3), it represents a third modification, due to the simultaneous shortening of both the supra- and sub-intestinal cords. It would appear, therefore, that S. zonata approximates, both in its general anatomy and in the minutiæ of its nervous system, to those Naticoid forms which have been already examined.

Unquestionably, if judged by the nervous system alone, it would be placed, not only near, but within the genus Lamellaria; but, since it differs from this genus in its external form, and from all other Naticoid genera in the characters of its radular dentition, I think it will be well to keep it in a genus by itself, which is to be regarded as closely related to, but distinct from, Lamellaria. Unquestionably Spekia can no longer be regarded as having any relation whatever to the members of the family to which the genus Lithoglyphus belongs, and with which S. zonata was unhesitatingly placed by the conchologists. We have here, therefore, obviously one more example of the impossibility of making correct determinations from the shell structure alone, while at the same time it forms an equally striking instance of the marine nature of the halolimnic fauna of the lake in which it lives.

Turning now to the question of the affinities of the genus Tanganyikia, it will be seen that the nerves, no less than the general anatomy of this genus as represented by T. rufofilosa, correspond to those of a number of well-known molluscous forms. I showed in the descriptive part of this paper that, but for the ventro-median protuberance on the cerebral ganglia, the nervous system of rufofilosa corresponds almost exactly to that of Melania amarula, as described by Bouvier (loc. cit.), and with that of the closely allied species of Melania represented in Pl. 15, fig. 5. There is nothing in the general anatomy, or in the reduced radula of T. rufofilosa, which need lead us to suppose that it is not really closely related to both these types. The only marked anatomical difference presented lies in the possession by T. rufofilosa of the curious accessory reproductive apparatus which I have described. But I have

already shown that this apparatus is undoubtedly to be regarded as indicatve of an archaic character in those forms which possess it, and the absence of the complete structure in these Melanias is therefore no indication that both they and the genus Tanganyikia are not direct descendants of the same approximate phylogenetic stock.

If, however, we were to assume from these considerations that T. rufofilosa and the amarula type of Melania are representative of the whole heterogeneous assemblage of organisms which are at present regarded by the conchologists as belonging to the single family of the Melaniadæ, we shall be speedily disillusioned. In Pl. 15, fig. 3, I have represented the nervous system of Melania episcopalis, and in fig. 4 that of Melania aspirata, while in fig. 10 the similar nervous system of Faunus is shown in the relation to the gills and the surrounding parts. All these nervous systems are obviously constructed on a similar plan, and they are all completely different from that type of nervous system which M. amarula and T. rufofilosa both possess.

But not only do these types of so-called Melanias differ in the character of their nerves; both series are easily distinguished by other anatomical features which they possess. In M. episcopalis, M. aspirata, and Faunus, the radular dentition, as represented in Pl. 15, fig. 14, is obviously of that Littorinoid character which is so marked in the numerous American forms, such as Io, and all the representatives of the genus Pachychilus.

The conclusions to which the extensive researches of Bouvier into the character of the nervous systems of the Cerithidæ and the Melaniidæ led him were briefly these: that the simpler dyaloneurous Ceriths form the marine starting-point for what may be called the Cerithio-melanias on the one hand, and for such forms as the Potamids and the genus Tympanotomus on the other. Secondly, that the Planaxids had nothing to do with the Ceriths, but were to be referred to a Littorinoid ancestry. This view of the matter, I believe, is in the main correct, but it does not account for the characters which a

number of the Melaniidæ possess, for there are, as we have seen, two distinct types of so-called fresh-water Melanias; one characterised by the possession of a Cerithio-planaxoid type of radula, the other by the dentition of the modern Littorinoid group.

Now if Bouvier's view that the Planaxoidæ are related to the Littorinidæ be correct, the modern Littorinas must have become modified in their teeth, for the Planaxoid radula is the older of the two (see p. 174). It follows consequently that those Melanias which possess a Cerithio-planaxoid radula are relatively the older fresh-water types; but they may be considered as having arisen directly either from the marine Ceriths themselves, or from a Littorino-planaxoid of the older type. In whatever way we view this matter, it is at any rate obvious that the Melaniidæ are no real family group, but can certainly be split into two broad divisions, one of which originated as a fresh-water stock from the more modern Littorinas, and may for present purposes be called the Littorino-melanoid group. The other has a Planaxoid radula, and the exact origin of its constituent forms is much more difficult to determine, for in the characters of their radulæ these species are equally similar to both the Cerithidæ and the Planaxidæ, nor are there any other features at present known by which, in the majority of cases, it would be possible to determine satisfactorily from which oceanic stocks such Melanoids originally sprang. Unquestionably the shell and the internal anatomy of many Melanias correctly indicate a Cerithioid ancestry, as Bouvier supposed; but there are others, such as M. episcopalis, which might equally well be supposed to have originated from the older types of Littorinas which possessed a Planaxoid radula, or even from the Planaxidæ themselves. Until further investigation has been prosecuted it seems, therefore, best to group all those Melanias with a Planaxoid radula, whatever their origin, into one series, which we may describe as the Melanio-planaxoid group.

In T. rufofilosa we have a form which in many ways would typify the marine ancestry of many Melanio-planaxoids, and the exact relation of this form to the living oceanic Planaxidæ

needs further working out. In the meantime, however, it could certainly be regarded as a representative of that ancient Littorino-planaxoid stock from which a portion of the Melanio-planaxoids appear to have originated. This view appears to me to receive a certain amount of support from the fact that T. rufofilosa possesses a reproductive apparatus fully developed, which is at present only known in its complete form in some of the Littorino-melanoids; for this would appear to indicate that both rufofilosa and some of the Littorino-melanoids still possess characters which were once common to their ancestral Littorinoid stock. Unquestionably the possession of the complete reproductive apparatus present in rufofilosa stamps a mollusc as an ancient form, for in this feature of its organisation we have seen (p. 160) that it harks back to a time and to those types which were in existence before the Opistho-branchs had become separated from the primitive Tænio-glossate stock. I conceive, therefore, that in T. rufofilosa we have before us a surviving example of a form that is closely akin to that ancient Littorina with a Planaxoid radula, out of which the modern Littorinas and the modern Planaxidæ with their respective fresh-water and terrestrial derivatives have individually sprung. To illustrate the relationships of these forms, we may refer to the diagram given on Plate 19.

To recapitulate: we have seen that there are indications that the Melaniidæ are certainly capable of being split into at least two groups which have no proximate relation to each other. But there is yet a third type of Melania represented by the genus Melanopsis, and this third group is in many ways more interesting than either of the other two. As Bouvier showed, there is very little in common between the zygoneurous nervous system of the genus Melanopsis and that of any of the other forms associated together at present in the family of the Melaniidæ. Nor yet does this nervous system approximate to that of any of the marine Ceriths or their derivatives that we know. On the other hand, there is a certain amount of resemblance both in the nerves and radula of the genus Melanopsis to the similar parts of the Nassopsis of Tanganyika. In

another paper[1] I have shown that two of these halolimnic genera, Paramellania and Nassopsis, are conchologically indistinguishable from the genus Purpurina of the old Jurassic seas; and although the genus Melanopsis does not come at all sufficiently near Nassopsis to be placed within it, I think there is some evidence for regarding this Tertiary genus as having arisen as a modification of the old Purpurine stock which died out in the sea during the later Secondary formations, but which it is possible still lives in that part of the old Jurassic seas to which Lake Tanganyika appears to correspond.

As I have shown elsewhere, the most remarkable feature about the genus Nassopsis is the very curious way in which the nervous systems of the forms belonging to it foreshadow those of the terrestrial Cyclophoridæ. Hitherto no satisfactory explanation has been given as to the origin of the Cyclophoran nervous system; and it is in the highest degree probable, from the close similarity which it bears to that of the Jurassic Nassopsis, that the Cyclophoridæ originated from some true fresh-water derivative of the Purpurinas, such as the genus Pyrgulifera, which is found in the fresh-water deposits of the chalk. In the table (Pl. 19) I have represented the Purpurina of Tanganyika as the direct continuation of the similar forms found in the old Jurassic seas; while it is suggested that the more modified Pyrguliferas of the chalk may represent a true fresh-water non-halolimnic development of these forms.

It will, I think, be obvious that the observations here collected in no way finally dispose of the interesting problem of the origin of the heterogeneous constituents composing the Melaniidæ as the family at present stands; but the splitting of this group into three broad divisions, based on the anatomical characters of such of them as have hitherto been investigated, is unquestionably in accord with the morphological facts of the case. What is immediately required is a more detailed

[1] 'Quart. Journ. Micr. Sci.,' Vol. 41, pp. 314—317, 1898.

knowledge of the morphology of a large number of the tropical forms. When this is obtained it is in the highest degree probable that the Melaniidæ will be still further split up, for we are at present in complete ignorance of the nature of the animals contained in a large proportion of the shells belonging to this so-called group. As Bouvier so justly remarks, shells which are identical may harbour animals that are quite distinct; and if any shell is referred to in existing conchological works as belonging to the Melaniidæ, one is naturally led to inquire to which of the heterogeneous groups lumped together in that family it should be referred, for there is as much difference between these forms as that subsisting between the members of such distinct families as the Aphoridæ, the Chenopidæ, and the Strombidæ, and to all such questions no answer is in the nature of the case to be obtained. What I have endeavoured to do has been to obtain the materials wherewith it may be possible to view a certain portion of the subject from a phylogenetic standpoint, since in dealing with such a series it will certainly not be contested that this is the only rational method it is possible to adopt. The conchological method of grouping like shells together may, as Bouvier remarked, be useful for the compilation of museum catalogues, but it is without meaning from a broader zoological point of view; and the extensive use of this purely conchological method of classification, which has recently been so much in vogue, is all the more mischievous because the same terminology is used in it as that which is applied to the classification of animals the morphology of which is fully known, and it thus lends an appearance of completeness to the existing molluscan system of classification that in reality is merely an illusion and a snare.

DESCRIPTION OF PLATES 14—19,

Illustrating Mr. J. E. S. Moore's paper on "The Molluscs of the Great African Lakes."

Reference Letters.

B. P. Brood-pouch. *œ.* Œsophagus. *st.* Stomach. *g. g.* Genital gland. *g. d.* Genital duct. *g. v.* External genital groove. *Sp. d.* Spermatic duct. *g. a.* Genital aperture. *os.* Osphradium. *rad.* Radular sac. *c. st.* Crystalline style. *r.* Renal aperture. *int.* Intestine. *ant. ch.* Anterior stomachic chamber. *c. g.* Cerebral ganglion. *Sb. int. g.* Subintestinal ganglion. *Sup int. g.* Supra-intestinal ganglion. *pl.* Pleural ganglion. *p. g.* Pedal ganglion. *pl. p. com.* Pleuro-pedal commissure. *C. p. com.* Cerebro-pedal commissure. *c. com.* Cerebral commissure. *x.* Zygoneurous connection. *a.* Anus. *Int. g. g.* Internal genital groove. *d.* Dyaloneurous connection. *M. p. c. g.* Median process on cerebral ganglia. *B. m.* Buccal mass. *V. n.* Visceral nerve. *V. g.* Visceral ganglion. *V. d.* Vas deferens. *R. g.* Rectal gland.

PLATE 14.

Fig. 1.—Shells of Tanganyikia rufofilosa, showing the character of the operculum.

Fig. 2.—Tanganyikia rufofilosa with the mantle cavity opened up so as to show the relation of the mantle organs.

Fig. 3.—The radular apparatus and upper portion of the nervous system of rufofilosa.

Fig. 4.—Dissection of the viscera of rufofilosa.

Fig. 5.—A single row of teeth from the radula of rufofilosa.

Fig. 6.—The stomach of rufofilosa, showing the anterior chamber and crystalline style.

Fig. 7.—The gills of Melania episcopalis; at *a.* the gill filaments are simple and triangular, while at *b.* they have the filamentous character of the Littorinoids.

Fig. 8.—Right and left lateral views of the anterior portion of the nervous system of rufofilosa.

Fig. 9.—The large extremity of the intestine of rufofilosa, showing rectal gland.

Fig. 10.—Semi-diagrammatic view of the reproductive apparatus of the female rufofilosa.

Fig. 11.—The pericardial cavity and heart of rufofilosa.

Fig. 12.—Rudimentary spermatic groove in the male Melania episcopalis.

Fig. 13.—Dissection of the female Melania episcopalis, showing the relations of the super-œsophageal brood-pouch. A bristle is passed through the opening of the brood-pouch, *x.*, into its interior cavity.

Fig. 14.—The mantle cavity of a female Tanganyikia rufofilosa, showing brood-pouch containing embryos similar to that in Melania episcopalis (see previous figure), but subœsophageal and on the left side.

PLATE 15.

Fig. 1.—Partial dissection of Littorina litteria.

Fig. 2.—Posterior portion of the mantle cavity of a Faunus species (?).

Fig. 3.—Nervous system of Melania episcopalis.

Fig. 4.—Nervous system and radular sac of Melania aspirata.

Fig. 5.—Nervous system of Melania (?).

Fig. 6.—Nervous system of Tanganyikia rufofilosa.

Fig. 7.—Melania aspirata, showing gills and genital groove.

Fig. 8.—Buccal mass, cerebral ganglia, and radular sac of Melania episcopalis.

Figs. 9 and 10.—Gills and radular sac of Faunus species (?).

Fig. 11.—Lower portion of mantle cavity of Faunus, showing flab-like covering of the internal genital groove, *int. g. g.*, and the external genital groove, *g. v.*

Fig. 12.—Nervous system of Littorina litteria.

Fig. 13.—Mantle organ of the Melanias, species (?).

Fig. 14.—A single row of teeth in the radula of Melania episcopalis.

Fig. 15.—Male Littorina litteria, showing the rudimentary spermatic groove, *g. v.*

PLATE 16.

Fig. 1.—Semi-diagrammatic view of the reproductive apparatus in the female Tanganyikia rufofilosa, showing the relation of the external genital groove, *g. v.*, to the internal subœsophageal brood-pouch, *B. p.*

Fig. 2.—Similar view of (*a.*) the female Melania episcopalis; (*b.*) reproductive apparatus of the male.

Fig. 3.—Semi-diagrammatic view of the reproductive apparatus in a male Strombus, showing the relation of the spermatic groove to the permanent penis.

Fig. 4.—The same parts in a Buccinum, showing the enclosed spermatic duct, *v. d.*

Fig. 5.—Diagrammatic representation of the reproductive apparatus in Pelta, showing the similarity in position (in relation to the genital groove) of the eversible penis to the brood-pouch of the Prosobranchs, figs. 1 and 2 (after Pelseneer).

Fig. 6.—The relation of the ducts in Auricula myosotis. Spermatic duct, *sp. d.*, is here enclosed.

Fig. 7.—The relation of the ducts in Acteon, showing similar modification of the penis to that observed in Buccinum, fig. 4.

PLATE 17.

Diagrams showing—I, different modifications in reproductive apparatus among the Gastropods. II, the different modifications in the nervous systems of six Naticoids. Refer to pp. 163 and 175.

PLATE 18.

Fig. 1.—Two views of the shell of Spekia zonatus.

Fig. 2.—The gills and mantle organ of Spekia zonatus.

Fig. 3.—Single row of teeth in the radula of Spekia zonatus.

Fig. 4.—The snout and tentacles of Spekia zonatus.

Fig. 5.—Dorsal and left lateral views of the nervous system of Spekia zonatus.

Fig. 6.—The relation of the visceral organs of Spekia zonatus.

Fig. 7.—Animal of Spekia zonatus removed from the shell.

Fig. 8.—Section through the genital groove of Spekia zonatus.

PLATE 19.

Diagram to show relationships of various genera.

The Molluscs of the Great African Lakes.

IV. Nassopsis and Bythoceras.

By

J. E. S. Moore.

With Plates 20 and 21.

Among the numerous forms of molluscs that appear to be peculiar to Lake Tanganyika at the present time, there are three very marked genera—Paramelania, Nassopsis, and Bythoceras, which, owing to the substantial similarity existing between their shells, might, and have hitherto been regarded as closely associated forms. The first of these to be discovered, Nassopsis, was originally classed by S. P. Woodward[1] among the Melanias, and as a member of the sub-genus Melanella, his determination being made from some empty shells which had been brought from Tanganyika by Captain Speke, during Burton's celebrated journey to that lake. The second genus, Paramelania, was formed by Smith,[2] in 1881, to include a somewhat similar form of empty shell which had been brought from the same locality by the missionaries; and to distinguish the members of this genus from the original Melanella nassa of Woodward, Smith[3] placed the latter form in the new genus of Nassopsis.

In 1896 I[4] dredged in the deep water of Tanganyika

[1] 'Zool. Soc. Proc.,' 1857.

[2] Ibid., 1881, p. 559.

[3] 'Ann. Mag. Nat. Hist.,' 1890, vol. vi, p. 93.

[4] The genus was first named in my paper in the 'Proc. Roy. Soc.,' vol. lxii, p. 451. The full diagnosis is contained in the 'Proc. Mall. Soc.,' 1898.

another form, which although it presents, when adult, the peculiar horns above and below the mouth represented in Pl. 21, fig. 3, still bears a most remarkable resemblance to both Paramelania and Nassopsis. For this new form, and before I was acquainted with more than the shells of any of the three, I proposed the third generic name, Bythoceras.

Now, without further knowledge, anyone judging from the appearance of the shells of these three different genera would certainly regard them as being in all probability closely related to one another. Therefore, holding the opinion that the conchological method of determining molluscan relationships is utterly unsound, it is with the greatest satisfaction that I am here enabled, by a detailed account of the anatomy of Nassopsis and Bythoceras, to show that even in their wider phylogenetic sense these genera bear no relation to each other. The characters of both, however, are of far greater importance to morphologists than that of affording them material wherewith to exhibit the futility of attempting to determine the nature of molluscan affinities from empty shells. The anatomical features of Nassopsis are in many ways quite unlike those of any forms hitherto described; and I shall immediately make it clear that this form presents us with an Archi-tænioglossa of an entirely new type. This being so, when we consider the unquestionably vast antiquity of the lake in which Nassopsis lives, and the fact that its shell, along with numerous others still living in Tanganyika, is specifically indistinguishable from forms which once abounded in the old Jurassic seas,[1] it will be readily accorded by those interested in prosobranchiate morphology that in Nassopsis we are presented with an animal which in the future will probably constitute one of the most important prosobranchiate archetypes of which we are in search.

[1] "On the Hypothesis that Lake Tanganyika represents an old Jurassic Sea," 'Quart. Journ. Micr. Sci.,' vol. 41, 1898, p. 303.

(A) Nassopsis nassa.

During life this mollusc inhabits the surface rocks of Tanganyika, and its shells are always richly encrusted with the green algæ which clothe the rocks for a considerable depth. It is sluggish, and appears to browse within a very limited area, like the Patellas of the ocean beach. The foot is broad, somewhat pigmented, and quite white in places; the snout is broad, black, and wrinkled, not protrusible, but retractile. The tentacles are short and black, and the eyes are not carried

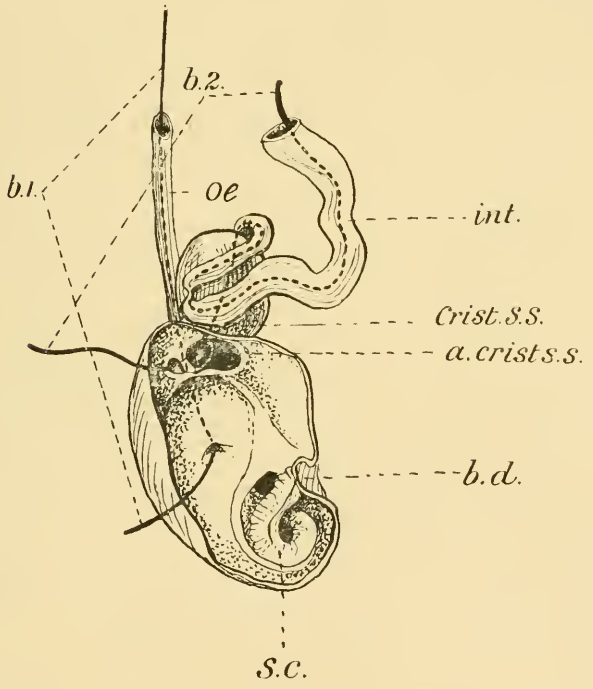

Fig. I.—Stomach of Nassopsis nassa opened from above, showing relation of crystalline style-sac to main chamber of stomach. *b* 1. Bristle passed from aperture of œsophagus along its interior. *b* 2. Bristle passed from aperture of intestine to the cut end of same. *Crist. s. s.* Crystalline style-sac. *a. crist. s. s.* Opening between stomach and crystalline style-sac. *b.d.* Aperture of digestive gland. *S.c.* Spiral cæcum.

on the tentacles themselves, but on secondary papillæ at their posterior bases (Pl. 20, figs. 5, 12, 6). There is a well-developed mucous gland in the mantle cavity (Pl. 20, fig. 5, *m. g.*), and the animal, unlike the genus Spekia, is viviparous. On opening up the body from before there is found to be a tolerably well-developed buccal mass (Pl. 20, fig. 12, *b. m.*) ; the radular sac is of average length, and the salivary glands are somewhat tortuous, simple saccular organs (fig. 12, *s. g.*). The radular dentition is strong and of the Littorino-planaxoid type (Pl. 20, fig. 8).

From a portion of the radula obtained by Smith, Guatkin referred it to the types approximating to the genus Cerithium, while Smith himself remarked upon its similarity to the radula of the Planaxidæ. The œsophagus is long, narrow, and simple, and leads into a large stomachic chamber (Pl. 20, figs. 9 and 11, also Fig. I, page 189), on the walls of which there are numerous glandular folds (fig. 11), and a very curious and striking double hemispherical cæcum on the floor (fig. 11, and Fig. I, p. 189), between the folds of which the curiously rectangular orifice of a single bile-duct opens (fig. 11, *a. h. d.*). Besides this stomachic chamber there is an anterior diverticulum into which the large stomach opens by a tubular aperture, through which a bristle is represented as passing in fig. 11, *w. x.*, and in this anterior stomachic chamber there lies an almost spherical crystalline style. The intestine passes out of the stomach beneath the tubular aperture between the posterior and anterior stomachic chambers, as indicated by the bristle represented in fig. 11, *y. z.*, also Fig. I, p. 189. The intestine is simple, almost straight, and towards its rectal extremity it contains a number of glandular folds and striæ.

The liver is large, and occupies the lower two thirds of the last two whorls of the animal's body. There is a single bile-duct, opening, as has already been stated, in the posterior chamber of the stomach.

The heart has the normal tænioglossate characters, and consists of a thin-walled auricle, a thick-walled ventricle, and a short aortic trunk (Pl. 21, fig. 1). Between the auricle, ven-

tricle, and aortic trunks there are the usual valves. The gill of Nassopsis is of average length, very simple in structure, and consists of a large number of low, broad, triangular leaves, the apices of which are not produced into filamentous processes, nor ornamented in any way. The osphradium is long and simple; it lies in a groove at the base of the gill, and shows no tendency to become pectinated or modified in any way either before or behind (Pl. 20, fig. 6, *o. s.*).

The nervous system of Nassopsis is extremely interesting, and is certainly one of the most archaic tænioglossate types at present known. The cerebral ganglia are widely separated from one another (Pl. 20, fig. 7, *c. g.*); and the pleural ganglia are not only separated from the cerebral ganglia (fig. 10), but quite below the œsophagus, the cerebro-pleural connectives being consequently of great relative length. The supra-intestinal cord springs directly from the right pleural ganglion, passes up over the œsophagus, and carries the supra-intestinal ganglion (fig. 7, *sup. int. g.*). From the left pleural ganglion there passes a fine nerve towards the supra-intestinal ganglion, which appears to form a dyaloneurous connection with a derivative of the supra-intestinal nerves. Towards the right the subintestinal connective passes from the left pleural ganglion beneath the œsophagus straight to the subintestinal ganglion (fig. 7, *sub. int. g.*). This ganglion is directly connected with the right pleural ganglion by a thick cord (fig. 7, *x.*), and the nervous system is therefore strongly zygoneurous on the right. Above, the cerebral ganglia give off a number of anterior nerves, which are distributed to the buccal mass and the parietes of the head. Among these there are conspicuous the tentacular nerves which pass separately to the tentacles and ocular papillæ. The buccal ganglia are situated on the lateral walls of the buccal mass, and are united to the cerebral ganglia by connectives (Pl. 20, fig. 7, *b. g.*). Near the origin of the buccal nerves there arise two fine nerves, one from each cerebral ganglion, which pass forward along the walls of the body, and then bend down, uniting with each other below the mouth (Pl. 20, fig. 7, *L. com.*). This connection appears, therefore,

to be unquestionably the labial commissure described by Bouvier as characteristic of a number of the Archi-tænioglossate and Rhipidoglossate types.

The cerebro-pedal connectives are very long (Pl. 20, figs. 7 and 10), and altogether the length of the cerebro-pleural, cerebro-pedal, and pleuro-pedal connectives gives to the nervous system the longi-commissurate character described by Haller.

The pedal ganglia are united by a rather small connection, and are prolonged into the foot along the course of two well-developed scalariform pedal cords. Between these pedal cords there exist ladder-like connections similar to those found between the pedal cords of Cyclopherus.

The otocysts in Nassopsis are relatively immense (Pl. 20, figs. 7 and 10, *ot.*). They are situated well up on the course of the pedo-pleural connectives, and the otocyst nerves pass obliquely from them towards the cerebral ganglia, and are not quite correctly indicated in figs. 7 and 10. The otoliths are small, numerous, and rectangular, with the faces slightly convex (fig. 10).

The reproductive apparatus in Nassopsis is similar in many ways to that of Typhobia, both male and female apparatus occupying the same general position. In the male the genital gland occupies the upper surface of the apical whorl in the body, and is connected by several channels with a nearly straight vas deferens, represented in Pl. 21, fig. 2, *v. d.* This latter structure opens without any modifications along its course by the slit-like aperture represented in Pl. 21, fig. 5. In the female the ovary occupies the same position as the male gland, and in like manner it is connected with the nearly straight oviduct, the lower portion of which, or that which lies within the mantle cavity, forming a brood-chamber where the eggs go through their later stages of development (Pl. 20, fig. 6, *b. s.*).

(B) Bythoceras.

Like Nassopsis, the genus Bythoceras, so far as is at present known, is exclusively restricted to Tanganyika, and as a

member of the halolimnic fauna of that lake is of considerable interest. Firstly, because, although conchologically so similar to the genera Nassopsis and Paramelania, it has, as we shall immediately see, no morphological relation to the former of these types. Bythoceras is interesting, secondly, because it presents us with more numerous points of correspondence with forms such as the genus Tympanotamus, which exists elsewhere, and the anatomy of which is known, than is the case with the majority of the halolimnic forms. Bythoceras is at present represented in Tanganyika by a single species, B. iridescens, which I dredged living at great depths[1] in the southern portion of the lake. When young the shell does not possess the characteristic spines represented in Pl. 21, fig. 3 (compare Pl. 3, fig. 4). Nor has it the peculiar pearly thickening of the mouth invariably present in the older forms. In the young condition (Pl. 21, fig. 4) the shell is extremely similar to that of Paramelania, and I am inclined to think that the figure of Paramelania crassilabrio given by Professor E. von Martens in his work 'Beschalte Weichthiere, Deutsch. Ost-Afrikas' (Pl. vi, fig. 38), is, in reality, that of a young Bythoceras iridescens.

The outward appearance of the animal is extremely similar to that of Cerithium vulgatum, with the exception that there is less pigmentation of the foot, which is nearly white in the Tanganyika species.

The snout is short, wrinkled, richly covered with black pigment, and non-protrusible. The tentacles are short, and the eyes are situated on the posterior bases of these organs, and not separated from them on subsidiary papillæ as in Nassopsis. The buccal mass is small, and the radular sac short, being reduced, as in the case of Typhobia and Tanganyikia, to a small swelling on the floor of the œsophageal tube (Pl. 21, figs. 7—11, *r. s.*).[2]

The radular dentition is extremely interesting, a single row

[1] From 300 to 1000 feet.

[2] Compare figures of Tanganyikia rufofilosa, preceding article (loc. cit.).

of teeth being represented in Pl. 21, fig. 9. The outer and inner lateral teeth distinctly resemble those of the Melanoplanaxoid type which I[1] described in considering the relationships of the genus Tanganyikia, and are something similar to those of the "Neomelanian" group of the brothers Saracin;[2] while the admedian tooth is peculiar, owing to the presence of two small subsidiary denticles on the inner face (Pl. 21, fig. 9), a very peculiar feature, and one which is only exemplified in the radula of Tympanotamus. The presence of this peculiarity, together with the general character of the radula, should certainly be regarded as of weight in diagnosing the nearer affinities of this form. And, as we shall see in placing it along with Tympanotamus, it is certainly in accord with the rest of the animal's morphological peculiarities.

The œsophagus and salivary glands (Pl. 21, fig. 7, *s.g.*) are in all ways similar to those of Typhobia, but these characters are common to so many different kinds of Gastropods that they are of little value from a special morphological point of view.

The stomach has two chambers, the anterior of which (Pl. 21, fig. 13) contains a style. The intestine is simple, and takes the course represented in Pl. 21, fig. 13. The rectum is not dilated, nor beset with any accessory gland. The bile-ducts seem to open by two very small apertures upon the base of the posterior stomachic chamber. Stomachic valves are feebly if at all developed. The liver is large, and occupies much the same position as in Nassopsis (loc. cit.). The excretory organ occupies a place in front of and above the heart, and opens by a minute pore at the extreme upper end of the mantle cavity (Pl. 21, figs. 5 and 13, *r.a.*).

The heart has the usual tænioglossate characters, consisting of an auricle, ventricle, and aortic trunk; but the last structure is much less developed in Bythoceras than in many forms,—as, for example, in the genus Typhobia.[3]

[1] Loc. cit.

[2] "Die Süsswasser-Mollusken von Celebes" (Wiesbaden, C. W. Kreidel's 'Verlag'), 1898.

[3] Loc. cit., 'Quart. Journ. Micr. Sci.,' vol. 41, 1898, p. 190.

The nervous system in Bythoceras is very interesting, since it is absolutely unlike that possessed by the genus Nassopsis, and closely simulates the type described by Bouvier [1] as typical of the genus Cerithium. It also strongly resembles that of the genus Tanganyikia. Viewed from above (Pl. 21, fig. 6), the cerebral ganglia are seen to be closely fused together, while the left pleural and subintestinal ganglion, as in Cerithium, form a single massive trunk, which at its hinder extremity gives rise to the subintestinal and visceral nerve-cords, and to the right pallial nerve (fig. 6). From the right pleural ganglion a nerve passes out to the mantle, and a branch from this anastomoses with a branch on the pallial nerve just described. In like manner on the left the pleural ganglion gives birth to a nerve on that side (fig. 6), which passes out and probably anastomoses with a twig given off from the supra-intestinal ganglion, but I was not able to trace this nerve throughout its entire course.

Unlike the subintestinal ganglion, the supra-intestinal ganglion is carried on a very long supra-intestinal connective (Pl. 21, fig. 6, *sup. int. g.*) exactly as it is in Cerithium or Aphorais. Viewed from the side (Pl. 21, fig. 10) the cerebro-pedal and pleuro-pedal connectives are seen to be of considerable length, rather longer than the same structures in Voluta, but not so long as those in Nassopsis or in Strombus. The pedal ganglion has the usual bulbous form as in the true Cerithidæ, and in like manner there pass from the lower extremity of each pedal ganglion two predominant foot nerves (fig. 10).

The otocysts lie behind the pedal ganglia, and the otocyst nerves pass directly between the cerebro-pedal and pleuro-pedal connectives to the cerebral ganglia on each side (Pl. 21, fig. 10). The otocysts are not large, and are round, as distinguished from those of Nassopsis. The otoliths are small, rectangular or barrel-shaped, and numerous.

The reproductive apparatus is very simple, and in both sexes consists of a genital gland which occupies the upper

[1] 'Ann. Dis. Sci. Nat.,' 1887, pp. 131—155, pl. vii, and figs.

surface of the last two whorls of the animal's body. This gland is put into connection with a large non-convoluted oviduct or vas deferens, as the case may be, by a number of fine tubes; and both ducts pass beneath the intestine and open just behind the anus in a large slit (Pl. 21, figs. 5 and 13, *g. a.*).

The genital duct in both sexes is much enlarged within the mantle cavity, somewhat in the manner of the same structure in the genus Typhobia. But in Bythoceras it is quite destitute of the singular organ which I described as an evertible penis in the male Typhobia.[1]

Comparative.

In considering the phylogenetic relationships of Nassopsis and Bythoceras, it will be needless after the foregoing description to insist further upon the fact that these genera bear no relation whatever to each other. Taking Nassopsis first, it will have already been clearly seen that the characters of this singular genus place it unquestionably among the archi-tænioglossate forms. Whatever opinion one may hold as to the value of the characters of the radula, it will have been seen that they too place it among the more primitive portion of the great melano-planaxoid group; and beyond this it is doubtful whether the radula can be used in a diagnostic sense at all. The large buccal mass, long radular sac, and the characters of the salivary glands certainly recall the littorinoid group, and at once dissociate Nassopsis from the early Stromboid or Xenophoran type to which Typhobia, Bathunalia, and Tanganyikia all appear to bear more or less distinct affinities. The extremely archaic and simple condition of the whole digestive tract in Nassopsis requires particular attention, for it will have been seen by any one acquainted with my former accounts of the anatomy of Typhobia, Tanganyikia, Bythoceras, and even Spekia, that the digestive apparatus, and especially the stomachic portion of the digestive apparatus in all these molluscs is built upon the same general plan. They all possess

[1] 'Quart. Journ. Micr. Sci.,' vol. 41, 1898, p. 191, loc. cit.

anterior stomachic diverticula which contain crystalline styles, similar to those of the genera Strombus, Pteroceras, Rostellaria, Murex, and Trochus; and it has already been shown in a former paper [1] that there is no reason whatever to doubt that these structures, when they appear in the Prosobranchiata, are like the heart when perforated by the rectum, as in the Trochidæ, to be considered as the retention of a condition common to the ancestors of both Prosobranchs and Lamellibranchs alike.

The presence of these similar arrangements in so many of the halolimnic Gastropods, which in all other ways are so widely separated from each other, is thus only intelligible if we suppose that peculiar stomachic apparatus was once universal among the ancestors of the Prosobranchiata of the present day, and that in the remnant of an old fauna, such as that now existing in Tanganyika, we encounter a more abundant representation of an archaic state of things.[2]

The presence of a well-developed mucous gland in Nassopsis, the extremely simple character of the gills and the osphradium, the simple reproductive apparatus, are features which all further dissociate Nassopsis not only from the hitherto known fresh-water forms, but also from the more modified Tænioglossa at present existing in the sea.

All the preceding structural peculiarities which demonstrate the archaic character of Nassopsis are, lastly, fully substantiated by the curious condition of the nerves. The great relative length of the cerebro- and pleuro-pedal connectives suggests the condition found in Haller's so-called "longi-commissurate forms," such as Strombus, Pteroceras, and their allies; while in the presence of a labial commissure we are confronted

[1] 'Quart. Journ. Micr. Sci.,' loc. cit., pp. 198, 210.

[2] In the case of Nassopsis we can go, however, further than this; for comparing the spiral cæcum in the stomach of this genus with the well-known and similar structure in the stomachs of the Rhipidoglossa, the two are found to be apparently identical, and the retention of this typically primitive feature in a form which also in other ways is so distinctly archi-tænioglossate is a morphological fact of the highest interest.

with a feature which, like the cæcum, connects Nassopsis on the one hand with the Rhipidoglossa, and on the other with the Archi-paludines. In like manner the wide separation of the cerebral ganglia from one another, and the comparatively great length of the cerebro-pleural connectives, are certainly features which recall the primitive scattered condition of these parts. In fact, the position of the pleural ganglia in Nassopsis represents a condition of development which is really halfway between what I may term the hypo- and the epi-athroid types, the nervous system of Nassopsis marking, in fact, a third, intermediate, or dystenoid type.

Again, in the scalariform pedal cords with which Nassopsis is provided we have another most important feature, showing that this form is not far from the border-land between the older groups of Tænioglossa and their Rhipidoglossate ancestry.

Nassopsis in many ways is more primitive than Paludina, but at the same time, as will have been seen, it bears no very approximate relation to this form; neither is it very near the ancient group of Littorinas, nor indeed to any of the individuals which at present are regarded as constituting the archi-tænioglossate group.

The morphological interest of Nassopsis lies, therefore, in this, that it presents us with a new starting-point from whence to study the inter-relationships of the great Prosobranchiate order.[1]

Nassopsis, therefore, presents us with a type of organisation which there are conchological reasons for believing is similar to that possessed by several species of a genus which was once abundant in the sea, but which has long since become extinct outside the confines of Lake Tanganyika, where,

[1] I shall elsewhere emphasise more fully the importance of Nassopsis as an archetype, and it is therefore needless for me here to do more than point out the fact that the above conclusions respecting the great morphological antiquity of this form are fully substantiated by the very unexpected similarity which three of the rather marked varieties of the shells of Nassopsis present to the genus Purpurina from the old Jurassic seas.

strangely enough, it appears to have lived on, not alone, but in company with several other usually extinct marine Jurassic forms.

Turning now to the characters of the new genus Bythoceras, we find no such peculiarities as those I have just described as distinctive of Nassopsis nassa. But nevertheless this genus is not without much interest from a morphologist's point of view. Nowhere, so far as I am aware, have we a better instance of the fact that shell structure as a means of classification is a "delusion and a snare;" for in the case of Bythoceras it is not only that the shell in no way foreshadows the animal inside it, but that its surprising similarity to Nassopsis is absolutely misleading, and, were we ignorant of the animals contained in both, would lead to a profound error in the close conchological association of the two genera.

Who would have dreamed, when contemplating the heavy, thick, and highly ornamental shell of Bythoceras Howesii, that the animal it contained bore any close resemblance to that enclosed in the strangely different shell of the genus Tympanotamus? Yet in the most peculiar features of its radula, nerves, gills, and viscera it strongly resembles Cerithium, Tympanotamus, and their close allies.

The reproductive organs of Bythoceras are simple and peculiar, and in many other ways the structure of this organism will afford ample material for conjecture concerning the final identification of the really primitive cerithoid type. For which is more ancient, Bythoceras or Cerithium? I do not feel competent to answer this question, and I would refer the reader to the broader discussion of this subject in my longer paper on "The Prosobranchiate Mollusca" (in the hands of the editor of the 'Quart. Journ. Micr. Sci.').

This much, however, may be affirmed with certainty, that Bythoceras is a form closely related to our general conception of the genus Cerithium, with some of the minor features in its radular dentition peculiar to and distinctive of the genus Tympanotamus.

In conclusion it may be interesting to reflect how all the evidence which has been collected concerning the nature of the halolimnic Gastropods invariably points to the vast antiquity of these forms. First we have the wide dissimilarity of their empty shells from those of any living types; next their rigid isolation to a solitary great lake, which, judged from whatever standard we may choose to adopt, is unquestionably of an enormous age. Next we have the wonderful similarity of the halolimnic shells now living in Tanganyika to those which have been left fossilised at the bottom of the old Jurassic seas; and lastly, there are the morphological characters of the halolimnic animals themselves, whereby they become mentally depicted like nothing so much as the incompletely developed embryos of numerous living oceanic types.

EXPLANATION OF PLATES 20 and 21,

Illustrating Mr. J. E. S. Moore's paper on "The Molluscs of the Great African Lakes."

Reference Letters.

a. Anus. *g.g.* Genital gland. *g.a.* Genital aperture. *m.g.* Mucous gland. *Int.* Intestine. *os.* Osphradium. *col. m.* Columellar muscle. *op.* Operculum. *r.s.* Radular sac. *s.g.* Salivary gland. *B.m.* Buccal mass. *cryst. s.* Crystalline style. *œ.* Œsophagus. *a.h.d.* Aperture of hepatic duct. *r.* Kidney. *R.* Renal aperture. *b.s.* Brood-sac. *g.a.* Genital aperture. *st.* Stomach. *ft.* Foot. *c.g.* Cerebral ganglion. *pl.g.* Pleural ganglion. *p.g.* Pedal ganglion. *l.com.* Labial commissure. *b.g.* Buccal ganglion. *ot.* Otocyst. *sub.int.g.* Subintestinal ganglion. *sup. int. g.* Supra-intestinal ganglion. *os.g.* Osphradial ganglion.

PLATE 20.

Fig. 1.—Two views of a variety of the shell of Nassopsis.

Fig. 2.—Two views of another variety.

Fig. 3.—Embryonic shells and protoconch of Nassopsis.

Fig. 4.—Animal of Nassopsis removed from shell, showing the operculum, *op.*

Fig. 5.—Male Nassopsis, showing the mucous gland, tentacles, and genital aperture.

Fig. 6.—Mantle cavity of female Nassopsis, showing character of gill and osphradium.

Fig. 7.—The nervous system of Nassopsis dissected from above.

Fig. 8.—Dissection of Nassopsis, showing relations of stomach, crystaline style, kidney, œsophagus, and intestine.

Fig. 9.—The radular elements of Nassopsis.

Fig. 10.—Nervous system of Nassopsis dissected on the right side.

Fig. 11.—Details of the alimentary tract of Nassopsis.

Fig. 12.—The relations of the buccal mass, radular sac, and salivary glands of Nassopsis.

PLATE 21.

Fig. 1.—The relation of the heart, pericardial cavity, and reno-pericardial connection in Nassopsis.

Fig. 2.—The reproductive apparatus of a male Nassopsis.

Fig. 3.—Two views of the shell of Bythoceras Howesii.

Fig. 4.—Young shell of Bythoceras Howesii, showing the absence of the spines above and below the mouth.

Fig. 5.—Dissection, showing the gill and mantle organs of Bythoceras.

Fig. 6.—The nervous system of Bythoceras dissected from above.

Fig. 7.—The buccal mass, radular sac, and salivary glands of Bythoceras.

Fig. 8.—The nervous system of Cerithium vulgatum dissected from above.

Fig. 9.—The radular elements of Bythoceras.

Fig. 10.—The nervous system of Bythoceras dissected from the right side.

Fig. 11.—Right side view of the buccal mass, salivary glands, and radular sac of Bythoceras.

Fig. 12.—The operculum of Bythoceras.

Fig. 13.—Details of the alimentary apparatus of Bythoceras.

Further Study of Cytological Changes produced in Drosera.

Part II.

By

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With Plate 22.

This research is an expansion of that previously undertaken, of which an account has been given in this Journal, vol. 39.

Aims.

The present aim was to ascertain—

(1) Whether substances which differ chemically will produce specific alterations in either the cytoplasm or the nucleus.

(2) To what extent the cytoplasm and the nucleus are interdependent.

(3) Whether the rapidity with which changes occur depends on the ease with which substances are absorbed.

(4) The relation between cytological changes and the irritability of leaves, the latter being calculated from the rate of closure of the tentacles.

(5) What effects can be produced by bringing healthy cells in contact with supposed waste products of ordinary metabolic activity.

Methods.

To deal with these questions satisfactorily necessitates that no reliance should be placed on any single fixing method, and therefore the whole of my previous work has been repeated with chemically very diverse fixatives, e.g. 5 per cent. and 10 per cent. trichloracetic acid, 5 per cent. and 10 per cent. phosphotungstic acid, osmic acid vapour, picric acid, picro-corrosive sublimate with tannin added, absolute alcohol, Hermann's fluid, Flemming's strong mixture, and Flemming's weak mixture. All these methods fully confirmed what I have stated in my previous paper, so that in pursuing my new research I had every confidence in employing again the methods I formerly used, viz. Mann's aqueous picro-corrosive fixative and Mann's eosin and toluidin blue stain.

The following substances were placed on vigorous fully expanded leaves:—Paraffin, white of egg, Kühne's pure amphopeptone, Witte's peptone (albumoses), ammonium sulphate, fibrin, Grübler's fibrin peptone, globulin, casein, nuclein and nucleic acid, milk, calcium phosphate, glutin, leucin, creatin, urea.

In all cases except that of casein the species of Drosera used was D. rotundifolia, as plants reared in greenhouses cannot be considered normal.

The leaves after being fed were fixed at intervals varying from one minute up to the time of reopening of the leaves. As, however, in the case of white-of-egg feeding the changes produced after one minute are so remarkable,[1] shorter periods varying from 5 to 60 seconds were tried with white of egg and also with pure peptone.

Unfed leaves were fixed at the same time to serve as controls, and to avoid all errors caused by irregular staining sections of control leaves were always mounted alongside those of fed ones on the same slide.

It may be well to quote here again a definition of the terms applied by me to the nuclear organs.

[1] See previous paper.

Chromosomes are the organs which show the well-known affinity for alkaline dyes. They are situated at the periphery of the nucleus, and consist of nuclein.

Nuclear plasm is the material which, in the resting state, forms the main bulk of the nucleus. It is neutrophile in character, and is frequently called the nuclear sap, but corresponds to "œdematin" globules.

Nuclear sap is the more watery fluid inside the nucleus, in which is suspended the nuclear plasm, the latter being precipitable by $HgCl_2$ and other reagents.

Nucleoli are the spherical organs which show a special affinity for acid dyes, and have a more or less central position, and they consist of Flemming's paranuclein.

Resting Gland Cells (fig. 1).—The gland cells in their resting state, when fixed and stained by Mann's methods, appear as follows:

The cell wall is pale blue; the cytoplasm is arranged in an open foam-like manner; it stains pure blue with a coarse granulation embedded in it, which appears of a deeper blue and represents the zymogen.

The nucleus is situated just below the middle of the cell. It may be spherical or oval. It stains purplish, and is sharply defined against the cytoplasm, partly because of the difference in tint, and also because of the peripherally placed, very minute, dark blue chromosomes.

The nuclear plasm is granular, always very dense, and of a purplish tint. One (sometimes more) very distinct, deep red nucleolus is always present, surrounded by a narrow Frommann's clear zone.

Experiments.

Paraffin (fig. 2).—The rapid changes which can be produced by certain foods suggested the idea of ascertaining whether purely physical factors, such as contact with insoluble substances, would not also produce effects. When pieces of well-boiled cork were applied to the leaves vacuolation of the

gland cells resulted; but as this alteration might have been due to traces of soluble substances it was thought necessary to use paraffin of a high melting-point (58° C.), which had been carefully cleaned by boiling. Shavings of it were laid on the tentacles, and pressed into thorough contact with them by means of a brush. About one hour after the application of the paraffin the alteration in the cells reaches its height. It consists in a vacuolation of the cytoplasm, but with no alteration in colour reactions. The nucleus remains quite unchanged. In the course of twenty-four hours complete recuperation has taken place.

White of Egg [stains Red] (fig. 3).—My former research having dealt minutely with the changes produced in the gland cells by white of egg, I have now only to add those resulting in the exceedingly short time of five seconds. They are as follows:

Cell wall very pale blue.

Cell plasm deep purple, frequently red round the nucleus, vacuolated in the upper third of the cell, densely aggregated below.

Nuclear plasm unaltered in arrangement, but staining dark purple or red like the cytoplasm surrounding the nucleus.

Chromosomes as in controls.

Nucleolus staining somewhat less brightly.

Peptones (figs. 4—9).—The experiments were made on a large number of leaves, which were fed and fixed on their native moor. It is important to note this, as plants which were taken home did not show the characteristic precipitates about to be described, but only vacuolation.

Histological Changes.—In all cases the peptone appeared as greenish-blue droplets adhering to the exterior surface of the gland cells. In the cells of that layer which in my former paper I called the third layer of gland cells, the cell sap is particularly rich in tannin, and here is formed a heavy greenish-blue precipitate encrusting the peripheral walls. When viewed with a low power the dark blue line thus formed gives a perfectly characteristic appearance to all

the sections of material used in this experiment. The changes in the apical gland cells are as follows:

Five Seconds after the Application of a 10 per cent. Solution (fig. 4).—A precipitate (?) resembling minute blue droplets covers the interior of the cell walls, especially the lateral ones.

The cell walls stain very faintly blue, or show no colour.

The cell plasm stains much more deeply blue than in controls. It appears more dense and homogeneous, being apparently almost free from vacuoles. The reticular arrangement seen in controls has given place to what appears like a thick, evenly distributed granular mass. Thus instead of vacuolation there is found a direct increase in bulk. At the periphery of the plasmic body are seen dark blue droplets or granules resembling those on the walls.

The nucleus is normal as to position, size, shape, and clearness of definition.

The nuclear plasm is often also bluer than in controls, though it frequently stains normally. Its arrangement is unaffected.

The nuclear chromosomes are unchanged.

The nucleolus is normal as to size, and also as to colour in those nuclei in which the plasm stains normally. In the bluer nuclei it stains more of a purple. Frommann's zone remains clear.

The nuclei of the third layer remain unchanged.

Ten to Thirty Seconds.—The above description holds good. I have, however, observed a slight shortening of the nuclei of the third layer of gland cells in leaves fed for thirty seconds.

Specimens fed for one minute require no separate detailed description, as they resemble the bluer types about to be described.

Five Minutes after Feeding (fig. 5).—The precipitates appear as in earlier stages. The cell walls stain blue with extreme faintness. The cytoplasm remains as shown in fig. 4, or is more vacuolated, and stains less deeply (fig. 5). The

vacuoles appear to be filled with a substance which stains pale blue. In a few tentacles there is a reddish tinge in the apical half of the cells. The nucleus is normal as to position, size, and shape. The nuclear plasm stains occasionally bluer than in controls (fig. 4); or again in those cells which show a tendency in the cell plasm to stain red, the nuclear plasm takes on the same tint. There is, therefore, at this stage a decrease in the basophile affinity which was so marked a characteristic of the earlier stages. The nuclear plasm is not diminished in quantity or altered in arrangement.

The chromosomes are not perceptibly increased in size, but appear to be slightly more numerous. In some nuclei they appear as if united by fine threads of a substance which also stains dark blue. The nucleolus is normal.

The nuclei of the third layer are either normal or very slightly shortened.

One Hour after Feeding (figs. 6—8). — The droplets adhering to the exterior of the tentacle and those on the lateral walls of the gland cells generally stain more faintly and are less conspicuous than in earlier stages.

The cell walls stain extremely pale blue.

The cytoplasm is paler blue and less dense than in earlier stages, but is quite as full or fuller than in most controls, and its mass is often outlined with dark drops or granules.

The nucleus is sometimes normal in size and shape, and sometimes shrunken. The nuclear membrane has quite disappeared. The nuclear plasm stains like that of controls, and in those nuclei which have not shrunken it is arranged as in controls, while in the shrunken nuclei its arrangement cannot be made out.

The chromosomes have increased enormously. In those nuclei which have not shrunken they are seen to be ribbon-shaped. I have not found it possible to determine whether or not they exist in any fixed number.

The nucleolus is smaller than normal. In the unshrunken nuclei it is surrounded by a clear zone.

In the third layer the precipitate is as heavy as before.

The nuclei vary in shape from being only slightly shorter than normal to forms such as are shown in figs. 32 and 33 of my previous paper.

One Day after Feeding (fig. 9).—After the leaves had been fed it was unfortunately necessary to remove the plants from their natural habitat. They were placed in a cool greenhouse, where the leaves that were allowed to remain growing reopened healthily in two to four days, but it is possible that the transplanting may have affected the cytological appearances about to be described. It is remarkable that there was nothing to be seen resembling the precipitates of the earlier stages either externally or internally.

The cell walls are very pale blue, almost colourless.

The cytoplasm is purple, very granular, greatly vacuolated in the apical half of the cells, dense in the basal half, though showing here also small vacuoles. In the neighbourhood of the large vacuoles dark blue granular matter collects.

The nucleus is normal in size and shape, and is surrounded by a membrane, which facts suggest that recuperation has begun in the nucleus, though not as yet in the cytoplasm.

The nuclear plasm is of the same tint as the cytoplasm, and is irregularly disposed, instead of being regularly distributed as seen in controls and in the earliest stages of peptone feeding.

The chromosomes are large and angular, drawn out into points, which frequently gives them a stellate appearance. I have not ascertained whether their number is constant.

The nucleolus is not very much smaller than normal.

The nuclei of the third layer are globular or irregular in shape, pale pink, nearly destitute of nuclear plasm, with a few conspicuous chromatin granules and a diminished nucleolus. They resemble fig. 32 of my former paper.

Ammonium Sulphate. — As Kühne's amphopeptone is prepared with ammonium sulphate, it was thought advisable to ascertain whether the precipitates formed in the cells by feeding with amphopeptone might be due to traces of this salt. Ammonium sulphate in 10 per cent., 100 per cent., and 200

per cent. solutions in distilled water was therefore applied to the leaves. No precipitates resulted, and vacuolation of the cytoplasm was the only effect produced in the cells.

Some experiments were also tried with Witte's peptone, which is very rich in albumoses. The tentacles did not close rapidly, but in from one to four hours they had all closed. The bending caused was more rapid than that induced by the fibrin peptone. In about three days the leaves reopened. The material fed with Witte's peptone was accidentally spoiled and rendered useless for histological examination.

Before describing, however, the changes induced by fibrin-peptone it is best, for comparison, to detail alterations caused in the gland cells by Grübler's fibrin.

Fibrin (stains Red).—Maximum change shown in fig. 11. The fibrin was reduced to a powder by crushing, and was then applied either dry or after being moistened with distilled water. If all the tentacles were loaded they closed in about ten minutes, otherwise only those that were touched closed, and did so more slowly. The leaf itself did not double up as after feeding with white of egg. Cytological changes :

Five Minutes after Feeding.—The only observable changes are that the cytoplasm is vacuolated in the upper half of the cell, and the nucleolus somewhat diminished.

After One Hour (fig. 10 *a*).—The cell wall is paler blue than normal. The cytoplasm pale blue, much vacuolated ; frequently the cell is almost emptied. The nucleoplasm is aggregated. The chromatin is normal. The nucleolus has diminished to about one half its original diameter.

After One Day (fig. 10 *b*).—The cell wall is almost colourless. The cytoplasm is reduced to a scanty pale blue reticulum. The nucleoplasm is normal as to colour, or redder, aggregated and diminished in amount, showing empty spaces. The chromosomes have enlarged. The nucleolus is always diminished, sometimes reduced to a mere point.

After Two Days (maximum change, fig. 11).—The cell walls are colourless or slightly pink. The cell plasm is blue, extremely scanty. The nucleoplasm is thin, pale purple. The

chromosomes are enlarged. The nucleolus is small and dull red.

After Two to Four Days.—Recuperation takes place as described for leaves fed with white of egg, but much more slowly, and is complete in four to seven days.

Grübler's fibrin-peptone stains red and produces the maximum change in one hour after feeding, as shown in fig. 12. The food appears as pink drops adhering to the outside of the gland. The cell wall is nearly colourless, or faintly greyish blue. The cytoplasm is very scanty. In different leaves it varies in colour from pale blue to pinkish grey. The nuclei are more or less shrunken ; the nuclear plasm reduced to a little granular cloud which stains violet. The chromatin has increased enormously, and forms large segments with rounded ends. Whether their number is constant or not I have not determined. The nucleolus is small and of a dull red tint.

Milk (stains Blue).—A small drop was placed on the centre of the leaf or drawn over the marginal rows of tentacles. In the first case the leaf itself folded up. In the second case the tentacles closed regularly inwards in ten minutes or less, and after an hour or two the entire leaf doubled over as in the first case. In from one to three days the leaves reopened perfectly uninjured unless the tentacles had been here and there glued together by the milk drying. Milk may be absorbed twice or thrice and the leaf open as clean as a control.

Five Minutes after Feeding (fig. 13).—The cell walls are somewhat deeper blue than normal. The cytoplasm has contracted into dense strands, appearing coarsely granular and staining a much deeper blue than normal. The nuclear plasm is somewhat redder than in controls, and the individual granules have fused (aggregated) to form dark masses alternating with clear spaces. The nuclear chromosomes are normal. The nucleolus appears more of a purple.

One Hour after Feeding.—The cell wall is normal. The cytoplasm disappears completely except at the periphery of the cell and round the nucleus. It stains pale blue. The nuclei are sometimes slightly shrunken. The nuclear plasm is red-

dish and becoming more scanty. The chromosomes are normal. The nucleolus is diminished.

One to Two Days after Feeding (usual type, fig. 14).—The cell walls are very pale blue. The cytoplasm is extremely scanty, and stains very pale blue. The nuclear membrane is indistinct. The nuclear plasm is reddish and scanty. The chromosomes have slightly enlarged. The description just given is typical for the state of "maximum change" usually produced by milk in one to two days. It will be seen that the nuclear changes, especially those affecting the chromatin, are comparatively small. I have found this to be characteristic of milk. In a few cases, however, the nuclear changes have presented the appearance shown in fig. 15, which must be regarded as exceptional. The nuclear membrane is scarcely traceable. The nuclear chromosomes are much enlarged, angular, and very clear. They closely resemble those produced by calcium phosphate feeding (see fig. 16). The nuclear plasm has almost entirely disappeared. The nucleolus is pale red and very small. Recuperation proceeds in the usual way, and is very complete.

Calcium Phosphate (fig. 16).—The salt did not adhere to the tentacles after fixation, and so does not appear in stained material. When treated with a mixture of the two stains employed it seems to take up both, assuming a violet tint. The salt was applied as a dry powder. The leaves closed fairly rapidly. In two to three days they reopened, showing still the white powder apparently unaffected.

Changes induced in Five Minutes.—The cell wall stains a deeper blue than normal. The cell plasm shows a slightly increased vacuolation. The nucleus is contracted to about two thirds of its normal size. The nuclear plasm stains deep blue. The chromosomes and nucleolus are normal.

One Hour after Feeding.—The cell wall is blue. The cell plasm shows increased vacuolation. The nucleus is contracted. The chromosomes are normal. The nucleolus is diminished somewhat.

One to Two Days after Feeding (maximum change,

fig. 16).—The cell walls are pale blue. The cytoplasm is exhausted and very pale blue. The nucleus is contracted irregularly. The nuclear plasm is scanty—very pale violet or blue. The chromosomes are greatly enlarged. The nucleolus is diminished, sometimes excessively small, and stains dull red.

The maximum changes thus described closely resemble conditions produced by milk (fig. 16).

In Two to Three Days.—Recuperation occurs. The cytoplasm remains rather poor. The nucleus becomes quite normal.

Globulin (stains Red) (maximum change, fig. 17).—The leaves were dusted with the dry powder and closed rapidly, i. e. seven to ten minutes. The globulin was entirely absorbed, and the leaves reopened in about three days quite clean. Some of them were fed a second time.

Cytological Changes: Five Minutes after Feeding.—The cell walls stain very pale blue or purple. The cytoplasm is diminished in amount, and large vacuoles appear in it. It stains blue or slightly purple. The nuclear plasm shows slight aggregations. The chromosomes and nucleolus are normal.

One Hour after Feeding.—The cell walls do not stain at all. The cytoplasm is grey and scanty. The nucleus loses its sharply defined contour, and its outline is only indicated by the dark blue circle of the somewhat enlarged chromosomes. The nuclear plasm is aggregated, and has become more eosinophilous. The nucleolus has diminished, and stains less brightly.

One Day after Feeding (maximum change, fig. 17).—The food in immediate contact with the apex of the tentacle appears at this stage striated with colourless lines issuing in a radiate manner from the apical cells, and suggesting that secretory products pass out through the apical walls. At the same time the food seems to be passing in between the lateral walls of the cells, which seem to stand apart, apparently separated by the red-stained globulin. The cell walls stain red. The cytoplasm is pale blue, or grey and scanty. The nuclear

plasm is reduced to a few purplish granules. The chromatin is greatly increased, and is distributed in a few large angular segments, which, however, do not equal in size those resulting from feeding with white of egg. The nucleolus is small, and stains dull red.

In Two to Four Days.—Recuperation takes place as in leaves fed with white of egg.

Casein prepared by "Hammersten's" Method (stains Red).—The experiments, owing to the time of year, had to be made on Drosera capensis kept in a greenhouse, and I therefore do not regard them as satisfactory.

The powder was dusted on the tentacles, which closed slowly, taking several hours. The maximum change observed resulted in two or three days, when the cytoplasm had entirely disappeared with the exception of a very narrow red peripheral layer, and a scanty remnant round the nucleus. The nucleus remained normal in size and shape, but the nuclear plasm was red and aggregated. The chromosomes showed slight enlargement, and the nucleolus appeared to be slightly diminished.

Nuclein (stains Violet).—Nuclein was applied to the leaves dry, and also wetted with distilled water. It caused no movement of the tentacles and no increased secretion, i.e. apparently no effect at all.

Five Minutes after Feeding.—The cell walls stain a deep violet colour. The cell plasm is somewhat vacuolated. The vacuoles appear blue. The nuclear plasm is unaltered in arrangement, but is sometimes of a bluer tint than normal. The nuclear chromosomes and nucleolus are unchanged.

One Hour after Feeding (maximum change, fig. 18). —The cell walls stain deep violet. The cytoplasm stains very deep blue, and forms a network of thick strands enclosing large vacuoles, which appear blue. The nuclear plasm stains the same deep blue tint as the cytoplasm. It appears to be slightly aggregated. The nuclear chromosomes are normal. The nucleolus is somewhat smaller and more purple. No further changes occur, and the cells become completely normal

in one to two days. Fig. 19 shows such a cell nearly quite recuperated after twenty-four hours.

Nucleic Acid (stains Blue) (figs. 20—23).—The acid was prepared absolutely pure by Dr. Grübler. The powder was made into a paste, and a piece about the size of a pin's head placed on the centre of a leaf which folded up and secreted violently for one to two days. At another time dry powder, or minute particles of the paste, were dusted on the tentacles, which then bent inwards and secreted copiously for one to two days.

In both cases the leaves commenced to reopen in one to three days, and were perfectly uninjured.

Five Minutes after Feeding (fig. 20).—The cell walls stain a much deeper blue than in controls. The cytoplasm shows very great vacuolation, and has contracted into thick strands, forming a network which stains very deep blue, and has a coarsely granular or knotted appearance. The nucleus has shrunk considerably. The nuclear membrane and nuclear chromosomes are unchanged. The nuclear plasm stains bluer than normal, is somewhat aggregated and less dense. The nucleolus is normal in size, but stains purple.

One Hour after Feeding (fig. 21).—The cell walls and cytoplasm show no further change. The nucleus has become irregular in shape, and usually has elongated transversely to the long axis of the cell. The nuclear periphery is distinct, the membrane staining dark blue and remaining distended. The nuclear plasm has contracted away from the nuclear membrane, and forms a small, compact, purple sphere round the nucleolus, suspended in the nuclear cavity by fine radiating threads attached to the nuclear membrane. A homogeneous fluid which takes a pale blue stain appears to fill the space between nuclear plasm and membrane. The chromosomes are not distinguishable. The nucleolus stains dark crimson. Frommann's clear zone is obliterated by the closely contracted nuclear plasm.

One Day after Feeding (fig. 22, *a*, *b*).—The cell walls stain paler blue. The cytoplasm is very pale blue, and is

reduced to a mere vestige. The nuclei are frequently enlarged, and may be spherical, oblong, or distended irregularly. The nuclear plasm is absent or represented by a faint cloudiness in the homogeneous pinkish fluid (?) which fills the nuclear cavity. The chromosomes appear as extremely minute, peripherally placed, blue granules. In some very clear nuclei they appear to be connected by fine threads which also stain blue. The nucleolus is slightly smaller than normal.

One to Two Days (fig. 23).—The cell walls are pale blue. The cytoplasm is almost completely exhausted, very pale blue. The nucleus is small. The nuclear plasm stains as in controls, but deeper. The nuclear membrane is ill-defined and the chromosomes are normal. Nucleolus diminished and staining dull red.

One to Three Days.—Recuperation takes place in a perfectly normal way.

Glutin (stains Red).—In the first experiments glutin was crushed into a powder and moistened with distilled water. Minute particles of this were placed on the tentacles. The tentacles closed rapidly, in about five minutes. The whole supply of glutin given to each leaf did not exceed an ordinary pin's head in bulk. In half an hour the particles had swollen and become white and translucent like milk. Dry glutin, even if powdered, was not conveyed by the tentacles to the centre of the leaf with anything like the same rapidity; indeed, it appeared to be indigestible.

All the tentacles and parts of the leaf which had been touched by the moistened glutin, as just described, died, i. e. became brown and withered, though the leaf as a whole reopened. Therefore the experiments were repeated with still smaller quantities of glutin. It was moistened and softened with distilled water, and placed with great care on the tentacle heads. There resulted complete absorption, and when the leaves reopened they were perfectly clean and healthy.

These experiments show what very different results may follow dissimilar applications of the same stimulus.

Five Minutes after Feeding. — The cell walls are

normal. The cytoplasm is normal as to colour, much vacuolated in upper third. The nuclear plasm is reddish purple, aggregated. The chromosomes and nucleolus are unchanged.

One Hour after Feeding. — The cell walls are pale. The cytoplasm stains blue, and is much vacuolated. The nuclear plasm is reddish purple and aggregated. The chromosomes are more prominent than in controls. The nucleolus is duller red and smaller.

One Day.—The cell walls are colourless or slightly pink. The cytoplasm is very pale blue, extremely scanty, merely lining the cell wall and surrounding the nucleus. The nuclei are often shrunken. The nuclear plasm is almost absent, and stains faintly blue or violet. The chromosomes are more or less enlarged. The nucleolus is dull red and small. Recuperation is normal.

In the tentacles which died the cytological appearance was the same as that caused by death from feeding with urea (figs. 26 and 27).

Creatin (fig. 24).—Creatin was employed dry and also wetted with distilled water. The latter proved the better stimulant. The tentacles bent inwards slowly. Secretion was not profuse. Some leaves seemed quite unaffected, and their tentacles did not bend. Those that closed reopened in two days, and the white particles of creatin were adhering to their tentacles apparently unaffected. The examination showed conditions as follows:

One Hour after Feeding.—The cell wall is normal; the cytoplasm slightly pink, and greatly vacuolated in the upper half of the cell; the nucleus unaffected.

In the course of the one or two days in which the leaf remains closed the further cytological changes are not great.

The nuclear plasm diminishes in quantity. The chromosomes in some cases increase slightly. The nucleolus diminishes to some extent (fig. 24). Recuperation is complete in two to three days.

Leucin (fig. 25).—Did not adhere to fixed tentacles, and does not appear in stained sections. Leucin was applied

both dry and wetted with distilled water; the former proved most active. In about 15 per cent. of the leaves used some of the tentacles bent slowly, taking about twenty-four hours to close; but as a rule the tentacles did not move at all, though their secretory activity appeared to be stimulated. Some of the leucin was dissolved and hung from the leaves in large drops, which finally fell off. In some cases it was not dissolved, and fell off or was blown away. In any case the tentacles were to all appearance left quite uninjured.

Leaves were fixed at the following intervals after feeding:— Five minutes, one hour, one day, two days, three days.

The cytological changes seem to be produced very tardily. I find none during the first two periods. After one day there is considerable vacuolation of cell plasm and nuclear plasm, and slight diminution of nucleolus. In tentacles that had closed there is slight but quite distinct increase of chromatin three days after feeding (fig. 25). This has not been seen to occur in tentacles which remained unbent.

Urea did not adhere to fixed material, and so does not appear in the stained preparations.

Crystals were powdered, and dusted on the tentacles. The urea readily melted in the secretion, and seemed to increase the exudation, but caused no bending of the tentacles. At the end of half an hour the leaves looked as if they had been under a shower of rain. In twenty-four hours the leaves appeared yellow, flaccid, with weak and shrivelled tentacles. Death of the leaf invariably ensued.

One Hour after Feeding (fig. 26).—The cell walls are blue. The cytoplasm is still blue and granular in the lower half of the cells, but is vacuolated in the upper half. The nuclear membrane remains irregularly distended, but the nuclear contents have contracted to form a small dark purple body in the centre of the nuclear cavity, in which neither nuclear plasm, chromosomes, nor nucleolus can be distinguished.

One to Two Days after Feeding (fig. 27).—The withered tentacles show the cytoplasm reduced to a scanty

reticulum of threads, staining blue, purple, or red. The nucleus has almost lost its identity, having amalgamated with its surrounding cytoplasm to form a more or less crescentic body, which stains reddish purple, and exhibits no differentiation whatever.

Summary.

1. By feeding with chemically different foods very characteristic alterations are obtained, both in the colour reaction and morphology of the cell. For example, in five seconds white of egg causes both the cytoplasm and nuclear plasm to become more eosinophile, while pure amphopeptone increases their affinity for blue stains. The former food quickly causes great impoverishment of cytoplasm and nuclear plasm, while the first effects of pure peptone are to increase their bulk and density. Both the foods produce an enormous increase of the chromatin element of the nucleus, while other foods, e. g. nucleic and nucleic acid, produce no such result.

2. While the cytoplasm is the cell constituent most rapidly and most constantly affected by external stimuli, the nucleus is the seat of metabolic activity, and the state of the nuclear organs indicates whether or not the food supply was of service to the metabolism of the plant.

It is not surprising, therefore, to find a certain independence of cytoplasm and nucleus in their behaviour to external stimuli, coupled with great interdependence with respect to all resulting processes of metabolism. Thus, substances such as paraffin and nucleic acid act merely as stimuli to the secretive activity of the cells; and they cannot be regarded as foods, for they affect the nucleus only by causing a slight drainage on the nuclear plasm and nucleolus. If the stimulus is very transient the nucleus remains unaffected. On the other hand, with highly nutritious food like egg-albumin and peptone the nucleus is the seat of the greatest change, and the chromosomes, i. e. the nuclein, undergo an enormous

increase, independently of the state of the cytoplasm. With egg-albumin we do not get these great changes in the nuclear organs till the cell plasm is thoroughly exhausted, while in the case of 10 per cent. amphopeptone we get them while the cell cavity is full of cytoplasm.

In all cases the process of recuperation begins in the nucleus. The nuclear plasm first becomes abundant, and restoration of the cytoplasm begins in contact with the nucleus, and spreads thence to the remoter parts of the cell.

3. To answer the question, whether the rapidity with which changes occur depends on the ease with which substances are absorbed, we must deal only with such changes as are characteristic of foods. Changes that are produced also by mere contact with insoluble substances must not be taken account of.

Changes which are produced in five seconds are rapid, yet in this short space of time we get quite specific alterations for two foods so different in their diffusibility as egg-albumin and peptone. But then only the plasm is affected, that is that cell constituent which I have just shown to be most rapidly altered by external stimuli. Nuclear changes, e. g. the increase of chromatin, must depend for their rapidity on the constitution of the food, i. e. on the series of chemical changes the latter must undergo before it is converted into basophile chromatin. In the two cases just cited the length of time required to produce this change differs widely, being about twenty hours in the case of egg-albumin, while one hour suffices for peptone; and yet from the changes wrought in the cytoplasm in five seconds we might argue that both foods entered the cells with equal rapidity. We may conclude, then, that the rate of plasmic changes depends on the rate of absorption, but that the rapidity of nuclear changes is commensurate only with the digestibility of the food.

4. In determining the relation between cytological changes in the gland cells on the one hand, and the degree of irritability in the leaves calculated from their rate of closure on the other hand, cytoplasmic changes only constitute our legitimate criterion; for I have shown above that the cytoplasm is the

cell constituent directly influenced by external stimuli, and that nuclear changes are the secondary results of metabolism. A careful comparison of the action of the substances applied to the tentacles proves that there is a constant concord between the rate of closure of the tentacles and the degree of vacuolation produced in the cytoplasm. The citation of a few cases will illustrate this :

Paraffin and nuclein caused no closure, and very slight and transient vacuolation.

Pure peptone caused very slow inbending, and no vacuolation for one or two hours.

White of egg and milk caused rapid bending and rapid vacuolation.

Fibrin caused slower closure and slower vacuolation.

Creatin did not induce bending of all of the tentacles to which it was applied; and those that closed did so with extreme slowness. The vacuolation was only transient.

Leucin sometimes, but not invariably, occasioned bending, and this was extremely slow. Vacuolation of short duration ensued.

Urea caused no bending, but killed the leaves. Being therefore a poison, its action should not be compared with that of the other stimulants named.

5. By bringing healthy cells into contact with the waste products, creatin, leucin, and urea, urea was found to act as a poison, creatin as a mild stimulus to movement, and leucin caused active secretion without much movement. These two substances, therefore, do not injure the leaves, and they seem to be of some nutritive value.

At a future time I may ascertain the effects of feeding with various carbohydrates, should the results obtained from preliminary experiments give promise that such an investigation would be of any value.

In conclusion I desire to acknowledge the kindness of Professor Gotch in allowing me to work in the Physiological Laboratory at Oxford, and to express my indebtedness to Dr. Gustav Mann for his constant advice and help.

DESCRIPTION OF PLATE 22,

Illustrating Lily H. Huie's paper on "Further Study of Cytological Changes produced in Drosera."

All figures were drawn with Zeiss's camera lucida, Zeiss's $\frac{1}{12}$ apochromatic immersion objective, and No. 8 compensating ocular. The magnification equals 2000 diameters. All cells represent apical gland cells.

FIG. 1.—The resting condition (control). Vide p. 205.

FIG. 2.—After paraffin shavings, one hour. Vide p. 205.

FIG. 3.—White of egg, five seconds. Vide p. 206.

FIG. 4.—10 per cent. amphopeptone, five seconds. Vide p. 207.

FIG. 5.—10 per cent. amphopeptone, five minutes. Vide p. 207.

FIGS. 6, 7, and 8.—10 per cent. amphopeptone, one hour. Vide p. 208.

FIG. 9.—10 per cent. amphopeptone, two days. Vide p. 209.

FIG. 10 *a*.—Fibrin, one hour. 10 *b*. Fibrin, one day. Vide p. 210.

FIG. 11.—Fibrin, two days. Vide p. 210.

FIG. 12.—Fibrin-peptone, one hour. Vide p. 211.

FIG. 13.—Milk, five minutes. Vide p. 211.

FIG. 14.—Milk, one to two days, usual type. Vide p. 212.

FIG. 15.—Milk, one to two days, occasional type. Vide p. 212.

FIG. 16.—Calcium phosphate, one day. Vide p. 212.

FIG. 17.—Globulin, one day. Vide p. 213.

FIG. 18.—Nuclein, one hour. Vide p. 214.

FIG. 19.—Nuclein, recuperating one day. Vide p. 215.

FIG. 20.—Nucleic acid, five minutes. Vide p. 215.

FIG. 21.—Nucleic acid, one hour. Vide p. 215.

FIGS. 22 and 23.—Nucleic acid, one day. Vide p. 215.

FIG. 24.—Creatin, one to two days. Vide p. 217.

FIG. 25.—Leucin, three days. Vide p. 217.

FIG. 26.—Urea, one hour. Vide p. 218.

FIG. 27.—Urea, two days. Vide p. 218.

Remarks on some Recent Work on the Protochorda, with a Condensed Account of some Fresh Observations on the Enteropneusta.

By

Arthur Willey, M.A., D.Sc.

The eighth volume of the well-known 'Traité de Zoologie Concrète,' by Professor Yves Delage and M. Edgard Hérouard, published in 1898, is devoted to what the authors style the Procordés or Prochordata, a group which is made to include three classes, namely, Hemichordia, Cephalochordia, and Urochordia. It is a pleasure to turn to a text-book in which the Enteropneusta are treated on equal terms with the Cephalochorda and the Urochorda, especially when, as in this case, the distinguished authors have aimed at impartiality—a quality which in one or two places has led them beyond the bounds of discrimination. It goes without saying that the work is an excellent one, and admirably calculated, on the whole, to give a just idea of these animals; but on the present occasion I am neither concerned with its many excellences nor with its few blemishes, but merely with the subject-matter.

No doubt the classification employed by the authors of this text-book answers not only their purpose, but that of their readers also. At the same time it should be borne in mind that it is in no sense a final classification, nor even one which corresponds to the present state of our knowledge. By not including the Pterobranchia in the same volume with the

Enteropneusta, the authors have neglected the opportunity of pointing an interesting and instructive analogy.

What the Urochorda are to the Cephalochorda, such are the Pterobranchia to the Enteropneusta.

The perpetual domination of the notochord in classification constitutes a noteworthy example of the manner in which zoological knowledge moves along well-worn grooves. There is strong reason to suppose that the gill-clefts have the priority of the notochord, or at least equal antiquity with it; and if this supposition should prove to be correct in principle, there ought to be some indication of it in the classificatory system.

I have treated this subject in some detail in a memoir on the Enteropneusta collected by me in the South Pacific, which will shortly be published;[1] and the following is a simplified form of the table of classification there constructed.

PHYLUM BRANCHIOTREMA, n. n.

I. HEMICHORDA, Bateson, 1884.

Class 1. Pterobranchia, Lankester, 1885.
Class 2. Enteropneusta, Gegenbaur, 1870.

II. PROTOCHORDA, Balfour, 1882.

Class 1. Urochorda, Lankester, 1877.
Class 2. Cephalochorda, Lankester, 1877.

III. VERTEBRATA,[2] Lamarck and Cuvier.

Class 1. Acrania, Haeckel, 1866.
Class 2. Craniota, Haeckel, 1866.

In the above system the group containing Amphioxus appears under two different names, Cephalochorda and Acrania. I see no objection to this procedure, nor any other way out of the difficulty.

[1] In Part iii of A. Willey's 'Zoological Results' (Cambridge University Press).

[2] Vertebrata = Holochorda, Gadow, 1898.

The justification for the new collective name, Branchiotrema, is contained in my forthcoming memoir, where it is introduced in connection with a new theory of gill-clefts, to which I have been led by my study of the Enteropneusta. This theory may be barely and briefly stated as follows:

The gonads and gill-slits were primarily unlimited in number and co-extensive in distribution, the gonads having a zonary disposition and the gill-slits occupying the interzonal depressions.

The primary function of the gill-slits was the oxygenation of the gonads, their secondary function being the respiration of the individual—the change of function having taken place pari passu with an elaboration of the vascular system.

Correlatively with the progressive regional differentiation of the body, the gonads and gill-slits became limited both anteriorly and posteriorly. The anterior limitation of the gill-slits behind the collar region is constant in all Enteropneusta, but the posterior limitation is excessively variable.[1] The emancipation of the gonads from their topographical relations with, and functional dependence on, the gill-slits has taken place in several ways, but the resultant tends to be, and eventually actually is, the restriction of the gonads to a post-branchial genital region.

Such, in outline, is the theory to which I have committed myself. The Harmer-Brooks-Masterman theory of gill-clefts does not, in my opinion, account for their prime origin, but it does perhaps explain the retention of a single pair of gill-clefts in forms like Cephalodiscus and Appendicularia.

In recording the fact that Spengel divided the species of Enteropneusta into four genera, MM. Delage and Hérouard prefer to describe them "en bloc sous leur ancienne dénomination commune." This conservative method of treatment is somewhat foreign to the general spirit of the book, since an endeavour has been made to incorporate the results contained in the most recent publications. As a consequence, some

[1] Both anterior and posterior limits of the gonads are variable.

structures of first importance are relegated to foot-notes, e. g. the genital pleuræ (p. 5) and roots (p. 45) of Ptychoderidæ.

In a paper published in this Journal,[1] which our authors have overlooked, I have given reasons for supposing that a form like Ptychodera flava, in which the gill-slits open freely to the exterior and not first into gill-pouches, represents the most primitive existing type of Enteropneusta. Speaking from personal experience, I may at least say that this species has opened my eyes as to the significance of the enteropneustic organisation.

The figure of Ptychodera clavigera, given on pl. ii, facing page 64, is apt to be misleading. The pharynx in this figure appears to stand boldly forth as a cylindrical tube at the base of the open chamber formed by the arching genital pleuræ; and the parallel arcuate lines have the appearance which is actually presented by the true gill-bars in Ptychodera flava. The gill-bars of Pt. clavigera are, however, quite invisible externally, the genital pleuræ have a dorsal origin, the pharynx does not project beyond the level of the floor of the peribranchial space,[2] and the gill-pores are extremely small, lying at the base of the narrow branchial grooves.

The authors have very naturally followed Spengel in their explanation of the lateral septa of the Ptychoderidæ, as being the outer walls of a pair of cœlomic diverticula (p. 23, foot-note).

The prolongations of the truncal cœlom into the collar region (viz. perihæmal and peripharyngeal cavities) are intelligible facts; but how the truncal cœlom could project a portion of itself into itself was a mystery to my mind until I realised that there is no question of a diverticulum at all.

[1] A. Willey, on Ptychodera flava, Eschscholtz, 'Quart. Journ. Micr. Sci.,' vol. 40, 1897, p. 165.

[2] Excepting that the branchial tract or gill-area, i. e. the area enclosed within the branchial grooves is somewhat arched. The blue-lined structure in the figure above referred to is simply the gill-area or Kiemenfeld of Spengel.

The fact that Ptychodera flava clears away this difficulty is alone sufficient to entitle it to be regarded with particular respect. This remark no doubt applies to the sub-genus Chlamydothorax to which Pt. flava belongs.

In most Ptychoderidæ the lateral septa, as described by Spengel, have a limited anterior extension; the point of their proximal or mesial origin from the basement membrane gradually approaches that of their distal insertion into the same membrane until the two points coincide; and so the lateral septum on each side comes to an end in the posterior branchial region. In this way there actually exists a portion of the cœlom, bounded mesially by the dorsal mesentery[1] and laterally by the lateral septum, which ends blindly in front; and as long as this was all that was known on the subject there was perhaps no other alternative than to propound some such formal explanation as that put forward by Spengel.

In Ptychodera flava the lateral septa do not come to an end in the posterior branchial region, but they are co-extensive, in front and behind, with the genital pleuræ. It can therefore hardly admit of question that the genital pleuræ and lateral septa are causally related to one another.

Where the genital pleuræ are at their maximum the lateral septa are entire. As the genital pleuræ have become reduced, the reduction always taking place from before backwards, the the lateral septa have been subjected to the same process of limitation, and exhibit the effects of it in a more marked manner.

MM. Delage and Hérouard do not devote much space to the difficult subject of excretion in the Enteropneusta, being content to state that the essential organ of excretion is the glomerulus which forms part of the central complex of the proboscis, while the excretory products are said to be got rid of through the proboscis-pore.

[1] This holds good only for the post-branchial region. In front of the last gill-cleft on each side, the proximal origin of the lateral septum is transferred from the wall of the gut to the basement-membrane of the epidermis.

Bateson[1] found granules in nearly all the mesoblastic tissues, and he says (p. 526) "they may perhaps be excretory, and it is possible that they are more or less removed by the proboscis-pore and collar-funnels respectively. This does not explain their presence in large masses in the trunk body-cavity, from which no pore has been observed to open."

In the first place it should be remembered that it is not absolutely necessary that excretory products must be removed from the body; this is shown in the Ascidians, where there are no excretory ducts. The so-called pericardium (Herzblase) of the Enteropneusta which lies in the centre of the glomerulus, i.e. between the two halves of the latter, appears, from the curious way in which its endothelium proliferates into the cavity (to such an extent as sometimes to completely block up the cavity), to stand in functional as well as in topographical relation to the glomerulus. If this is so, it would, in its capacity as a closed sac associated with the renal function, be physiologically comparable to the organ of Bojanus of the Molgulidæ.

The glomerulus of the Enteropneusta is, so far as our present knowledge goes, a structure sui generis, and it is quite clear that it is the principal organ of excretion only in virtue of its having superseded something else, namely the paired excretory canals.

The proboscis-pores are highly variable; the collar-pores are constant; but neither the former nor the latter are any longer mere excretory pores. The collar-pores especially seem to promote locomotion by taking in water, and so causing the collar to swell (Spengel); this may happen also in the case of the proboscis-pores sometimes, but not always.

I have observed what I believe to be the vestiges of a pair of truncal canals and pores in two species of the genus Spengelia. In both Sp. porosa and Sp. alba, n. sp., there is a pair of canalicular extensions of the first pair of gill-pouches into the posterior end of the perihæmal cavities close to the level at which the latter pass into the truncal cœlom. They occur approximately at the same level as the collar-canals,

[1] 'Quart. Journ. Micr. Sci.,' vol. 26, 1886.

which likewise arise as canalicular extensions of the first gill-pouch into the collar cœlom on each side.[1] These truncal canals of Spengelia are such definite structures that I was for a long time perplexed as to their significance.

I have referred above to the fact that the collar-canals are actively functional; their walls consist of richly ciliated columnar epithelium, and they retain a uniform calibre from their external orifice to the wide semilunar funnel by which they open into the collar cœlom. The truncal canals, on the contrary, taper towards their internal ends, their walls contain ill-defined mucous cells, and, in short, they distinctly appear to be in a vestigial condition. The perihæmal prolongations of the truncal cœlom usually contain merely virtual cavities; in other words, their cavities are quite blocked up with muscular and connective tissue. This is the case in Sp. porosa, whereas in Sp. alba a true space appears in the posterior portion of the perihæmal cavities, namely, in the region in which the truncal canals occur.

I can neither state positively that there is an internal opening nor that there is not; one thing only is certain, namely, that the truncal canals are there. In Sp. porosa they are longer than in Sp. alba, but they present more the appearance of vestigial structures in a chronic state of mucoid degeneration in the former species than in the latter.

A minute terminal pore is always difficult to find in transverse section, or even in any kind of section, and it will be remembered that there was the same difficulty in the case of the atrio-cœlomic or brown funnels described by Professor Lankester in Amphioxus.

If the truncal canals of Spengelia[2] and the brown funnels

[1] This is Spengel's view. Morgan says the collar-pore and first gill-slit arise coincidently. I do not think this affects the present question. Bateson describes the collar-pores in B. kowalevskii as arising as thickenings of the outer "atrial" wall which become perforated. The so-called atrial cavities, formed by the overhanging lateral margins of the collar, are peculiar to B. kowalevskii (Spengel).

[2] It need be no cause for surprise that these structures only occur in one genus of Enteropneusta. Each species of Enteropneusta may and

of Amphioxus be regarded as vestigial structures, the importance of their possession of an internal opening is diminished.

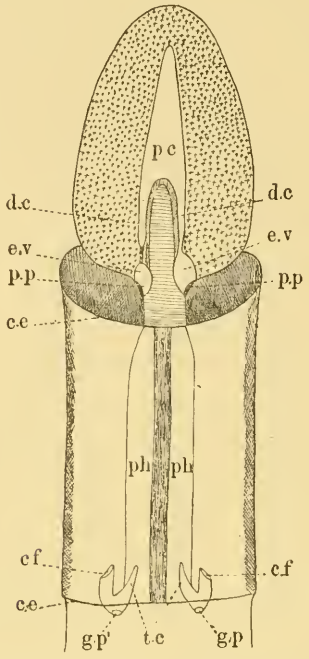

Fig. 1.—Diagram of anterior end of an Enteropneust, to show the regional canals and pores. The proboscis-pores are indicated as they sometimes occur in Ptychodera flava; the collar and truncal pores as in Spengelia. c. e. Anterior and posterior margins of collar. c. f. Collar funnels. d. c. Dorsal canals of proboscis cœlom. e. v. End vesicles of proboscis (Eichelpforten). g. p'. First gill-pores (only the most dorsal portion of the first gill-pouch is indicated). p. c. Proboscis cœlom. ph. Perihæmal cavities. p. p. Proboscis-pores. t. c. Truncal canals (opening with the collar canals into the first gill-pouch).

In Ptychodera flava there are always two proboscis-pores, one of which (either the right or the left) is smaller than the other, and its terminal vesicle is usually not in

usually does present peculiar vestiges of structures which were presumably associated together in the ancestral forms.

communication with the proboscis cœlom (see Fig. 1). Thus the internal opening of the proboscis canal (end vesicle or Eichelpforte) is lost, while the external opening remains; and it is probably a general rule that when these regional pores change their function, or lose their function and become vestiges, one of the first things to happen is likely to be the closure of the internal or cœlomic opening.[1]

In accordance with the above considerations, I regard the truncal canals of Spengelia and the atrio-cœlomic funnels of Amphioxus as the vestiges of a pair of functional truncal pores, which were homodynamous with collar-pores and proboscis-pores. It is therefore of great interest to point out that in Amphioxus there are also traces of the other regional pores.

[1] As I have referred to this loss of the internal opening of the end vesicle of the proboscis canal, I will briefly state here what I believe to be some of the potentialities of this structure.

i. The proboscis-pore is frequently well in front of the anterior neuropore.

ii. Sometimes it is closely associated with the neuropore.

iii. Sometimes it opens into the medullary tube of the collar behind the neuropore.

iv. Frequently the end vesicle is prolonged behind the pore, as a cæcal follicle lying below the medullary tube.

v. Combining what sometimes happens into one phenomenon, we see the neuropore leading into the medullary tube, and a subneural organ opening into the latter.

vi. The entire medullary tube of the collar of Enteropneusta corresponds to the cerebral vesicle only of Amphioxus and of the Ascidian larva. The spinal cord is represented in Enteropneusta by the dorsal nerve which lies in the skin, and is not closed in.

vii. The subneural gland of the Ascidian larva opens by the neuropore into the dorsally placed mouth, and at the other end into the cerebral vesicle.

viii. The inner or cerebral opening of the subneural gland of the Ascidian larva is thus seen to correspond to the proboscis-pore, which has lost all relation to the cœlom.

ix. Hence the peculiar mode of development of the tunicate subneural gland is explained, and the apparent absence of a proboscis-pore in the tunicate larva is accounted for.

x. The roots of Ptychoderidæ are related to the epiphysial complex of the thalamencephalon of Craniota.

The structure in the larva of Amphioxus known as Hatschek's nephridium,[1] which opens at one end into the buccal cavity, has been shown by MacBride[2] to be, at an early stage, in open primary communication at its other end with the left archenteric pouch, which he has suggestively named the left collar-cavity. In spite of differences in the method of development, I regard Hatschek's nephridium as being in principle the vestige of a pair of collar canals.

Bateson tentatively compared the collar-pores of the Enteropneusta both to Hatschek's nephridium and to Lankester's brown funnels. The comparison of the enteropneustic proboscis-pore with the orifice of the amphioxine præoral pit is of old standing, and likewise originated with Bateson, who further compared them both to the craniate pituitary body, without carrying the comparison into any great detail.

In the Enteropneusta the excretory function of the regional pores has been superseded by the specialisation of the glomerulus; in Amphioxus by the evolution of the nephric tubules which were discovered by Weiss and Boveri.

It may indeed be said that in the Enteropneusta the primordia of the nephric tubules are present in the form of a minute diverticulum at the dorsal medial angle of each gill-pouch, or in a corresponding position in those cases where the gill-pouches are confluent, as in Pt. flava. These structures are particularly well seen in sections through Spengelia alba. Whether this be so or not there is undoubtedly a special significance in the remarkable fact that Boveri's tubules are precisely co-extensive with the gill-clefts, and a renewed importance should be attached to the connecting vessels observed by Paul Mayer between dorsal aorta and sub-intestinal vein in embryos of Pristiurus, which were shown by Rückert to occur in the same segments with the pronephric tubules and to furnish the latter with rudimentary glomeruli.

[1] If I understand them aright, MM. Delage and Hérouard have completely misunderstood this structure (p. 121, foot-note).

[2] E. W. MacBride, "The Early Development of Amphioxus," 'Quart. Journ. Micr. Sci.,' vol. 40, 1897, p. 589.

If Boveri's tubules open the way to a perception of the subsequent potentialities of the excretory system, Lankester's brown funnels, Hatschek's nephridium, and the præoral pit furnish a clue to its past history.

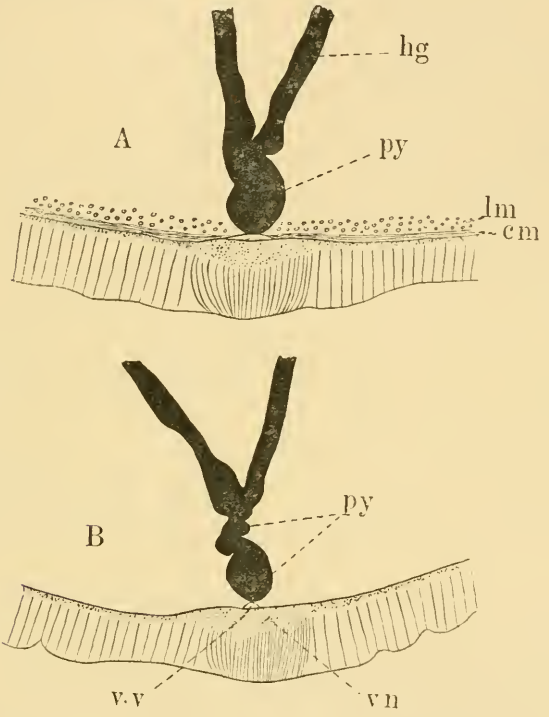

Fig. 2, A and B.—Portions of transverse sections through the caudal region of Ptychodera ruficollis, n. sp. A. Through the anterior caudal region. B. Through the mid-caudal region. cm. Circular muscles of body-wall. hg. Wall of hind gut. lm. Longitudinal muscles. py. Pygochord. vn. Ventral nerve-cord. vv. Ventral blood-vessel.

The substitution of nephric tubules in the truncal region for regional pores in the archimeric (Masterman) regions, which is displayed before our eyes in Amphioxus, is one of the most striking examples of the working of the principle of substitution that I can call to mind.

MM. Delage and Hérouard retain the designation notochord applied by Bateson to the diverticulum from the throat which projects into the proboscis, where it acquires a rigid consistency and sustaining properties. I prefer to call this structure by a non-committal name, and propose the term stomochord. The stomochord is not the only skeletal product of the gut wall in the Enteropneusta. There is another structure which finds no mention in the text-book under consideration, but which is hardly second in interest to the stomochord itself. It occurs along the entire length of the hind gut in the caudal region on the ventral side in many Ptychoderidæ; it is a solid keel-like, ribbon-shaped band, with dilated distal border abutting upon the ventral blood-vessel, and united at its dorsal edge with the median ventral epithelium of the gut.

This structure, which I propose to call the pygochord, was first seen by Spengel in Pt. minuta, then by Hill in Pt. hedleyi, and I have found it in Pt. flava, Pt. carnosa, n. sp., and Pt. ruficollis, n. sp.

In Spengelia alba, n. sp., it appears to be represented by a vacuolar thickening of the ventral epithelium, which, however, retains its epithelial position, and is not drawn out into a band.

I do not think that the enteropneustic stomochord corresponds to any definite part of the true notochord. The præoral extension of the notochord, far beyond the anterior limit of the neural tube in Amphioxus, is due to a forward growth of the notochord as such; whereas the præoral position of the stomochord in the Enteropneusta is due to a forward projection of a portion of the collar-gut or throat. Spengel calls it the Eicheldarm, but although he intended this name to be indifferent, it is capable of misleading interpretation, since it does not belong to the proboscis at all in its primary quality of integral constituent of the gut, but only in its secondary quality of a skeletally metamorphosed derivative of the gut. Moreover, whereas the notochord is essentially a uniform, single, indivisible structure,[1] the stomochord exhibits strongly

[1] The anterior tip of the notochord presents certain properties which may

marked regional differentiation (Fig. 3). It is therefore not sufficient to say that any structure in other forms is comparable to the enteropneustic stomochord, but it must be specified which portion of this structure is referred to.

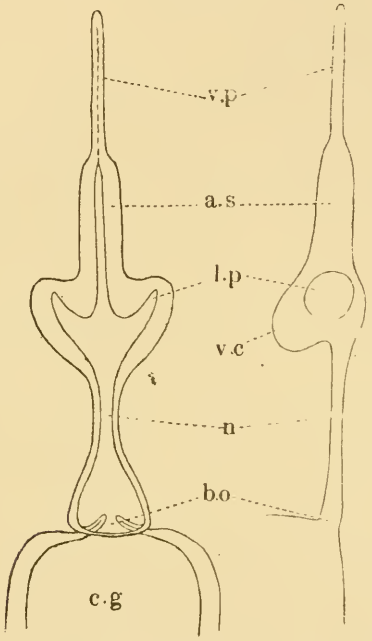

Fig. 3.—Diagram of a complete stomochord of an Enteropneust from the dorsal and lateral aspects, to show its regional differentiation. v.p. Vermiform process. a.s. Anterior region of body of stomochord. l.p. Lateral pouch. v.c. Ventral cæcum. n. Nuchal region. b.o. Buccal orifice of stomochord. c.g. Throat or collar-gut.

The lumen of the stomochord is highly variable; it may safely be said that it is not the same in any two individuals of a species. Sometimes it is in a fragmentary condition, some-

have an atavistic significance, but this does not affect my general proposition. The most that could be looked for in the embryos of higher Chordata would be a vestige of the buccal orifice of the stomochord, and perhaps this does occur.

times it is locally obsolete, and sometimes it is unusually capacious. In all cases it is patently vestigial. In more than one of my species (e. g. Pt. carnosa, n. sp.) the stomochord undergoes fragmentation in the nuchal region, owing to the invasion of skeletal substance. In Balanoglossus canadensis Spengel found that the entire nuchal region of the stomochord was lacking.

The structure of which the stomochord, in its capacity as a portion of the gut, is a vestige, must originally have been post-oral,[1] and I have convinced myself that the structures recently described by Masterman [2] in the Actinotrocha of the Bay of St. Andrews are capable of being explained on this basis.

In its middle region the stomochord is greatly dilated, both dorso-ventrally and laterally. Spengel only emphasises the ventral cæcum of the stomochord in this region; but often there is a pair of lateral pouches which are particularly well-marked. In some cases the lateral pouches are less pronounced than the ventral cæcum, and sometimes the exact reverse is the truth. Sometimes the lateral pouches do and sometimes they do not unite with one another across the middle line by the intermediation of the ventral cæcum.

The interest of the situation has been increased rather than diminished by the recently published observations of Roule on the Actinotrocha of Phoronis sabatieri. Instead of the paired lateral diverticula or pleurochords described by Masterman, there is in Roule's larva a single median anterior ventral diverticulum, whose cells likewise undergo vacuolar degeneration, which gives it a semi-rigid consistency. According to Roule this ventral diverticulum arises at the anterior end of the intestine (in the same region as Masterman's pleurochords), and projects forwards ventrally below the œsophagus.

[1] I think it is universally admitted that the stomochord is a secondary projection into the proboscis cavity pushing the cœlomic epithelium or splanchnotheca of Spengel before it.

[2] A. T. Masterman, "On the Diplochorda," 'Quart. Journ. Micr. Sci., vol. 40, 1897, p. 281.

Thus in Masterman's pleurochords we have, as I believe, the representatives of the lateral pouches of the enteropneustic stomochord; while in Roule's ventral diverticulum we have the representative of the ventral cæcum of the stomochord.[1]

With regard to the structure in Actinotrocha which Masterman homologises with the so-called notochord described by Harmer in Cephalodiscus and by Fowler in Rhabdopleura, but which he (Masterman) labels "subneural gland," it is not easy to suggest a final explanation. Harmer[2] has pointed out its resemblance to the vermiform process of the stomochord which occurs in the Spengelidæ, and it may no doubt be compared with this structure.

What is doubtful is its primordial significance. As a first step in the discussion, the name arbitrarily given to it by Masterman should be dropped, because it involves a simple begging of the question.

The following proposition may therefore be stated categorically:—The "notochord" of the Pterobranchia represents the vermiform process of the stomochord of the Enteropneusta (Spengelidæ); but there is not sufficient evidence upon which to found an opinion as to its antecedent history.

In the process of regional differentiation of the entire body, which is such a characteristic feature of the Enteropneusta, the part of this influence devoted to cephalisation has, as has been already mentioned, led to serious changes at the anterior end

[1] The above view gives an adequate explanation of Roule's ventral diverticulum sufficient at least to show that the extraordinary theoretical excursion which this author makes is quite superfluous. Roule compares Actinotrocha directly with the Vertebrates because the Enteropneusta are "quelque peu aberrant." As a fact there is, in my opinion, reason to suppose that the Enteropneusta are even nearer the direct line of Craniate descent than Amphioxus. To set aside the Enteropneusta as aberrant forms argues ignorance of the group.

Reference.—Louis Roule, "Sur la place des Phoronidiens dans la classification des animaux, et sur leurs relations avec les Vertébrés," 'C. R.,' t. cxxvii, 1898, p. 633.

[2] S. F. Harmer, "On the Notochord of Cephalodiscus," 'Zool. Anz.,' 1897, p. 342. (Masterman's reply thereto, ibid., p. 443.)

of the trunk. Not only does a portion of the gut become projected into the proboscis, with the result that its lumen has become vestigial and its walls rigid, but gill-slits have been abolished from the anterior portion of the gut which lies in the collar region.[1] Masterman's pleurochords lie in the collar or lophophoral region, and from his writings [2] they appear to be vestiges of the gill-clefts which still persist in Cephalodiscus.

As we have seen, the stomochord of the Enteropneusta is a derivative of the collar-gut, and retains vestiges of structures formerly serving another function in the post-oral collar region. Thus we may conclude, in accordance with the preceding considerations, that the pleurochords of Actinotrocha, the gill-clefts of Cephalodiscus, and the lateral pouches of the enteropneustic stomochord are the persistent vestiges of primitive gill-clefts belonging to that portion of the body which, in the Enteropneusta, is now specialised as the collar region. The great series of truncal gill-clefts is entirely lacking in the sessile forms, just as in the Ascidians there are strong grounds for the interpretation of the numerous branchial stigmata as having originated by the subdivision of a single pair of gill-slits[3] which persist in their undivided condition in Appendicularia.[4]

[1] In Amphioxus the first larval gill-slit closes up.

[2] A. T. Masterman, "On the Further Anatomy and the Budding Processes of Cephalodiscus dodecalophus," 'Trans. Roy. Soc. Edin.,' vol. xxxix, 1898, p. 507.

[3] From the mode of origin of the primary branchial stigmata in Ciona intestinalis I thought three primary gill-clefts were represented in the Ascidians, but a study of their formation in Molgula manhattensis convinced me that such an interpretation could not be upheld; and on this point I modified my views, and am now disposed to recognise the truth of Van Beneden and Julin's hypothesis as to the presence of one pair only of primary slits (see A. Willey, 'Amphioxus and the Ancestry of the Vertebrates,' 1894, p. 232).

[4] It follows from what has gone before that the anterior portion of the body of the stomochord in Enteropneusta, that is the part intervening between the vermiform process (when present) and the region of the cæcal pouches (Fig. 3, *a. s.*), corresponds to the functional œsophagus of Actinotrocha; not that Actinotrocha is itself an ancestral form, but it appears to

A word is necessary as to the development of the Enteropneusta and the significance of the direct and indirect methods of development. In the text-book before us the authors consider the direct development as the more typical. This may be so in a certain sense, but it is necessary to bear certain facts in mind.

Spengel showed clearly that the Enteropneusta are divisible into three families, but he only named one of them, namely, the Ptychoderidæ. I propose to call the other two families the Spengelidæ and Balanoglossidæ respectively. Spengel was of the opinion that the Balanoglossidæ comprise the most primitive forms, and the Ptychoderidæ the highest or least primitive forms. As I have before stated, I hold the Ptychoderidæ to be the primitive family, emphatically not the Balanoglossidæ; I think the anatomy of Ptychodera flava shows this conclusively.

The Balanoglossidæ are all northern forms (White Sea, Greenland, Canada, Massachusetts), with relatively large eggs (from $\frac{1}{3}$ to $1\frac{1}{3}$ mm. in diameter), and from the size of the egg alone we are justified in concluding that they develop directly in the manner described by Bateson in B. kowalevskii. The Ptychoderidæ[1] have small eggs (rarely more than $\frac{1}{10}$ mm. in diameter) which develop into a Tornaria larva, i. e. indirectly.

have retained some of the characters of a primitive creature, just as the Ascidian tadpole retains primitive features which have quite disappeared from the larva of Amphioxus.

The difficulty will naturally arise as to how the portion of the primitive gut, represented in the stomochord, could have been projected past the mouth. I do not think we are obliged to make an obstacle of this difficulty. The principle of segregation will temporarily account for it. That segregation has taken place is shown by the origin of such a complex structure as the stomochord from a simple primordium (the mode of development of the vermiform process of the stomochord in Spengelidæ is unknown).

There is so much on the surface which demands explanation that I have ventured on dangerous ground in the endeavour to collate the various facts.

[1] Probably also the Spengelidæ (Schizocardium, Spengelia, Glandiceps).

It is therefore a striking fact that the more primitive forms have the indirect method of development. There is probably a special significance in this seeming paradox, and in order to get at the meaning of it, it is important to call to mind a parallel case. The difference in the method of development followed by Peripatus capensis and by Peripatus novæ-britanniæ is, allowing for the intra-uterine environment, precisely the difference between direct and indirect development. The egg of P. capensis (·5 mm.) is about five times as large as the egg of P. novæ-britanniæ, and there are not wanting anatomical features which point to the more primitive character of the latter species.

Thus both in the Enteropneusta and in the Onychophora the most primitive forms pass through an indirect development; and in both cases it is the indirect development which yields information about the proximal relationships of the respective groups; while the direct development apparently instructs us in the matter of the primordial significance of the organisation (cf. blastopore of P. capensis and body-cavities of B. kowalevskii).

In their treatment of the development of Amphioxus, MM. Delage and Hérouard expose themselves to criticism at several points. They have apparently overlooked the works of Van der Stricht and Sobotta,[1] especially the latter, in which the question of the polar bodies of Amphioxus is practically settled. These authors found that the first polar body is extruded while the egg is still inside the ovary, and a portion of the egg-membrane is constricted off with the first polar body, so that the latter comes to lie quite outside the membrane. When the latter springs away from the egg at the time of fertilisation,

[1] Van der Stricht's paper, "La maturation et la fécondation de l'œuf d'Amphioxus lanceolatus" ('Arch. de Biol.,' xiv, 1895, p. 469), is quoted in the bibliography at the end of the work, but Sobotta's latest important paper on this subject is not quoted; perhaps it is too recent. J. Sobotta, "Die Reifung und Befruchtung des Eies von Amphioxus lanceolatus," 'Arch. f. mikr. Anat.,' vol. l, 1897, p. 15.

the first polar body is removed from the surface of the ovum and so is lost.[1]

In their account of the later development, the authors of the Traité have been seriously led astray by the recent work of R. Legros.[2] I regret to say that this author has produced a paper of a highly destructive character, from which it would appear, to the uninitiated, that his predecessors are incapable observers. As one of his principal results he seeks to show, by transverse sections through embryos preserved in a sublimate-acetic mixture, that the præoral pit, which is such a distinctive feature of the larva of Amphioxus, arises as a solid ectodermal proliferation which subsequently hollows out.

Hatschek's account of the origin of the præoral pit from the left head-cavity has recently been confirmed by MacBride (loc. cit.) by transverse sections through embryos preserved in osmic acid.

Hatschek's nephridium, according to Legros, arises as an outgrowth from the alleged ectodermal præoral pit, and the whole apparatus is subjected to an obvious and well-fitting comparison with the hypophysis of Ammocœtes, and hence with the hypophysis of Craniates in general; in fact, he makes it identical with the hypophysis of Ammocœtes, thus leaving no room for change of function nor for evolution.

The orifice of the præoral pit of Amphioxus, considered as a cœlomic cavity opening to the exterior, has generally been supposed to be related to the proboscis-pore of the Enteropneusta.[3] From what has been said above (p. 231) it follows that the præoral pit, like the mouth, has quitted its

[1] Sobotta describes two membranes round the egg, an inner and an outer, but makes no reference to the follicular membrane described and figured by Langerhasn.

[2] Robert Legros, "Développement de la cavité buccale de l'Amphioxus lanceolatus. Contribution à l'étude de la morphologie de la tête," 'Archives d'Anatomie microscopique,' tome i, No. 4, 1897; and tome ii, No. 1, 1898.

[3] For full treatment of this difficult subject see my memoir entitled 'Enteropneusta from the South Pacific, with Notes on the West Indian Species,' now in the press; Part iii, Zool. Results.

primary association with the neuropore, the notochord intervening. I knew this had happened in the case of the mouth, my views on this point being acceptable to MM. Delage and Hérouard, but I had not, until recently, realised that a similar change had affected the præoral pit.

It will therefore be seen that Legros has probably touched upon the fringe of a fundamental truth, so far as the morphology of the præoral pit is concerned, although led thereto by erroneous premises; moreover the same truth was broached by Bateson, to whose work the author makes no reference.

The contradictory result as to the origin of the præoral pit arrived at by Legros would, if true, very seriously discredit the work of Hatschek, but it has nevertheless been well received, Klaatsch,[1] for example, ingenuously and uncritically rejoicing at the "Correctur der Hatschek'schen Angabe;" and it has been adopted by MM. Delage and Hérouard.

Hatschek's discovery of the conversion of the left head-cavity into the præoral pit, which has been confirmed, let it be repeated, by MacBride, was a matter of unbiassed observation, and must have come as a serious shock to his sense of cœlomic propriety. To contradict Hatschek on this point in ignorance of the living transparent embryos, and to base the contradiction entirely upon sections through material preserved in sublimate and acetic, is surely very rash. The Belgian author has obviously, in this matter, been a victim of the microtome, but it is to be feared that his results will more or less dominate the subject throughout the next decade, since they have already found a home in a leading treatise.

The external orifice of the club-shaped gland is a minute pore below the anterior end of the larval mouth; it is invariably to be seen in all living larvæ before the metamorphosis, but is not always easy to find in transverse section. Of course Legros does not find it, and he denies its existence.[2]

[1] Hermann Klaatsch, "Ueber den Bau und die Entwickelung des Tentakelapparates des Amphioxus," 'Verh. Anat. Ges. (Anat. Anz.),' 1898, p. 184.

[2] See also E. Ray Lankester and A. Willey, "The Development of the

In his remarks dealing with the larval mouth he attempts to set aside my work on the later larval development altogether; but he does not refer to a single figure of mine, so that I do not know whether he doubts the accuracy of all of them or only of some.

During the transition from the lateral larval mouth to the median adult velum the mouth maintains its integrity, but alters its shape and rotates through an angle of 90°.

Legros wastes pages of ink in denying this rotation, i.e. in denying a self-evident fact.

He goes on to say that the larval mouth does not, in its entirety, become converted into the definitive mouth, but only its anterior portion; the posterior portion closes up by fusion of the lips; and the evidence which he brings forward in support of this assertion is neither furnished by section nor by direct observation, but by measuring the relative distance of the posterior angle of the mouth from the tip of the snout. It is quite true that this distance becomes somewhat shorter, a fact which my figures and description render completely intelligible by the change of shape and position which the mouth undergoes. No soldering of the lips whatever takes place, and to assert that it does so on the evidence which Legros adduces is mere trifling.

The Ascidians naturally take up the most space in this volume of the Traité, and the treatment which they receive on the whole leaves little to be desired. In the account of budding in the Botryllidæ the authors follow Pizon's work almost entirely. On many points Pizon's results are in opposition to the work of Hjort. It is a subject which requires more investigation.

The volume concludes with a useful summary of facts and of theories relating to the origin of the Vertebrata, from which I will make one quotation only. On the subject of the præ-

Atrial Chamber of Amphioxus," 'Quart. Journ. Micr. Sci.,' vol. 31, 1890, where the external orifice of the club-shaped gland is figured both as seen in toto and as seen in section.

oral lobe (p. 315) the authors say, "Chez les Tuniciers adultes, il n'existe rien de tel, mais chez leur larve on retrouve des dispositions tout à fait comparables à celles de l'Amphioxus."

I have been treated to harsh words for holding the view that the organ of fixation of the Ascidian larva represents the præoral lobe. It is therefore possibly a matter for satisfaction that this view finds favour with the authors, but I could have wished that they had been more explicit. It may be worth while to add, in order to ward off possible misunderstanding, that for my own part I am more than ever convinced of its essential truth.

Klaatsch (loc. cit.) has recently made the suggestion "mit allem Vorbehalt," "dass das kolbenförmige Drüse [club-shaped gland of the larva of Amphioxus] die Anlage des Tentakelskelets darstellen könnte."

This is an astounding suggestion to make, and it will not survive criticism. The skeletal elements of the buccal cirri commence to appear long before the disintegration and consequent disappearance of the club-shaped gland.[1]

[1] I venture to refer Dr. Klaatsch to my paper entitled "The Later Larval Development of Amphioxus," 'Quart. Journ. Micr. Sci.,' vol. 32, 1891; and to the figures on Pl. 15 accompanying the paper. He may there see for himself the vindication of what I have said above. If he doubts the accuracy of these figures, possibly in his next publication he will kindly inform us why he does so.

The Structure of Xenia Hicksoni, nov. sp., with some Observations on Heteroxenia Elizabethæ, Kölliker.

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With Plates 23—27.

Contents.

Introduction.

At the suggestion of Professor Hickson I undertook to examine a specimen of the Alcyonaceous coral Xenia, which he had collected on the reefs of Talisse Island, North Celebes, chiefly with the intention of working out the arrangement of the canals of the colony. As the investigation proceeded the preservation of the colony was found to be so exceptionally perfect that it seemed desirable to make a study of its histology, and as several new and interesting points were early observed I finally decided to work out in detail the complete anatomy and histology of the colony. Although many authors have described the external characters of various species of Xenia, few have paid any attention to their internal structure. Bourne (1895) has referred to the canal system, the mesoglœa, and the distribution of spicules in two species of Xenia, and in Heteroxenia Elizabethæ, and Kölliker (1874) described the anatomy of his new Heteroxenia Elizabethæ as far as its very imperfect preservation would permit. These two accounts contain practically the whole of our knowledge of the internal structure of these two genera, and hence, when the beautiful preservation of this colony of Xenia from Talisse was apparent, there was a strong inducement to attempt a more complete account of its detailed anatomy and histology.

The work has been carried on during the past two years in the zoological laboratories of the Owens College. My best thanks are due to Professor Hickson for the beautifully preserved specimen upon which most of my work has been done, and for advice and criticism given during the progress of the work. I am also indebted to Professor Lankester for a specimen of Heteroxenia Elizabethæ, to Mr. J. S. Gardiner for a specimen of Xenia from Rotuma, and to Dr. Arthur Willey for fourteen specimens of Xenia from various reefs in the Pacific.

External Characters of the Colony (Pl. 23).

The Xeniidæ are distinguished from all other Alcyonaria by their soft, fleshy consistency and non-retractile polyps. The

former character is due to the fact that their spicules are very minute rounded or oval discs, which have an organic basis impregnated with only a small quantity of calcium carbonate.

This colony arises from a single stem, which is slightly expanded at its point of attachment to the rock, and measures about 15 mm. in diameter at that point. This basal portion is very short and thick, and supports four main stems, all of which divide into two or more, producing altogether thirteen stems or branches ranging in length from about 10 mm. to 30 mm., and in breadth from about 4 mm. to 10 mm. The total height of the colony from the point of attachment to the tips of the highest polyps is 50 mm.

The polyps are, for the greater part of their length, bound together in bundles of about forty to sixty, each bundle forming one stem of the colony. The free portions of the polyps arise from the slightly expanded umbrella-shaped area at the distal end of each stem. Many of the polyps stand almost perpendicularly to the convex disc, but those near the edge of the disc hang downwards towards the base of the colony. The polyps are closer together near the edge of the umbrella, being here ·5 mm. to ·7 mm. apart, whereas in the middle of the umbrella they are 1 mm. to 2 mm. apart.

Polyps (Pl. 24, fig. 2).—The polyps, or, more correctly, the free portions of the polyps, are non-retractile and moderately long and slender. The tentacles are half to two thirds as long as the body of the polyp. Each tentacle bears on its inner side numerous short, conical elevations with rounded ends. These correspond to the pinnules found on the tentacles of other Alcyonaria.

The colour of the colony in spirit is light brown.

As mentioned above, the free portions of about forty to sixty fully developed polyps project from the umbrella-shaped area at the distal end of each stem, but besides these there are several younger polyps or buds in various stages of development, and these are invariably situated on the edge of the umbrella.

In fully developed specimens the following are the measurements:—"Body" of the free portion of the polyp 4 mm. to

7 mm. long, and 1·0 mm. to 1·2 mm. broad. Tentacles 2 mm. to 5·7 mm. long, and ·75 mm. broad. The total length of the adult polyps is thus 6 mm. to 12 mm.

The body of the polyp is cylindrical and its wall moderately strong. In several of the Xeniidæ the body-wall of the polyp is so weak that when the colony is taken out of spirit the polyps fall together into a mass. In this species, however, the body-wall is just strong enough to support the polyps in an upright position, so that on removing the colony from spirit the polyps do not hang limply, but remain standing approximately in their natural positions.

Tentacles and Pinnules (Pl. 24, figs. 2 and 3).—Each polyp bears eight tentacles, each of which is provided with numerous pinnules. The pinnules on each side of the middle line of the tentacle are arranged in three longitudinal rows, and they form also somewhat oblique transverse rows of three pinnules rising from the oral towards the aboral side of the tentacle (fig. 3, *D*). When the tentacle is viewed from the inner or oral aspect, all the pinnules are generally visible (fig. 3, *B*, *D*), but on the outer or aboral side of the tentacle only the outer longitudinal row of each side is, as a rule, seen (fig. 3, *A*, *C*). The pinnules are often clearly separated into the two series of three rows in each by a narrow area which extends along the middle line of the inner face of the tentacle, from the base to within a short distance of the tip (fig. 3, *D*). This area, free from pinnules, may be ·25 mm. across, and may often be traced to within 1 mm. of the tip of the tentacle. In other specimens, however, it is entirely obliterated, and the median pinnules of the two series are in contact with each other, at any rate at their bases. The width of this area varies, not only in separate individuals, but in the different tentacles of the same individual. These variations are probably due to the different degrees of contraction of the tentacles on killing, and the condition in which the free area is well marked is seen only in those tentacles which have been killed in an expanded condition.

At the tip of the tentacle the pinnules are smaller than those

in the middle. They are arranged more or less in two series, one on each side of the middle line of the tentacle, but three rows on each side are not distinguishable; at a distance of about 1 mm. from the tip of the tentacle, however, the typical arrangement of two series, with three rows of pinnules in each, is gradually assumed (fig. 3, *B*).

At the base of the tentacle the pinnules are much smaller but have the typical arrangement, except in the case of one or two of the proximal transverse rows. Here the pinnules appear to be in course of formation, the outer ones being formed first, the inner ones developing from without inwards (see fig. 3, *D*).

On looking at the tentacle from the outer side only the outermost row of pinnules is usually visible. These, which are about twelve to twenty in number on each side, are set close together and point towards the tip of the tentacle (fig. 3, *A*). At the tip of the tentacle the arrangement of the pinnules may be well studied, and, as in Alcyonium (Hickson, 1895), they are not paired (Pl. 24, fig. 2). The pinnules are conical elevations with rounded ends. Those in the middle portion of the tentacle are about ·5 mm. long and ·15 mm. to ·2 mm. broad, but those nearer the base and tip are smaller. The pinnules when fully expanded are about three times as long as they are broad, and each tapers gradually from its base to its blunt, rounded tip. When slightly contracted the pinnule is somewhat swollen at its base, and if further contracted becomes more swollen and globular at its base as its length decreases. Although the body of the polyp is non-retractile, the tentacles are often found slightly contracted, being in many cases curled inwards over the mouth. Several examples of tentacles in this condition are shown in fig. 1.

Diagnosis of the Species Xenia Hicksoni.

The species of Xenia are distinguished from each other by the general form of the colony, the size of the polyps and tentacles, the number of rows and shape of the pinnules, and the presence or absence of an area free from pinnules on the inner face of the tentacle.

After careful comparison with the accounts of all the hitherto described species, I am unable to refer this specimen to any of them, and therefore I have established for it a new species with the name Xenia Hicksoni. Its characters are as follow:

The colony consists of several cylindrical, usually branched stems, arising from a single thick stem or base. The stems range in length from 10 mm. to 30 mm., and in breadth from 4 mm. to 10 mm. From the arched or convex summit of each stem the free parts of the polyps arise. These are smaller and moderately close together near the edge of the summit, but larger and further apart in the middle of the arched end. The polyps (including tentacles) measure 6 mm. to 12 mm. in length, and 1 mm. to 1·2 mm. in breadth. The tentacles are moderately slender and 2 mm. to 5·7 mm. long. Each tentacle bears on the inner side two series of pinnules, each series consisting of three rows of twelve to twenty pinnules in each row. There is usually a narrow area free from pinnules extending along the middle line of the tentacle to within about 1 mm. of the tip. The pinnules are conical elevations with rounded ends. Those in the middle of the tentacle are about ·5 mm. long, and are about three times as long as they are broad; those nearer the base and tip of the tentacle are somewhat shorter. The body-wall of the polyps is moderately thick, and is strong enough to support the polyps in their natural position when the colony is removed from spirit. The spicules are round or oval discs measuring ·012 mm. to ·022 mm. in length, ·006 mm. to ·013 mm. in width, and about ·004 mm. in thickness. They are numerous in the ectoderm of the stem and of the body of the polyp, but are practically absent from the tentacles and pinnules.

Round the edge of the umbellate summit of each stem are a few buds or young polyps in early stages of development. The specimen is light brown in colour (in spirit).

Habitat.—The reefs of Talisse Island, North Celebes.

This species appears to differ from most, if not all other species of Xenia in the absence of spicules from the tentacles and pinnules. In general form of the colony this specimen

most resembles X. umbellata, Savigny, but it differs from the latter in possessing smaller polyps, with much shorter and stouter pinnules, which do not leave the axis of the tentacle free along its whole length (cf. Klunzinger, 1877, pl. 3, fig. 3*a*).

General Anatomy.

Stomodæum and Mesenterial Filaments.—In the centre of the oral disc of each polyp there is a funnel-shaped depression about one third of a millimetre in depth leading to the mouth. This depression is formed by partial contraction of the oral disc; if the polyp were fully expanded this depression would not exist, but the mouth opening would be level with the oral disc. The mouth (*Mo.*) leads into the stomodæum (*St.*), which is 1·8 mm. to 2·2 mm. long. The stomodæum is long compared with the length of the free portion of the polyp, and in longitudinal section presents a striking appearance, running down, as it does, so far into the cœlenteron. The stomodæum is oval in transverse section, being somewhat flattened from side to side. It has a well-marked ventral groove or siphonoglyph (*Si.*), the cells of the lower third of which bear long flagella (*F.*). The groove is not as well marked in the upper as in the lower portion of the stomodæum, and is scarcely discernible at the mouth opening. The columnar epithelial cells forming the siphonoglyph are, as is usual, longer than those of the rest of the stomodæum, and these cells bear very long flagella (·07 mm.), which in some examples extend almost to the centre of the cavity of the stomodæum. The epithelium of the rest of the stomodæum is smooth and not folded in any way. Many of these epithelial cells bear short cilia on the free surface, but among these are numerous cells (*G.*), which are, like goblet-cells, swollen or flask-shaped, due to the presence of some secretion to which they give rise. These cells generally appear to be empty, having discharged their secretion, which in some cases can be seen issuing from the cell into the cavity of the stomodæum (Pl. 26, fig. 18). These secreting cells occur chiefly in the

middle and lower portions of the stomodæum, and are most abundant on the lateral walls near the siphonoglyph. They

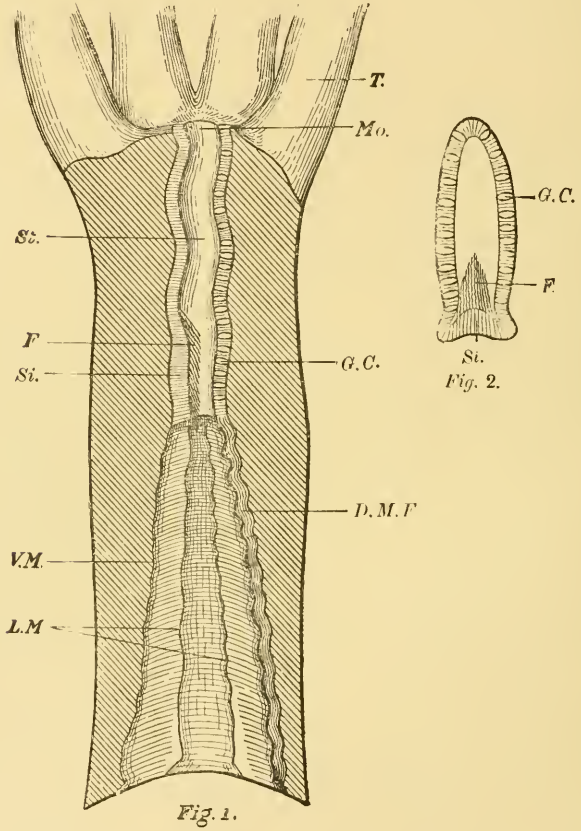

Fig. 1.—Semi-diagrammatic view of one half of a polyp which has been cut along the dorso-ventral line. Only the bases of the tentacles are shown ×20.

Fig. 2.—Transverse section through the lower third of the stomodæum (about the level of the reference letter *F* in Fig. 1) ×80.

D.M.F. Dorsal mesenterial filament on the edge of the dorsal mesentery; *F.* Flagella of siphonoglyph; *G.C.* Gland cells of stomodæum; *L.M.* Edge of lateral mesentery (mesenterial filament absent); *Mo.* Mouth; *Si.* Siphonoglyph; *St.* Stomodæum; *T.* Tentacle; *V.M.* Edge of ventral mesentery (mesenterial filament absent).

do not occur among the cells which form the siphonoglyph (Pl. 25, fig. 10). Secreting cells have not hitherto been noticed in the stomodæum of the Alcyonaria (Ashworth, 1898).

These goblet-cells of the stomodæum are the only secreting cells connected with the digestive cavity, as the six thick short ventral and lateral mesenterial filaments, which bear the gland-cells in other Alcyonaria, are absent in all polyps of this Xenia (see Woodcut, p. 252). Only the dorsal mesenteries possess thickened edges, forming two mesenterial filaments (*D.M.F.*) which have a similar course and structure to those of Alcyonium. The free edge of the ventral and lateral mesenteries is only very slightly thickened, and the thickening is due entirely to the greater amount of mesoglœa present at the edge of the mesentery. The cells which cover the edge differ in no way from those which cover the remaining portions of the mesentery.

New points in the anatomy of this Xenia are the presence of gland-cells in the stomodæum, and the absence of the six ventral and lateral mesenterial filaments usually present in the polyps of the Alcyonaria. Wilson (in Kophobelemnon, 1884) and Hickson (in Alcyonium, 1895) have shown that these mesenterial filaments bear the cells which produce the digestive secretion. I would suggest that the absence of these filaments in this Xenia is correlated with the presence of gland-cells in the stomodæum, and that the latter, judging from their appearance and position, perform some digestive function. As mentioned above, the cells are most abundant on the ventro-lateral walls of the stomodæum, near the siphonoglyph. As the flagella of the siphonoglyph create an inward current of sea water carrying food particles, which passes along the ventral groove, the greater abundance of secreting cells in the walls abutting on this groove is suggestive of the digestive function of the secretion, which can readily be poured out on to the ingoing food particles.

The siphonozooids which occur in some other Alcyonacea (e.g. Sarcophyton) and in Pennatulids are the only recorded examples of polyps in which the ventral and lateral

mesenterial filaments are absent. According to Wilson (1884), these siphonozooids derive their food supply from the autozooids or feeding polyps, the dorsal mesenterial filaments of the former creating upward currents which cause a flow of fluid from the autozooids to the siphonozooids, through the canals which connect them together. Food undergoes digestion in the autozooids, and some of the products are passed on to the siphonozooids, hence the latter do not require cells to produce a digestive secretion.

In this Xenia the secretion in connection with the digestive cavity is formed, not by endoderm cells, but by cells which are derived from the ectoderm, as from a study of the buds I have found that the stomodæum is ectodermic in origin in this as it is in other Alcyonaria (Wilson, 1883). Since the absence of ventral mesenterial filaments in Xenia Hicksoni was proved I have carefully examined all the other specimens of Xenia at my disposal. These are sixteen in number, viz. one from Talisse Island, North Celebes, one from Rotuma, Fiji Islands, and fourteen from various reefs in the Pacific. In all these the ventral and lateral mesenterial filaments are absent, there being only a very slight thickening of the free edges of the mesenteries, this thickening being entirely due to a slight increase in the amount of the mesoglœa along the free edges. In all the specimens the two dorsal mesenterial filaments are present, and have the typical course and structure. In Heteroxenia Elizabethæ the two dorsal mesenterial filaments only are present. The absence of ventral and lateral mesenterial filaments may, therefore, be considered as one of the characters which distinguish the polyps of the genera Xenia and Heteroxenia from those of other Alcyonaria. In two at least of the specimens of Xenia[1] from Dr. Willey's collection some of the cells of the stomodæum are goblet-like, and similar to those described above in the stomodæum of Xenia Hicksoni.

Cœlentera of Polyps.—The other parts of the colony present a structure similar, in the main features, to that

[1] X. crassa, Schenk, and X. viridis, Schenk.

of other Alcyonaria. The polyps are, for the greater part of their length, bound together in bundles of about forty to sixty, each bundle forming one stem of the colony. The external characters of the free portion of the polyp have been described above. The eight mesenteries of the polyp are arranged as in typical Alcyonaria. On their ventral faces they bear the retractor muscles, and on the opposite faces the protractor muscles, neither of which are well developed. This somewhat feeble development of retractor and protractor muscles accounts for the non-retractile nature of the polyps. The intermesenterial spaces are continued upwards into each tentacle and into each pinnule. In the free portion of the polyp, and particularly in the tentacles and pinnules, these spaces are to a large extent filled up by zooxanthellæ. The specimen is a male, and in the lower part of the free portion of the polyp, and in the upper part of the stem, the cœlentera are crowded with sperm-sacs containing spermatozoa in all stages of development, from the small masses containing only two or four primitive sperm-cells to the large mature sperm-sacs containing many hundreds of ripe spermatozoa.

Mesoglœa, its Canals and Cells.—In the stems the cœlentera of the polyps are bound together by a moderately large quantity of mesoglœa. The mesoglœa immediately round each polyp is slightly denser than that further away, so that in transverse sections of the stem, especially if the sections be taken through the upper part, one can distinguish the more deeply staining ring of slightly denser mesoglœa, which definitely belongs to the cœlenteron within it, from the less dense mass of mesoglœa between these rings, which cannot be assigned to any polyp or polyps (Plate 25, fig. 9). Traversing the mesoglœa of the stem are numerous canals, cords and strands of cells, which place all the parts in intimate communication with each other. The canals may be divided into two systems—a superficial system and an internal system.

The superficial canal system (figs. 8, 9, *Sup. Can.*) is formed by a plexus of numerous endodermic canals, which

are situated in the outer portion of the mesoglœa, just beneath the ectoderm of the stem. This system of canals extends all round the cylindrical stems, and also runs on the umbrella-shaped areas from which the free portions of the polyps arise (fig. 8). The cavity of this system of canals is invaded throughout by zooxanthellæ, which are especially numerous in the canals in the upper part of the stem.

The internal canal system consists of a series of longitudinal canals (figs. 8, 9, *Long. Can.*) which run generally in a sinuous or zigzag course in the mesoglœa, between the cœlentera of the polyps. These canals lie in the mesoglœa, almost equidistant from the surrounding cœlentera, and they run in this position from the top of each stem to the base of the colony. These canals are also endodermic, and have usually only a small lumen. They communicate with the cœlentera of the polyps, with the superficial canal system, and with each other.

At the base of the colony the canal system is exceedingly complicated, due to the numerous branchings and anastomoses of the canals. The cœlentera are continued down to the base of attachment of the colony, where they are in communication with the numerous branches of the canal systems. The cœlentera are readily distinguishable from the canals by their greater size, and the presence in them of eight small ridges, to which the mesenteries in this region are reduced. Besides the superficial and longitudinal canals briefly described above, there are in connection with the longitudinal canals numerous small lateral or transverse canals, with small lumen, which pass from the canals either to other neighbouring canals or to the cœlentera (figs. 8, 9). The cœlentera of the polyps do not open into each other directly, but are indirectly connected by these canals.

The base of the cylindrical main stem is somewhat flattened out, and this basal portion is hard and horny; and, being closely applied to the rock, provided a firm basis upon which the other parts of the colony were supported.

Ectoderm.

The ectoderm is a moderately thick layer, in which large columnar and smaller interstitial cells may with difficulty be distinguished. If a section of a tentacle or pinnule, which was well expanded at the moment of killing, be examined, the ectoderm is then seen to consist of a row of cells elongated at right angles to the free surface, below which are smaller rounded cells which probably correspond to the interstitial cells of the ectoderm of Alcyonium and other cœlentera. (Pl. 25, fig. 11).

The protoplasm of the ectoderm cells is very finely granular; occasionally cells are met with containing a few small vacuoles. Many of the ectoderm cells of the tentacles and pinnules are produced at their inner ends into muscle processes which lie in the outer portion of the mesoglœa, parallel to the free surface. These muscles are all longitudinal in direction. The ectodermic muscles of the tentacles are much more strongly developed on the oral than on the aboral side, especially at the base of the tentacles, where the muscles of the oral side form a strong band beneath the cells quite eight times as thick as the band of muscles on the aboral side. Nearer the tip of the tentacle the muscles of the two sides become almost equal.

In the body of the polyp ectodermic muscles are present only in the distal portion around the base of attachment of the tentacles and for a distance of about a millimetre below this point. Myo-epithelial cells are absent from the ectoderm of the stem. The absence of muscle-cells from the ectoderm of the stem and the greater portion of the body of the polyp is connected with the non-retractile character of these parts. Their presence in the tentacles, pinnules, and distal portion of the body of the polyp confers on these parts some power of contraction, and an examination shows that the pinnules and tentacles vary somewhat in length and shape, and that the tentacles are often turned inwards over the mouth, due to the contraction of the muscles of the oral side. The absence of

spicules from the tentacles and distal portion of the polyp renders the ectoderm softer and more pliable, and therefore more readily acted upon by the contraction and expansion of the muscle processes of its cells.

Among the ordinary columnar cells there are in the ectoderm of the stem and the body of the polyp large swollen cells which probably secrete the mucus which thinly covers the external surface of most of these parts (Pl. 26, fig. 17, *Muc. C.*). These mucus-cells are large and abundant in the angle of the Y-shaped piece formed where a stem divides.

The ectoderm cells present on their outer side a moderately plane surface, but on the inner side a more irregular one, as numerous processes pass from the cells inwards and establish communication either with the endoderm or with cells lying deeper in the mesoglœa (fig. 17).

On reaching the base of the colony the ectoderm curves inward, and was applied to the face of the rock to which the colony was attached.

The stomodæum is ectodermic in origin in this as in other Alcyonaria (Wilson, 1883), as may be seen from a study of the buds. The presence of a ventral ciliated groove and of gland-cells and other features of its structure have already been referred to (p. 251). The ectoderm of the stomodæum and the adjacent endoderm are connected by numerous cells or strands of cells passing through the thin mesoglœal lamina which separates the two cell layers (Pl. 26, fig. 18).

The ectoderm cells give rise to nematocysts and spicules.

Nematocysts.—The nematocysts are exceedingly numerous in the ectoderm of the tentacles and pinnules (Pl. 25, fig. 11, *Nem.*); there are large numbers in the ectoderm of the body of the polyp, rather fewer in the ectoderm of the stem, and a few in the ectoderm of the oral disc, in the funnel leading to the mouth, and also in the upper part of the stomodæum. The nematocysts are, as in other Alcyonaria, exceedingly small, their length being ·008 mm. and their breadth ·002 mm. to ·003 mm. They have bluntly pointed ends and are circular in transverse section. Each nematocyst is formed in a cnidoblast cell

(Pl. 25, fig. 12, *Cn.C.*), the nucleus and protoplasm of which lie flattened against the capsule on one side and a little nearer the inner than the outer end of the capsule. The filament or thread lies coiled up inside this capsule, there being about twelve coils distinguishable in the most favourable specimens. The thread appears to be quite simple, there being no barbs visible. Its length when shot out would probably be about 80 μ (·08 mm.). The nematocysts are usually placed with their long axes at right angles to, and their bluntly pointed ends level with or slightly projecting from, the free surface of the ectoderm. This can be especially well seen in sections of the tentacles and pinnules (see fig. 11). The nematocysts stain deeply with thionin, hæmatoxylin, and especially with iron hæmatoxylin (Heidenhain). Iron hæmatoxylin is exceedingly useful for staining the small nematocysts of Alcyonaria; in fact, without some good staining reagent it would be very difficult to find the minute capsules in many cases. Moseley (1881, p. 119) wrote that "no nematocysts were found in Sarcophyton," but by using the iron hæmatoxylin stain I have found them in sections passing through the ectoderm of the tentacles. They are very similar in shape to those of Xenia Hicksoni, but smaller in size, being only 6 μ to 7 μ long and 2 μ wide. The nematocysts of Alcyonaria are all very small, as may be seen from the following table:

Sarcophyton pauciflorum[1]	6 μ— 7 μ	long and	2 μ	wide (J. H. A.)
Alcyonium digitatum . .	7½ μ	„ „	2 μ—3 μ	„ (Hickson)
Xenia Hicksoni	8 μ	„ „	2 μ—3 μ	„ (J. H. A.)
Heteroxenia Elizabethæ .	9 μ	„ „	2½ μ	„ (J. H. A.)
Clavularia viridis . . .	9 μ—10 μ	„ „	2 μ—3 μ	„ (J. H. A.)
Heliopora cœrulea . . .	9 μ	„		(Moseley)
Clavularia prolifera . .	10 μ—15 μ	„		(v. Koch)

Spicules (Pl. 25, figs. 13—15; Pl. 26, fig. 16).—The spicules, the form of which was compared by Kölliker to that of red blood-corpuscles, are rounded or oval discs, which are, however, sometimes bilobed (fig. 13). They are ·012 mm. to

[1] S. pauciflorum, Ehrenberg = Lobophytum pauciflorum, v. Marenzeller, 'Zool. Jahrb.,' i, 1886.

·022 mm. long, ·006 mm. to ·013 mm. broad, and ·003 mm. to ·005 mm. thick.

Spicules are absent from the tentacles and pinnules of all the polyps examined except one. In the latter a few small spicules (about 8 μ to 12 μ long) are present in the ectoderm of the tentacles, but only in the proximal ·4 mm.

In the deeper part of the ectoderm, just below the point of attachment of the tentacles, spicules are present in small numbers, while in the middle and proximal portions of the body of the polyp they are very numerous, a tangential section of the body-wall in this region showing that the spicules form an almost complete layer in the deeper part of the ectoderm. Spicules are numerous in the ectoderm of the stem, and especially numerous in the angle of the Y-shaped piece formed at the point of division of a stem. In the lowest parts of the colony, i.e. in the portion attached to the rock, they are present in enormous numbers, practically filling the entire mesoglœa in that region. The largest examples of spicules are to be found in the basal portion of the colony. (Those shown in fig. 13 are from this region.) The absence of spicules from the tentacles, pinnules, and from the body of the polyp around the base of the tentacles is correlated with the power of contractility, slight though it is, which is possessed by these parts. The presence of an almost complete layer of spicules forming a more or less rigid cylinder in the middle and proximal portions of the body of the polyp and in the stem, together with the absence of muscle processes from the ectoderm cells of these portions, sufficiently accounts for the non-retractile character of these parts of the colony. The increased number of spicules in the angle between two branches gives the required rigidity to this part, and prevents flexure of the branches and consequent closure of some of their canals and cœlentera.

Besides the extraordinary number of spicules present in the ectoderm and mesoglœa of the last few millimetres of the base of the colony, this part is further strengthened by much of the mesoglœa becoming converted into a dense and horny substance,

so that the part of the colony attached to the rock is hard and quite different to the touch from any other part of the colony. This hard base would afford a firm attachment and a rigid support to the branches which arise from it.

The spicules are formed within cells which at first lie in the deeper parts of the ectoderm or have migrated into the outer parts of the mesoglœa (fig. 16). The arrangement of the spicules is not very regular, as they appear to present to the free surface their edge or their flat face indifferently. In nearly all preparations the nucleus and remains of the protoplasm of the spicule-forming cell can be seen. The spicules, which have a horny consistency, have an organic basis impregnated with only a very small amount of calcareous matter. They stain deeply with hæmatoxylin, especially with hæmalum. They do not dissolve when treated with acids, but shrink very slightly, probably owing to the solution and extraction of the small amount of calcareous matter which they contain. They do not offer any difficulties in section-cutting, as the razor cuts through them with little resistance, and sections $2\,\mu$ to $4\,\mu$ in thickness may be readily obtained, although the specimen has not been previously decalcified. On this account Xenia offers exceptional facilities for the study of the development of spicules. As mentioned above, each spicule is formed within a cell lying in the deeper part of the ectoderm. When the young spicule first makes its appearance in the cell its substance is scarcely distinguishable from the protoplasm of the cell; in fact, it is not until the spicule has attained a diameter of $3\,\mu$ to $4\,\mu$ that it is possible to clearly differentiate it (Pl. 25, fig. 15). The young spicule is then a small disc which stains with hæmatoxylin like the protoplasm of the cell, but its homogeneous structure enables the observer to distinguish it from the finely granular cell-protoplasm which surrounds it. From this stage the spicule grows regularly in length and thickness, and the protoplasm of the cell covering it becomes gradually thinner until, in a fully formed spicule, this protoplasmic sheath forms an exceedingly thin investment, in which at one part may be seen the small, somewhat flattened nucleus em-

bedded in a small mass of protoplasm (figs. 13 and 14). In all the specimens, many hundreds in number, which I have examined, the spicule develops in a single cell with one nucleus. I have not been able to find any examples which showed that two cells or two nuclei were concerned in the formation of the spicule (cf. v. Koch's account of the development of the spicules of Clavularia prolifera, 'Morph. Jahrb.,' vii, p. 473, 1882).

Mesenteries (Pl. 26).

Mesenterial Filaments.—The stomodæum leads into the cœlenteron of the polyp, which is subdivided by the usual eight mesenteries (Pl. 25, fig. 10). Of these only the two dorsal ones possess thickened edges or mesenterial filaments (Pl. 26, fig. 19). The free edge of the remaining six mesenteries is only very slightly thickened, this being due entirely to the presence of a slightly greater amount of mesoglœa near the free edge of the mesentery. The cells which cover this thickened portion differ in no way from those covering the other parts of the mesentery (Pl. 26, fig. 20). The six ventral and lateral mesenterial filaments usually present in the polyps of the Alcyonaria are not found in this genus. The dorsal mesenterial filaments arise from the lower edge of the stomodæum and run in a sinuous course along the dorsal side of the cœlenteron. In the primary polyps they may be traced to the base of the colony. In transverse section the filament is slightly bilobed, i. e. there is a groove (of slightly varying depth) extending all the way down the middle of the free surface of the filament (fig. 19). The cells on each side of this groove bear long cilia (·0075 mm.). The dorsal mesenterial filaments are quite typical, and agree well with the accounts given of those of other Alcyonaria by Wilson and Hickson. They are probably ectodermic in origin, as they appear to be formed as two downgrowths from the inner end of the stomodæum. The cells of the filaments agree in structure with those of the stomodæum, being finely granular and non-vacuolated, and differing markedly from the much vacuolated neighbouring endoderm cells.

Muscles.—The retractor muscles are situated on the ventral faces of the mesenteries and the protractor muscles on the dorsal faces, as in Alcyonium. These muscles are somewhat feebly developed, as might be expected from the non-retractile nature of the polyps. Shortening of the retractor muscles produces a slight contraction of the oral disc and consequent formation of the funnel-like depression leading to the mouth, to which reference has already been made (p. 251).

Cells in Mesoglœa of Mesenteries.—On examining a transverse section through the mesenteries, there is seen to be a considerable quantity of mesoglœa between the two endodermic lamellæ covering the mesentery (fig. 20). In this mesoglœa there are cells which have the reticulate protoplasm and general appearance of endoderm cells. These cells migrate into the mesoglœa from the endoderm covering the surface of the mesentery, and even in a young polyp ·8 mm. long a few cells have already taken up their position in the mesoglœa. In older polyps there is a larger number of these cells in the mesoglœa of the mesentery, though they are not equally numerous in all parts. In the upper portion of the polyp, about the level of the stomodæum, the mesoglœal cells are few in number and small in size, but from this part downwards their number and size gradually increase, until in the mesenteries in the upper portion of the stem they are large and numerous, and in some cases completely fill up the mesoglœa, so that the mass of cells is in close contact on both sides with the endoderm covering the two sides of the mesentery. Towards the base of the stem the cells become fewer in number and slightly smaller in size. These cells are found in the mesoglœa of all the mesenteries, but they are less numerous in the dorsal mesenteries than in the remaining six. Many of the cells are at first somewhat elongated or pear-shaped, with one or more processes in connection with, or pointed towards, the endoderm; but later many of them become rounded and larger, and their nuclei become much larger.

The primitive genital cells are derived from these large rounded cells in the mesoglœa of the mesenteries at the base

of the polyp and in the upper portions of the stem. That this is the case is shown by the following:

(1) These cells are most numerous in those parts of the colony where gonads occur in greatest numbers.

(2) In many cases one of these cells, surrounded by a thin film of mesoglœa, may be seen enclosed in a follicle of endoderm projecting from the edge of the mesentery. These are exactly similar to the neighbouring follicles which contain spermatozoa in various later stages of development.

(3) The nuclei of most of these cells are large and spherical, more vesicular than the nuclei of the adjacent endoderm cells, and resemble the nuclei of the genital cells (see Pl. 27, fig. 30).

(4) The ripe spermatozoa are situated in a follicle covered by a thin mesoglœal lamina, as well as by the endoderm cells outside this, i. e. the ripe spermatozoa are situated in the same layer as these cells, viz. in the mesoglœa.

The migration of genital cells from the endoderm of the mesenteries into the mesoglœa is similar to that described by O. and R. Hertwig in Actiniæ ('Die Actinien,' Jena, 1879, p. 95, and pl. 7).

Endoderm (Pl. 26).

The endoderm cells lining the cœlentera and the cavities of the tentacles have a similar structure throughout the colony. They are cubical or columnar, and contain many small vacuoles which give the protoplasm a reticulate appearance.

Cells which bear Flagella (figs. 20—25, 27).—Among the ordinary endoderm cells there are numerous cells, the inner or free end of which is produced into a long process, which is from four to eight times as long as the basal portion of the cell. This process may be slender or moderately stout, and its length may vary in different specimens from ·015 mm. to ·12 mm. The basal part of the cell from which the process arises has the reticulate protoplasm of an ordinary endoderm cell, and the nucleus of the cell is situated in this portion. The process is not vacuolated, and for the greater part of its length its protoplasm exhibits a homogeneous or very finely

granular structure. Its basal part, i. e. the part in continuity with the vacuolated portion of the cell, stains deeply with hæmatoxylin, and in most cases shows very faint longitudinal striations which are visible only in the proximal third of the process.

The processes usually taper towards their free end, but in one instance this end is slightly broadened and flattened (fig. 25, *A*). In one case the process, which is a very large one, bears a short branch near the middle of its length (fig. 25, *B*). This is the only branched process found among many hundreds examined. The processes of most of the cells project outwards almost at right angles to the free surface of the endoderm (figs. 20, 21, 23, 24), but there are many similar to the one drawn in fig. 22, in which the process is strongly curved and apparently moderately flexible.

These curious processes are very numerous, and are found in all parts of the endoderm lining the cœlenteron and tentacles, but are most abundant in the portion of the cœlenteron situated in the body of the polyp and in the upper part of the stem (Pl. 25, fig. 9).

The nature of these processes is difficult to determine. In the preliminary note to the Royal Society (1898, written in February) I called them pseudopodia, but further investigation shows that the word flagella would probably better express their nature. The processes appear to be permanent, to have a moderately definite shape gradually tapering from base to tip, and to be flexible. The word pseudopodia implies more temporary structures with many different and continually changing shapes, while the term flagella implies tapering whip-lash-like processes of more permanent and definite shape. The homogeneous structure of the greater portion of these processes, differing so markedly from the vacuolated granular protoplasm of the rest of the cell, is also more in accord with their being flagella, as pseudopodia have the same structure as the body of the cell from which they are protruded.

It is difficult to suggest the probable function of these giant flagella. They are evidently motile organs, as they may be

found in all stages of flexion, some being practically straight, while others are bent almost into a semicircle. Their action probably serves to keep the liquid in the cœlenteron in slow motion, thereby securing a more equal distribution of the nutrient substances contained therein to the cells in their vicinity.

Many of the endoderm cells are provided with "muscle processes," but these processes are not numerous in the pinnules and in the stem; they are more numerous in the endoderm of the tentacles and of the free portion of the polyp. The muscle-fibres (except those forming the retractors and protractors) have a circular direction, and are similar to those of Alcyonium. In Alcyonium, however, the endoderm cells of the tentacles do not possess muscle-fibres (Hickson, 1895, p. 376).

In teased preparations the muscle-fibres of the ordinary endoderm cells may be clearly seen (fig. 26). On looking at the flagella-bearing cells in the same preparations, it is seen that most of these cells bear at their inner ends two processes which appear to be less stiff than the muscle-fibres of the ordinary endoderm cells, and which are never in a straight line with each other, but are invariably bent more or less towards each other (fig. 27). It is possible that these are the modified muscle-fibres of the cell, as they appear to be homogeneous, and, when treated with fuchsin or iron hæmatoxylin, stain similarly to, though rather less deeply than, the muscle processes of ordinary endoderm cells. The bending inwards of the two processes from the inner end of the cell causes them to become rather more deeply embedded in the mesoglœa than the muscle processes of the ordinary endoderm cells, and the cell is therefore provided with a firmly fixed base upon which the giant flagellum can work as on a fulcrum.

It is worthy of note that throughout the whole of the colony the muscle processes of the endoderm cells (except the protractor and retractor muscles on the mesenteries) are circular in direction, whereas the similar processes (where present) of the ectoderm cells are longitudinal in direction.

Zooxanthellæ are exceedingly numerous in the endoderm of the pinnules, so numerous that, in many cases, the lumina of the pinnules are entirely closed. They are also numerous in the endoderm of the tentacles and of the free portion of the polyp, but in the cœlentera of the stem there are few, except near the upper end. There do not appear to be any large gaps between the endoderm cells in the lower parts of the cœlentera, as described by Hickson in Alcyonium, but the endoderm cells in this part are more or less spherical in shape, and are only loosely connected together.

Mesoglœa.

1. Of the Free Portion of the Polyp.—The mesoglœa of the body of the polyp varies in thickness from ·02 mm. to ·06 mm., while that of the tentacles is much thinner, averaging ·013 mm. The mesoglœa of the pinnules is exceedingly thin, especially when they are expanded (cf. figs. 11, 17).

Cells which connect the ectoderm and endoderm may be seen crossing the mesoglœa in all parts of the tentacles and body of the polyps. In the tentacles, however, these cells are few in number, but in the body of the polyp, where the mesoglœa is thicker, the cells are more numerous. They are usually elongated or fusiform cells, having their outer ends embedded in or connected with the ectoderm, and their tapering inner ends passing into the endoderm (fig. 17). In most cases the connection between the two cell-layers is established by means of a single cell, but in some cases two or more cells are placed end to end to form the connecting cord. In most cases the larger portion of the cell and its nucleus are situated in the ectoderm, and this, together with the nature of the cell,which closely resembles an ectoderm cell in appearance, points to the fact that these cells in the mesoglœa are derived from the ectoderm. Besides these moderately large cells, the protoplasm of which is finely granular and often contains a few small vacuoles, there are other cells of very much smaller size which bear several or many processes. Some of the cells

lie close to the ectoderm, while others are near the endoderm. They are connected together by their exceedingly slender processes, which traverse the mesoglœa and unite with each other. These cells resemble, and are probably homologous with, the nerve-cells and nerve-fibres of Alcyonium and the Actiniæ. They will be further described below (see p. 277). The mesoglœa of the mesenteries and its included cells have already been described (see p. 263).

2. Of the Stem (see Pl. 25, figs. 8 and 9).—On examining transverse sections of the upper portion of the stem there is seen a slightly denser ring of mesoglœa (*Mg. D.*) around each of the cœlentera. This ring of denser mesoglœa is itself moderately free from cells, being crossed only at intervals by a cell or thin cord of cells, but it is bordered by an almost complete cordon of cells, interrupted only for the passage of endodermic canals. The canals of the stem are moderately large and very numerous, being much more highly developed than those of Alcyonium. The canals may be divided into the two systems described below.

The Superficial Canal System.—This system of canals is formed by numerous endodermic canals (*Sup. Can.*, figs. 8 and 9) which are situated in the stem about ·1 mm. beneath the ectoderm. This system is really a fine network of numerous canals, which have a similar structure and appearance in all parts of the colony. The canals are about ·08 mm. in diameter. In the intervals between these canals there are usually cords of ectoderm cells (*Ect. Str.*) which pass from the ectoderm to cells in the deeper parts of the mesoglœa. In many cases the superficial endodermic canals are themselves closely connected with the ectoderm by strands of cells passing across the mesoglœa from the ectoderm to the outer walls of the canal lying beneath. From the inner wall of these canals cords of cells frequently pass inward into the mesoglœa, and are connected with other cells, with the cœlentera, or with longitudinal canals.

The superficial canals are also present on the convex summit of the stem (see fig. 8), and form there a plexus of canals,

with similar relations to those above described in the cylindrical portion of the stem. In this portion of the stem the canals embrace or pass round each cœlenteron at a distance of about ·2 mm., and communicate by means of branches with the cœlenteron and with the neighbouring longitudinal canals. The superficial canals on the convex summit are continuous at the edge of the summit with the corresponding canals of the cylindrical portion of the stem. In the stem the superficial canals frequently communicate with the neighbouring cœlentera and longitudinal canals. Thus, by means of branch canals, this superficial system of canals is placed in communication with the remaining cavities lined by endoderm, and by means of strands of cells is placed in communication with the ectoderm and with the neighbouring cells in the mesogloea.

Where a stem divides there are extra canals which establish thorough communication between the superficial canals of the two branches.

As the base of the colony is approached there is a tendency for the superficial canals to send inwards wide branches, and in the lowest 2 mm. of the stem such branches are given off in large numbers, and unite with the complicated anastomosis of longitudinal canals present in that part of the stem.

This system of canals is of great importance, as all the young buds produced in the colony are formed by enlargement and growth outwards and inwards of one of these canals, the endoderm and lumen of the canal forming respectively the endoderm and cœlenteron of the young polyp (see also p. 291).

Histology of the Superficial Endodermic Canals. —The endoderm lining the cavity of the superficial canals is always much thicker on the outer side of the canal than on the inner side (Pl. 27, fig. 29). This is caused by the cells on the outer side being columnar and longer than the cubical or slightly flattened cells of the inner side of the canal.

The cells lining these canals resemble the endoderm cells of the cœlentera, but their protoplasm is somewhat less vacuolated, and therefore does not so markedly present the reticulate

appearance which is so usual in the endoderm of the cœlentera. None of the cells of these canals bear flagella. Among the bases of the cells there are small cells which are probably stages in the formation of the larger ones. Some of the cells of the canals appear to be provided with very slender muscle processes. There are numerous zooxanthellæ in the lumen of the canals and embedded in the endoderm lining the cavity.

The Internal Canal System.—The canals forming the main portion of this system are chiefly longitudinal in direction, and commence in the umbrella-shaped portion at the top of each stem. Each canal runs in a sinuous or zigzag course in the mesoglœa, about equidistant from the surrounding cœlentera.

The longitudinal canal communicates with the superficial canals lying around its origin (fig. 8). During its course down the stem the longitudinal canal very frequently communicates by small transverse canals with the neighbouring cœlentera and canals, and the longitudinal canals in the outer portion of the stem communicate also with the superficial canals. Owing to the frequent occurrence of branches, the longitudinal canals are nearly always angular in transverse section, one (or more) of the angles being usually produced into a small branch canal. The branch canals vary greatly in size; in the upper and middle portions of the colony being small, and their lumen very small or obliterated altogether, while near the base of the colony the branches are almost as large as the main canal. At the base of the colony these longitudinal canals give off several rather larger lateral branches on all sides, some of which unite with similar branches from adjacent canals, while others open into neighbouring cœlentera. Owing to the presence of so many canals in this region of the stem, the mesoglœa is penetrated in all directions by a complicated network of canals, which place all the cavities lined by endoderm in intimate communication with each other. Very close to the base of attachment of the colony, a canal may usually be seen passing from each side of the lowest portion of each primary cœlenteron, so that in longitudinal

section the cœlenteron and its two canals appear ⊥-shaped. The canal opens into the base of a neighbouring cœlenteron. These canals are probably the representatives of the original stolon from which all the primary cœlentera grew out.

The well-developed longitudinal canals running parallel to and between the cœlentera of the polyps of Xenia remind one of the cœnenchymal tubes of Heliopora cœrulea, described by Moseley (1881) and by Bourne (1895). The resemblance is more striking when we consider that in both cases the longitudinal tubes are lined by endoderm, and are connected near the upper surface of the colony with a network of superficial endodermic canals.

The differences between the canals of Xenia and the cœnenchymal tubes of Heliopora are chiefly due to the fact that in the latter only the outer portion of the coral is living, the internal parts consisting of calcareous skeleton only, whereas in Xenia the whole colony is penetrated by living cells. In Heliopora, therefore, the cœlentera of the polyps and all canals running into the colony must terminate within about 2 mm. of the surface, as this is the lowest limit of the living substance. The cœnenchymal tubes of Heliopora have an exactly similar course to the longitudinal canals of Xenia, as they run parallel to the cœlentera from their point of origin from the superficial canal system (just beneath the ectoderm covering the free surface) to the base of the living portion of the colony, where they and the cœlentera terminate blindly.

Moseley (1881) believed the cœnenchymal tubes of Heliopora to be degenerate siphonozooids from which the mesenteries had disappeared, but Bourne (1895) has shown that they cannot be so regarded.

In order to find a parallel to these cœnenchymal tubes of Heliopora, Bourne thought it necessary to go outside the Alcyonaria and compare the tubes with certain longitudinal canals of Millepora, described by Moseley (1876). After carefully studying the canal system of Xenia, it appears to me that this course is not now necessary, as the comparisons instituted above between the cœnenchymal tubes of Heliopora

and the longitudinal canals of Xenia are perfectly justifiable. It is true the cœnenchymal tubes of Heliopora are more numerous than the longitudinal canals of Xenia, but this also is probably due to the different modes of growth of the two corals. In Heliopora the living part is, as it were, spread out in a thin film, and the polyps are a considerable distance (1 mm. to 2 mm.) apart, several cœnenchymal tubes being therefore required to place the cœlentera in communication with the intervening ectoderm, calicoblasts (or skeleton-forming cells), and mesoglœa. In Xenia each stem is an elongated, cylindrical, compact mass of living tissues, and the polyps are much closer together, the cœlentera being only about ·25 mm. to ·4 mm. apart in the stem, and therefore one longitudinal canal, situated in the mesoglœa between adjacent cœlentera (together with the auxiliary strands and cords of cells which are so well developed in this Xenia), is sufficient to provide efficient communication between the cœlentera and all parts of the narrow mesoglœal column between them.

Histology of the Longitudinal Endodermic Canals. —These canals are lined by a single layer of cells of equal thickness on all sides (cf. the superficial canals). The cells are more or less cubical in shape, and closely resemble the endoderm cells lining the cœlentera. None of the cells in the canals bear flagella, but some bear slender muscle processes which, like those of the endoderm of the cœlentera, are chiefly circular in direction. In the canals near the base of the colony many nematocysts, each in its cnidoblast cell, may be seen lying among the endoderm cells. In the upper portions of the colony nematocysts are seldom seen in the canals. Zooxanthellæ are present only in those longitudinal canals which are situated in the circumferential portions of the stem and in the upper portion of the canals, where they approach the summit of the stem.

Cells in the Mesoglœa.—On examining a transverse section of the upper end of a stem of the colony, an almost complete chain of cells is seen surrounding the denser ring of mesoglœa round each cœlenteron (fig. 9, *Ect. Ch.*). Each

ring of cells is situated at a distance of about ·06 mm.—·07 mm. from the endoderm of the cœlenteron within. These cells are the ectoderm of the portion of the polyp enclosed in the stem, as can be seen on examining a longitudinal section through the upper part of the stem (see fig. 8), when this cylinder of cells is seen to be continuous and in line with the ectoderm of the free portion of the polyp. That these cells are ectodermic is further shown by the fact that spicules and nematocysts are found in them in moderate numbers, especially in the upper portion of the stem (figs. 8 and 9, *Sp. Nem.*). Adjacent cylinders of cells are placed in intimate communication by numerous cords of cells which traverse the mesoglœa between them.

Sections taken further down the stem show that the ring of cells becomes less complete and less definite, and each ring widens and recedes into the mesoglœa a little further from its cœlenteron. As they recede further and further, the rings of cells often coalesce in the mesoglœa at a point almost equidistant from their cœlentera; and, as the longitudinal canals also lie in this region, the cells come to lie in relation with, and form a plexus around, the canals. There is, therefore, still a cylinder of cells round each cœlenteron, but the cylinder is not quite so regular as in the upper portions of the stem, being interrupted at frequent intervals, and is further away (·15 mm. to ·2 mm.) from the enclosed cœlenteron.

In the upper portion of the stem the cylinder of cells has the same relation to the endoderm of the cœlenteron within it, as have the ectoderm and endoderm of the free portion of the polyp to each other. Crossing the denser ring of mesoglœa between the cylinder of cells and the endoderm are cells (viz. the vacuolated and granular cells, and the small nerve-cells) exactly like those observed in the mesoglœa of the free portion of the polyp.

Lower down the stem the cylinder of cells has been pressed further away from its cœlenteron, probably by the later growth of the mesoglœa, and is interrupted at intervals for the passage of canals and cords of cells which place all parts of the

mesoglœa in intimate communication with the cœlentera, the canals, and the external ectoderm. Bourne (1895) has shown that in Xenia umbellata these rings of cells are ectodermic on account of their connection with, and general resemblance to, the ectoderm of the free part of the polyp, and the occurrence of spicules and nematocysts in some of them.

The cords of cells in the mesoglœa are then chiefly ectodermic, as they arise from the cylinders of cells around the cœlenteron, or, in the outer portion of the mesoglœa, migrate inwards from the inner irregular surface of the external ectoderm. The cells all present a similar appearance, being either rounded or rather elongated in shape, with somewhat vacuolated protoplasm. The elongated cells frequently taper at their ends into long slender processes which become connected with similar processes of adjacent cells.

Some of the cords of cells are, however, obviously formed by obliteration of the lumen of a small canal; these cells are, of course, endoderm.

Spermatogenesis (Pl. 27, figs. 30—35).

The specimen is a male, and shows beautifully all the stages in the development of the spermatozoa.

Gonads are most numerous in the upper portion of the stems of the colony, but many sperm sacs are found in the basal part of the free portion of the polyps, and a few also in the lower portions of the colony. Sections through the upper portion of the stems show that sperm sacs are so numerous that they practically fill up the cavity of the cœlentera of several of the older polyps (Pl. 25, fig. 8, *S. S.*). In these cases most of the sperm sacs are no longer spherical, but by mutual pressure have become angular, being usually pentagonal or hexagonal in section.

The genital cells are derived from the cells which lie in the mesoglœa of the mesenteries near their inner or free edge. These cells, as shown above (see p. 263), have migrated to their present position in the mesoglœa from the endoderm, so

that the gonads are, in this as in other Alcyonaria, endodermic in origin.

Each sperm sac originates as a slight projection at the side or free edge (except in the dorsal mesenteries) of the mesentery, and consists of one of these genital cells covered by a thin sheet of mesoglœa, and by a single layer of endoderm cells continuous with the endoderm of the sides of the mesentery. The genital cell, the protoplasm of which is finely granular, or sometimes contains small vacuoles, is spherical and about ·01 mm. in diameter, and in the centre has a large spherical nucleus whose diameter is about half that of the cell.

The nucleus and protoplasm of the genital cell undergo division, which at first is apparently regular, as many cases of four or eight cells so produced may be seen (fig. 30). The divisions of the genital cell are accompanied by divisions of the endoderm cells covering it, and very soon the increase in size of the sperm sac causes it to project as a spherical or oval body from the mesentery, to which it always remains attached by a stalk consisting of a thin cord of mesoglœa surrounded by endoderm. When the sperm sac has reached a diameter of about ·06 mm. to ·08 mm. there appears in the centre a small cavity, free from nuclei, but containing some coagulable substance (fig. 31, *Cg.*). This central cavity continues to enlarge for some time, along with the growth of the sperm sac (fig. 32), and attains its maximum size in sacs about ·2 mm. to ·25 mm. in diameter, in which the central cavity reaches a diameter about one fourth that of the sperm sac. After reaching this size it is gradually encroached upon by the heads of the ripening spermatozoa (fig. 33, *Spz.*), and in the fully developed sperm sac, which is about ·35 mm. in diameter, the cavity has completely disappeared, the whole of the interior of the sperm sac being filled with a mass of somewhat loosely packed spermatozoa, many hundreds in number, produced by the continued division of the single primitive genital cell. There are no examples of karyokinesis in the many hundreds of sperm sacs I have examined.

The head of each ripe spermatozoon (fig. 34) consists

of a blunt, conical, anterior piece fixed to the spherical nucleus. The length of the head is 7 μ, the nuclear portion being 4 μ in diameter. The tail is a slender filament about 27 μ long.

The spermatozoa closely resemble those of Alcyonium in their structure and development (Hickson, 1895). The endoderm covering the sperm sac appears to undergo certain changes as the sperm sac grows, and in thin sections from two to five nuclei may be counted in many of the endoderm cells. One of these nuclei is sometimes larger than the others. In the left upper cell of fig. 35 the large nucleus near the centre occupies the position of the original nucleus of the cell. The other four nuclei have probably been produced from it by division, but there has been no corresponding division of the protoplasm. The cell with four nuclei which was figured by Hickson (1895, pl. 39, fig. 45, f.) from the teased preparations of the sperm sacs of Alcyonium, was probably one of the cells of the endodermic follicle.

At first it appeared that the sperm sacs were situated on ventral and lateral mesenteries only, although, as pointed out above (see p. 263), the cells in the mesoglœa, from which the genital cells are derived, are found in the dorsal mesenteries as well. After examining a large number of sections, I have found only two clear cases of sperm sacs occurring on the dorsal mesenteries. In each case the sperm sac is situated on the side of the mesentery a little distance from the free edge, so that, although the sac is of considerable size (·15 mm. in diameter), it does not push the dorsal mesenterial filament out of position, and, judging from the sections, would not impede its action.

Empty sperm sacs the walls of which are collapsing may be seen in several sections. The spermatozoa are discharged into the cœlenteron by bursting of the follicle of the sperm sac, and are then swept out to the exterior through the stomodæum. In sections of two polyps in which spermatozoa were escaping, the spermatozoa are found along the dorsal side of the stomodæum, being doubtless driven out by the upward or outward

current produced by the cilia of the dorsal mesenterial filaments. Although escaping in a mass they are not enclosed in the follicle, but lie loosely aggregated in the dorsal portion of the stomodæum. These two examples support the conclusion expressed above that the spermatozoa are discharged into the cœlenteron by rupture of the follicle, the collapsed remains of which retain for some time their attachment to the mesentery.

As spermatozoa are present in all stages of development, it is likely that the discharge of ripe spermatozoa continues over a considerable period,—in fact, probably throughout the year. This is perhaps due to the fact that, living on reefs in the shallow waters of tropical seas, this coral is not subject to any great variations in temperature and food supply. In this respect Xenia differs from Alcyonium digitatum, which occurs in the colder seas of Northern Europe. In the latter all the sperm sacs of a colony have reached a similar stage of development and are all ripe about the same time of the year, viz. December, and therefore the discharge of ripe spermatozoa occurs only over a limited period, probably over about a month (Hickson, 1895).

Nervous System.

In several sections there is a plexus of fine fibrils in the mesoglœa connected with very small cells in relation with the ectoderm and endoderm. This plexus appears to be homologous with the similar plexus described by Hickson in Alcyonium (1895, p. 371), and compared by him to the "Nervenschicht" of the Actiniæ.

In this Xenia the plexus is best seen in sections of a polyp in which the ectoderm is cut slightly obliquely. On examining in such a section (Pl. 26, fig. 16) the part where the ectoderm passes into the mesoglœa, very fine fibrils (*N. F.*) may be seen, forming an open network, upon which cells (*N. C.*) are situated at intervals. The fibrils can be best seen in the mesoglœa, but can be traced close to the ectoderm and endoderm. The cells of the nervous system are exceedingly small,

and usually fusiform, triradiate, or stellate in shape, the angles of the cell being produced into nerve fibrils.

On tracing the fibrils outwards from the mesoglœa into the ectoderm, they are seen to be in connection with small cells which are situated in the deeper part of the ectoderm. Owing to the irregularity of the inner face of the ectoderm, and to the presence of spicules in the portion of the layer where the nerve-cells are situated, it is not possible to obtain a section which shows the ectodermic nerve plexus clearly, but small portions of it may be seen where the spicules are slightly less numerous.

In the case of the endodermic nerve plexus this difficulty does not exist, and an oblique section through the wall of a polyp shows that the plexus of fibrils in the mesoglœa is connected with minute stellate cells situated upon the outer face of the muscle processes of the endoderm cells. Hæmatoxylin (especially Heidenhain's iron hæmatoxylin and Meyer's acid hæmalum) stains the nerve-cells and fibres most clearly.

History of our Knowledge of the Buds of the Xeniidæ.

Besides the fully developed polyps, the description and measurements of which are given above, there are on most of the branches of the colony young polyps or buds in various stages of development. These buds are invariably found at the edge of the umbrella-shaped area at the end of the stem. On one stem (fig. 1, left) there are ten small buds varying in length from ·5 mm. to 3 mm., and about fifty larger polyps from 5 mm. to 10 mm. in length. The smallest buds have simple tentacles devoid of pinnules, and all stages between these and the adult polyps may be found. As the polyps (both young and old) of Xenia are non-retractile, this genus offers considerable advantages for the study of the development of the polyps, and the young polyps have been noticed by many observers.

Quoy and Gaimard (1833) first observed these small polyps in

the Xeniidæ. They noticed them in Cornularia viridis, which appears to belong to the genus Xenia, and they suggested that these small polyps, the tentacles of which were devoid of pinnules, were young forms which had not yet attained the adult characters.

In 1874 Kölliker described Heteroxenia Elizabethæ, in which small individuals are very numerous. He regarded these small individuals as being of two kinds, some being young polyps in various stages of development, others being "zooids." Heteroxenia, therefore, is dimorphic. According to Köl-liker, there are several essential differences between these two kinds of individuals.

I. The Polyps.—These are of large size, the adults measuring 20 mm. to 55 mm. in length, and about 3 mm. in breadth. There are also obviously younger polyps, 5 mm. to 20 mm. long, all of which are situated round the edge of the disc. The tentacles of the adult polyps bear two series (in each of which four rows are distinguishable at the base of the tentacles) of long cylindrical pinnules, one on each side of the middle line of the tentacle. The cœlentera of these polyps extend a considerable distance into the stem of the colony, and are crowded with ova.

II. The Zooids.—These are much more numerous than the polyps and much smaller, measuring only 3 mm. to 5 mm. in length, and from ·7 mm. to 1 mm. in breadth. The tentacles are eight short simple lobes, ·14 mm. to ·2 mm. in length, and they bear no pinnules. The cœlentera of these zooids extend at most only 3 mm. into the stem, and contain no gonads.

It should be noted that Kölliker saw two kinds of small individuals, and described the differences between them in size, structure, and position, viz.: (1) the young polyps, 5 mm. to 20 mm. long., found only round the edge of the disc; and (2) the "zooids," 3 mm. to 5 mm. long, found all over the disc among the bases of the larger polyps.

Klunzinger (1877), who described the Xeniidæ of the Red Sea, saw small polyps in a specimen of Xenia umbellata; he called them bud-like polyps, and said that their tentacles,

which are at first simple, very soon show indentations or pinnules. He regarded such individuals rather as young polyps than as zooids. They were more numerous in the outer part of the arched end of the stem. In a new species, Xenia fuscescens, Klunzinger described the small polyps as very numerous, outnumbering the large polyps, and filling up the intervals between the bases of the latter. He wrote that these small individuals do not appear to develop into fully formed polyps, but to remain in the bud-like stage, with short, simple, mostly incurled tentacles. They are 1 mm. to 2 mm. long and ·5 mm. broad, and are cylindrical or club-shaped. On account of the large numbers of these small individuals, Klunzinger placed his Xenia fuscescens near the Heteroxenia Elizabethæ of Kölliker. There are no transition stages between these small individuals which do not appear to develop into larger polyps and the adult polyps, and from the description and figures they appear to be quite as distinct as the two kinds of individuals described by Kölliker in Heteroxenia.

Haacke (1887), who examined some of the Xeniidæ in Torres Straits, says that the small individuals are merely young polyps, and all stages of development between them and the adult polyps may be met with. Therefore he denies the occurrence of heteromorphism in the Xeniidæ.

Wright and Studer in the 'Challenger Report' (1889) record the observations of Klunzinger and Haacke noticed above. They agree with Haacke, and therefore propose the provisional abandonment of Kölliker's genus Heteroxenia.

Bourne (1895) observed in his new species, Xenia Garciæ, numerous imperfect polyps or buds in all stages of growth at the edge of the polyp-bearing summits of the stems. He remarked that "these are not siphonozooids, but stunted or developing polyps."

Bourne also described a specimen which he referred provisionally (being unable to procure Kölliker's original description) to the species Heteroxenia Elizabethæ. In his description were noted—

(1) The larger polyps with well-developed tentacles, with three rows of lateral pinnules on their margins, their cœlentera continued to the bottom of the stem or nearly so, and filled with ova. At the edges of the arched end of the stem there were numerous young polyps in all stages of development, many of which showed distinct pinnules on their tentacles.

(2) Closely applied sterile zooids which have no tentacles, but only eight radiate lobes round the mouth. The cœlentera of these individuals extend only a little way into the stem, and then communicate with the cœlentera of the polyps by anastomosing endodermic canals. Among these zooids there are never any individuals which show signs of pinnate tentacles nor which contain gonads. Bourne concludes that in this form there is distinct dimorphism.

Schenk (1896) mentions the occurrence of buds in eight new species of Xenia from Ternate, which he has described. He briefly describes the external characters of the buds in Xenia viridis, and figures three stages of development. The first figure represents a small bud about 3 mm. long, in which the tentacles are simple finger-shaped lobes. The other two are drawings of slightly larger polyps the tentacles of which bear, in one case six, and in the other about twelve pinnules on each side of the tentacle (seen from the outer side). In all the examples mentioned by Schenk the small individuals are young polyps in course of development, as pinnules have already appeared on the tentacles of many of them. Schenk (loc. cit., p. 53) remarks that he believes the zooids of Kölliker are merely young polyps, and therefore Heteroxenia Elizabethæ does not exhibit dimorphism; hence he renames it Xenia Elizabethæ, and places it near X. fuscescens, Klunzinger. Thus Schenk supports Haacke's view that there is no dimorphism of the polyps of Xenia.

From this short account of previous observations it will be seen that the nature of these small individuals in the Xeniidæ is not yet determined. That they are all buds or young polyps is strongly affirmed by Klunzinger, Haacke, Wright and Studer, and Schenk; while the opinion of Kölliker and

Bourne is that they are of two kinds, some being young polyps and others quite different individuals, termed zooids.

With a view to coming to some definite conclusion regarding the nature of these small individuals, I have examined them in the specimen of Xenia Hicksoni in great detail. I have also examined the sixteen other specimens of Xenia at my disposal, and a specimen of the Heteroxenia which Bourne has described and figured. These will be briefly described below.

External Characters of the Buds of Xenia Hicksoni.

In Xenia Hicksoni the small individuals are all buds or young polyps, as in this one specimen every stage in development may be seen, from the youngest polyp only ·32 mm. in length to the large adult polyp 12 mm. long; and the series is perfectly complete, there being no break at any point which would justify the division of the individuals into two kinds.

The buds are invariably found on the edge of the arched end of the stem. Their numbers vary on different stems, there being usually from six to ten buds less than 3 mm. long on each stem (Pl. 23, fig. 1, left). The smallest bud (Pl. 24, figs. 4, 4A) measures ·32 mm. in length and ·44 mm. in width. It is situated just under the edge of the umbellate summit of the stem. It is a short cylindrical outgrowth, at the distal end of which the developing tentacles are indicated as eight small rounded lobes about ·1 mm. long, divided from each other by shallow furrows. A slight depression in the centre of the distal end indicates the position of the future mouth, which is not yet open to the exterior. The tentacles increase in length rather more rapidly proportionately than the body of the polyp. In the smallest specimen they are less than one third the total length of the polyp, but in rather larger polyps they form from two fifths to one half the total length of the polyp. The tentacles remain simple lobes until the polyps attain a length of nearly 1 mm. In a specimen ·95 mm. long (fig. 5) the

tentacles have reached a length of ·34 mm., and the tip of each tentacle is distinctly trilobed when seen from the outer side, i.e. there is an indication of the formation of the first two pinnules, one on each side of the axis of the tentacle.

From this point onwards the formation of pinnules takes place regularly as the tentacles increase in size, as may be seen from the table below. When the polyp (fig. 6) has reached a length of 1·42 mm. the tentacles, which are ·6 mm. long, are seen from the outer aspect to bear four pinnules on each side. If one of the tentacles be examined from the inner side it will be seen that an inner row of pinnules has already been formed (fig. 6A). The polyp from which fig. 7 was drawn was 2·27 mm. long, and its tentacles 1·30 mm. long. Seen from the outer aspect the tentacles show eight pinnules on each side. For a distance of about two thirds of a millimetre from the tip of the tentacle, there are on the inner face two rows of pinnules on each side of the middle line, but a little nearer the base of the tentacles the three rows of pinnules on each side, characteristic of the tentacles of the adult, may be seen (fig. 7A).

After the earliest stages of growth are passed the polyps grow quite regularly, and the length of the tentacles bears to the total length of the polyp an almost constant proportion. From the appended table of measurements it will be seen the tentacles form rather more than two fifths of the whole length of the polyp. With the increase in length of the tentacles there is a corresponding increase in the number of pinnules which the tentacles bear, and, as the following table shows, there is a perfect series of examples, beginning with the very young specimens, in which the tentacles are devoid of pinnules, and ending with the largest polyps, whose tentacles show from the outer aspect nineteen or twenty pinnules on each side of the middle line. The gradual transition from the youngest to the oldest polyps shows that the small individuals in this colony are undoubtedly young buds in various stages of development. They are all "polyps," using the word in the sense in which it was used by Kölliker.

Reference Number.	Total length of Polyp (Body and Tentacles).	Width of Polyp.	Length of Tentacles.	Number of Pinnules on each side of Tentacle (outer aspect).	
	mm.	mm.	mm.		
I.	·32	·44	·10	0	Pl. 24, figs. 4, 4A.
II.	·43	·4	·12	0	In section, Pl. 27, fig. 28.
III.	·56	·37	·19	0	—
IV.	·8	·4	·31	trace of first	—
V.	·95	·35	·34	Trilobed = 1	Pl. 24, fig. 5.
VI.	1·0	·34	·43	Trilobed = 1	In section, Pl. 25, fig. 8.
VII.	1·1	·5	·53	2	—
VIII.	1·42	·87	·6	4	Pl. 24, figs. 6, 6A.
IX.	1·6	·96	·7	4—5	—
X.	2·0	·86	·86	6	—
XI.	2 1	·8	1·03	7	—
XII.	2·27	·6	1·3	8	Pl. 24, figs. 7, 7A.
XIII.	2·8	·86	1·4	8	—
XIV.	3·2	·9	1·4	8—9	—
XV.	4·0	1·0	1·8	10	—
XVI.	6·1	·9	2·4	10—11	—
XVII.	6·5	·9	2·5	11—12	—
XVIII.	6·8	1·1	2·85	13	—
XIX.	9·1	1·2	4·2	14—15	—
XX.	12·0	1·2	5·1	17	—
XXI.	11·65	1·2	5·7	19—20	Pl. 24, fig. 2.

I find in all the sixteen other specimens of Xenia at my disposal, young buds in all stages of growth, similar to those described and figured in Xenia Hicksoni. Most of these young buds occur on the arched end of the stem, but in two of the colonies examined a few buds are found in the middle portion of the summit between the bases of the larger polyps. These buds, however, differ in no way from those round the edge of the summit.

External Characters of Heteroxenia Elizabethæ (Pl. 27, fig. 37).

Bourne (1895) has already described the external characters of a colony similar to this one, but a brief description will be given here. The colony is somewhat triangular in shape, the base of the triangle being formed by the polyp-bearing summit of the stem. The stem is slightly flattened, and is narrow

at its lower end, gradually widening from below upwards. The total length of the stem is about 30 mm., its breadth at the base 12 mm. by 6·5 mm., its breadth at the top 23 mm. by 6 mm.

The non-retractile polyps (*A.*) are long and slender, and measure on an average about 15 mm. in length (including the tentacles), though a few specimens reach almost 30 mm. in length. The polyps are 1 mm. to 2 mm. broad. The tentacles of the polyps measure 4 mm. to 5 mm. in length, and bear on the inner side two series of rather slender pinnules, each series consisting of three rows of about sixteen to twenty-four pinnules in each row. In one or two of the tentacles examined, the pinnules at the base are so arranged that it is difficult to say whether they are in three or four rows on each side of the middle line. The pinnules in the middle of the tentacle measure about ·5 mm. in length, and are about four times as long as they are broad. The middle line of the tentacle is free from pinnules along the greater part of its length. The polyps are 1 mm. to 3 mm. apart, but they are closer together round the edge of the summit of the stem than on the middle portion of the summit. Around the edge of the summit of the stem there are many small polyps in all stages of development,—in fact, all the polyps near the edge of the summit are small ones, the large ones being situated in the middle portion of the polyp-bearing area.

The walls of the polyps are thin, and when the colony is removed from spirit the polyps fall together and form a confused mass on the end of the stem.

Between the bases of the polyps there are many closely apposed small zooids (fig. 37, *S.*), quite different in appearance from buds or young polyps. These siphonozooids are from six to ten times as numerous as the polyps or autozooids, and they occur all over the summit of the stem. The siphonozooids are all similar in appearance, being cylindrical or club-shaped, 2 mm. to 5 mm. in length and ·5 mm. to 1 mm. in diameter. The tentacles are eight small rounded lobes arranged round the mouth; they are never longer than ·25 mm., and never possess pinnules.

Bourne provisionally referred this specimen to Kölliker's species Heteroxenia Elizabethæ, and, after comparing the specimen with Kölliker's original account and figures (1874), I can confirm his conclusion. This specimen is much smaller than Kölliker's, its polyps being only about half the size, but there are many points of agreement between them, viz. general anatomy and proportion of parts, size, and structure of zooids; size, colour, and distribution of spicules; canals, &c.

After carefully examining the two kinds of small individuals (viz. young polyps and zooids), I have no doubt that they are entirely different in nature. The external characters are quite different, and there are important differences in their internal arrangements, to which reference will be made.

External Characters of the Young Polyps of Heteroxenia Elizabethæ.—The youngest polyp, which is also by far the smallest individual on the colony, is ·64 mm. in length (fig. 38). Its tentacles are finger-shaped lobes ·24 mm. to ·3 mm. in length. The tip of one of the tentacles is slightly three-lobed, but all the other tentacles have simple rounded ends.

A polyp 1·4 mm. long, the tentacles of which have attained a length of ·4 mm., are trilobed at their ends, i.e. the first pinnule on each side of the axis is being formed. The formation of pinnules proceeds regularly with the growth in length of the tentacles, and the adult size and characters are gradually attained. From the appended table it will be seen that there is a complete series of stages from the smallest polyp, ·64 mm. long, with simple tentacles devoid of pinnules, to the largest polyp, nearly 30 mm. long, the tentacles of which show on the outer face twenty-four pinnules on each side of the middle line. In this Heteroxenia the ratio between the length of the tentacles and the total length of polyp is not quite as constant as in the Xenia considered above. The tentacles form about one fourth to one third the length of the whole polyp.

Measurements of the Autozooids of Heteroxenia Elizabethæ.

Reference Number.	Total Length of Polyp.	Width of Polyp.	Length of Tentacles.	Number of Pinnules on each side of Tentacle (outer aspect).	
	mm.	mm.	mm.		
A 1 . .	·64	·3	·24—·3	0—1	Pl. 27, fig. 38.
A 2 . .	1·4	·5	·4	Trilobed = 1	—
A 3 . .	2·0	·7	·9	3—4	A 3, fig. 37.
A 4 . .	2·8	·9	1·0	5	—
A 5 . .	4·4	·7	1·3	7	A 5, fig. 37.
A 6 . .	5·8	1·1	1·7	8	—
A 7 . .	6·0	1·1	2·2	12—13	—
A 8 . .	9·1	2·0	3·1	15	—
A 9 . .	12·8	1·2	3·5	15—16	—
A 10 . .	14·0	2·4	4·3	16	—
A 11 . .	20·2	2·0	5·0	18	—
A 12 . .	28·4	2·4	5·2	24—25	—

External Characters of the Siphonozooids of Heteroxenia Elizabethæ.—The siphonozooids, like the autozooids, are non-retractile. These vary in length from 1·8 mm. to 5·0 mm., and are ·5 mm. to 1·0 mm. wide. Their tentacles are all in the same condition, and are mere lobes from ·2 mm. to ·25 mm. in length, and never show any traces of the formation of pinnules. It will be seen from the appended table that there is no development of the tentacles corresponding to the increase in length of the individuals, so that the tentacles of a zooid 5 mm. in length are very similar to those of a specimen less than half its size (compare S 1 and S 7 in table).

Table of Measurements of the Siphonozooids of Heteroxenia Elizabethæ.

Reference Number.	Total length of Zooid.	Width of Zooid.	Length of Tentacles.	
	mm.	mm.	mm.	
S 1 . .	1·8	·7	·2	—
S 2 . .	2·0	·8	·2	—
S 3 . .	2·3	·8	·23	—
S 4 . .	3·0	·8	·24	—
S 5 . .	4·5	1·0	·2	—
S 6 . .	4·8	·6	·25	—
S 7 . .	5·0	·8	·2	Figs. 37, 39.
S 8 . .	5·0	·9	·25	—

By means of their rudimentary, short, rounded tentacles the zooids may be readily distinguished from young polyps of the same size, whose tentacles are longer, pointed, and bear pinnules. Compare, for example, the young polyp A 5 (see table, p. 287) with the siphonozooid S 5. They are both about the same length, but the tentacles of the former are 1·3 mm. long, and, examined from outer aspect, show seven pinnules on each side of the middle line, whereas the tentacles of the siphonozooid are only ·2 mm. long, and are simple rounded lobes. Now if, as urged by Schenk and others, these two individuals are both young polyps, how can the differences in the size and character of their tentacles be accounted for? If the zooids are stages in the development of polyps, it is very difficult to account for so many being in the same stage of development (as the tentacles of all the specimens examined are in the same simple condition), when, on the same colony, other individuals of the same length, or even smaller, have already acquired some of the adult characters, viz. the pinnate tentacles. On the colony drawn in fig. 37 there are over two hundred zooids, all of which are similar in appearance, having simple round tentacles. The examples indicated in the above table are chosen haphazard from this large number, being arranged in order of length merely for convenience of reference. If these were young polyps we should

expect to find a more or less constant increase in the length of, and alteration in the character of, the tentacles, with the increase in length of the individuals; but this is not the case. We should also expect them to resemble other individuals of the same length on the colony which are undoubtedly young polyps; but, as we have seen, they are very different.

It must be concluded, then, that these zooids are different in nature from young polyps, and that there are in Heteroxenia two kinds of individuals, polyps and zooids, or, to use Moseley's terms, autozooids and siphonozooids.

The Internal Anatomy of Heteroxenia Elizabethæ.

Owing to the very imperfect preservation of the specimen, it is impossible to give a detailed account of the internal structure of the colony, and therefore attention will be chiefly directed to the points in which Heteroxenia differs from the Xenia described in detail in the former part of this paper.

Autozooids.—Spicules are numerous in the ectoderm of all parts of the body of the autozooid, and are very numerous in the ectoderm of the outer face of the tentacles. They are also present in the pinnules. They are similar in size and shape to those of Xenia Hicksoni. They are whitish or bluish white by reflected light, but of a reddish-brown tinge by transmitted light. Nematocysts are exceedingly scarce and difficult to find. They measure 9 μ in length and $2\frac{1}{2}$ μ in width. The ectodermic muscles of the tentacles are well developed; the muscle band of the oral face is very much thicker than that of the aboral face.

The oral disc of each polyp is slightly contracted, producing a funnel-shaped depression leading to the mouth. The stomodæum is only 1 mm. to 1·5 mm. long, and is wrinkled or folded transversely. It bears a ventral groove or siphonoglyph, the cells of the lower half of which bear moderately long flagella. There are the usual eight mesenteries, bearing rather feebly developed retractor muscles on their ventral faces. Only the

dorsal mesenteries bear mesenterial filaments, and these have a typical course and structure. Owing to imperfect preservation it is impossible to say anything definite about the cells of the other mesenteries, but no ventral or lateral mesenterial filaments are visible.

The cœlentera of the polyps may be traced a considerable distance into the stem, those of the primary polyps being continued down to the base of the colony. In the upper portion of the stem the cœlentera contain many ova, each of which is surrounded by an endodermic follicle attached to the mesenteries. The largest of these ova measure ·3 mm. to ·4 mm. in diameter.

Siphonozooids (fig. 39).—Spicules are not so numerous in the ectoderm of the zooids as in that of the polyps.

The stomodæum is very badly preserved in most of the specimens. A few of the zooids situated near the edge of the summit, however, are rather better preserved, and two of these (S 3 and S 7 in the table, p. 288) have been sectioned and the stomodæum examined. In the zooid 2·3 mm. long the stomodæum measures ·6 mm., and shows a well-marked siphonoglyph, the cells of the lower ·2 mm. of which bear flagella. The other specimen (S 7 in table, see also fig. 39) is 5 mm. long. Its stomodæum measures ·8 mm. long, and the cells of the lower ·4 mm. of the siphonoglyph bear flagella.

Thus both autozooids and siphonozooids of this specimen possess a siphonoglyph, the cells of the lower half or third of which bear flagella. This does not agree with the observations of Hickson (1883, p. 696) on a specimen of Heteroxenia, in the siphonozooids of which he found a well-marked siphonoglyph, but in the autozooids a complete absence of siphonoglyph.

The siphonozooids possess the usual eight mesenteries, but they are extremely thin, and retractor muscles are not visible upon them. The dorsal mesenteries bear mesenterial filaments (fig. 39, *D. M. F.*), which run in a sinuous course down the dorsal side of the polyp, and a short distance into the portion of the cœlenteron contained in the stem. The cœlen-

tera of these siphonozooids cannot be traced more than two millimetres into the stem. At this depth the cœlentera terminate in connection with one or more of the numerous endodermic canals. Ova are not present in any of these cœlentera.

Stem.—No definite superficial endodermic canal system is distinguishable on the summit of the stem, except at its edge, where the young buds appear to originate from it. It is, however, present in the cylindrical portion of the stem, and has a similar appearance and relations to the corresponding canal system of Xenia Hicksoni.

The longitudinal canals are developed to a much less extent than in Xenia Hicksoni. They are small, and their lumen is often almost obliterated. Canals are most numerous in the upper part of the stem, especially in and immediately below the portion penetrated by the cœlentera of the siphonozooids. In this region of the stem there are very numerous short transverse canals which place the various cœlentera in intimate communication with each other.

The Origin and Internal Structure of the Buds of Xenia Hicksoni.

In this specimen the buds or young polyps arise on or just under the edge of the umbrella-shaped area at the end of the stem. In most other species of Xenia the buds generally arise in a similar position, but in two of the colonies I have examined, and in three of the new species described by Schenk (1896), buds occur not only round the edge, but a few also on the middle portion of the expanded end of the stem. The buds are much more numerous on the edge of the summit of the stem and in Xenia Hicksoni, and, in fact, in most specimens the buds are found only in this position.

On examining a transverse section of the upper portion of a stem of the colony, the cœlentera in the peripheral portion of the stem are seen to be smaller than those in the central portion, and some of them are obviously the cœlentera of very young polyps (Pl. 25, fig. 9, right).

1. The buds arise in connection with that portion of the superficial endodermic canal system situated at the edge of the summit of the stem. When a bud is about to be formed, the superficial canal becomes enlarged and the outer wall of the canal becomes pushed outwards towards the surface of the stem. This produces a small tubercle upon or immediately under the edge of the expanded end of the stem. The tubercle increases in size, and its distal end soon becomes divided into eight small pouches which become the tentacles of the polyp (Pl. 24, fig. 4). The divisions between the pouches are the mesenteries of the polyp, and they gradually grow inwards into the cœlenteron. The eight mesenteries are already formed in the young polyp shown in fig. 4.

In the centre of the free end of the bud there is a slight depression, and a small darker area which marks the position of the future mouth (fig. 4, *Mo.*), and also the ingrowing plug of ectoderm which forms the stomodæum. Sections of this bud show that the stomodæum is ·14 mm. long, and rather flattened laterally. Its oral end is still solid, but the inner portion has become tubular, and its cavity opens into the cœlenteron of the polyp. The distance from the depression indicating the position of the mouth to the innermost portion of the cœlenteron is ·4 mm. Mesenteries are distinguishable only in the upper three fifths of the cœlenteron. In other respects this polyp resembles the one described below, and drawn in fig. 28.

2. A slightly older polyp (No. II in table, p. 284, and drawn in section, fig. 28), ·43 mm. long, possesses tentacles which are not all of the same size. The dorsal and ventral ones are largest (about ·12 mm. long), the two lateral ones a little smaller, and the remaining four still smaller. These differences in size are very well seen in transverse sections through the polyp at the level of the mouth. A second specimen of the same size was cut longitudinally, and shows several interesting points (fig. 28). The stomodæum is about ·32 mm. long, and is tubular along the greater part of its length. The mouth is, however, closed partly by approximation of the walls

of the stomodæum and partly by a small plug of mucus. The siphonoglyph is not yet differentiated. The distance from the mouth to the inner end of the cœlenteron is about ·7 mm. The mesenteries may be traced almost to the inner end of the cœlenteron, and their retractor muscles are just distinguishable. The dorsal mesenteries are slightly thicker than the others, but their mesenterial filaments have not yet been formed. The cœlenteron is formed by enlargement of the superficial endodermic canal. An evagination of the outer wall of the canal forms the cœlenteron of the free portion of the polyp, and pushes outwards the mesoglœa and ectoderm, thus giving rise to the protuberance which forms the free portion of the young polyp. A diverticulum produced by the bulging inwards into the mesoglœa of the inner wall of the canal forms the inner part of the cœlenteron.

The endoderm of the outer wall of the superficial canal is much thicker than that of the inner wall (fig. 29). This difference enables one to see in section (fig. 28) that the endoderm of the whole of the free portion of the polyp is derived from the thick outer wall of the canal. The endoderm of the portion of the cœlenteron situated in the outer part of the stem is formed directly from that of the canal, while the endoderm of the inner portion of the cœlenteron is derived from that of the thin inner wall of the canal, which has grown inwards into the mesoglœa. The cœlenteron gives off a large canal at its base, which opens into the adjacent longitudinal canals. During its short course in the stem the cœlenteron communicates several times by means of short canals with the neighbouring superficial and longitudinal canals.

Among the endoderm cells of the middle portion of the cœlenteron there are a very few flagella-bearing cells. The flagellum is a short conical or finger-shaped process which in these sections is never more than 15 μ in length.

3. A slightly longer bud (No. III in the table, p. 284), ·56 mm. long, possesses, like the preceding example, tentacles of different sizes. In this specimen also the dorsal and ventral tentacles are largest, the lateral ones being next in point of

size, and the four intermediate ones somewhat smaller. Does this indicate the order of development of the tentacles? This bud resembles the preceding one in all essential particulars.

4. There are several important new structures noticeable in a young polyp ·8 mm. in length (No. IV in table, p. 284). The tentacles are very slightly indented a short distance from the tip; this is the first indication of the formation of pinnules upon the tentacles.

The stomodæum is ·57 mm. long, and oval in transverse section. It is open throughout its whole length, thus placing the cœlenteron in communication with the exterior. There is now also a marked ventral groove, which, however, is not distinguishable in the outer third of the stomodæum. The cells of the lower two thirds of the groove bear flagella, except for a very short distance near the inner end. Some of the cells of the lower half of the stomodæum contain a large cavity, and are similar to the goblet-cells described in the stomodæum of the adult. They are more numerous on the lateral walls of the stomodæum, and do not occur among the cells forming the siphonoglyph. The ectodermic muscles of the tentacles and the retractor muscles on the mesenteries are now quite obvious. The endoderm covering the mesenteries and lining the body-wall is very thick in the free portion of the polyp, (as in fig. 28), and the intermesenterial spaces are consequently very small. The mesenteries may be traced nearly to the end of the cœlenteron, i. e. about 1 mm. below the lower end of the stomodæum. Flagella-bearing cells are present in the middle and lower portions of the cœlenteron, but the flagella are still few in number and of small size, never excceding 20 μ in length.

The most novel feature in this polyp is the presence of dorsal mesenterial filaments, which may be traced more than halfway (·6 mm.) down the free edge of the dorsal mesenteries. They have already acquired their typical structure, i. e. each is a band of ciliated cells on the somewhat thickened edge of each of the dorsal mesenteries, and there is a longitudinal groove down the middle of the band, so that in transverse section

it is V-shaped. The filaments have a very sinuous course, and are therefore cut across two or three times each in the same section. These filaments are undoubtedly derived from the lower end of the stomodæum, with which they are perfectly continuous. Their cells are exactly like the cells of the stomodæum, being small and having homogeneous or finely granular deeply staining protoplasm and dark nuclei. Their cells differ markedly from the cells of the surrounding endoderm, which are larger, have reticulate protoplasm, stain more lightly, and have less distinct nuclei.

There is another very interesting feature worthy of note in this polyp, viz. that sexual cells are already clearly differentiated. Sections through the mesenteries in the lower part of the cœlenteron show that a considerable number of the endoderm cells covering the mesenteries are more spherical, and two or three times as large as their neighbours. They have clearer protoplasm than the ordinary endoderm cells, and their nuclei are large (5 μ in diameter) and vesicular, and possess a well-marked, deeply staining nucleolus (fig. 36 shows them in a slightly older polyp). These cells occur in all the mesenteries, but are more numerous in the ventral and lateral than in the dorsal ones. Some of the cells have already migrated into the mesoglœa of the mesenteries, but most of them still retain their position in the endoderm. These modified cells are present only in the portion of the mesenteries situated in the lower two thirds of the cœlenteron.

On comparing these cells with the primitive sperm cells of the adult the resemblance is so striking that I am convinced these modified endoderm cells are the sexual cells which have become differentiated at this early stage in the development of the polyp. This view is supported by the following facts :

a. They are at first endoderm cells covering the mesenteries, which, on becoming differentiated, migrate into the mesoglœal lamina of the mesentery. This agrees with the origin, migration, and position of the sexual cells traced in the adult polyps (see p. 263).

b. These cells are found in the mesenteries some distance

below the lower end of the stomodæum. They are not present in the mesenteries of the free portion of the polyp, but in the portion enclosed in the stem. They are therefore in the position in which the genital products of the adult polyps are most numerous (see p. 263, and fig. 8).

c. They agree in size and are similar (especially their large vesicular nuclei) in appearance to the primitive genital cells of the adult.

Fig. 36 shows a section through a ventral mesentery of a slightly older polyp (No. V in table, p. 284). The genital cells are still more clearly marked than in the preceding polyp, and, as shown in the figure, are readily distinguished by their large vesicular nuclei. Many of them have already taken up their position in the mesoglœa.

On comparing the polyp ·8 mm. long with one about two thirds its size (·56 mm. long) it is seen that during the period in which the elongation of ·24 mm. was accomplished, many important adult structures were acquired, viz. the first indication of pinnules on the tentacles, the open mouth, the siphonoglyph, the goblet-cells in the stomodæum, the dorsal mesenterial filaments, and the primitive genital cells. A polyp which has reached this stage of development would probably be quite capable of supporting itself, as, by means of its siphonoglyph and dorsal mesenterial filaments, it would be able to create the currents necessary for its nutrition and for keeping up the circulation of liquid in the cœlenteron.

In a polyp ·95 mm. long (V in table, p. 284) the first two pinnules are distinguishable upon each tentacle. The stomodæum is ·6 mm. long, and the siphonoglyph extends along the ventral side of the lower, ·34 mm. The lips of the stomodæum are widely open below. Flagella-bearing cells are much more numerous in this polyp than in any of the young polyps previously described. They are fewer in number in the upper part of the cœlenteron than in the deeper portions. Their flagella are still short, not exceeding 30 μ in length (fig. 36, *G. F.*).

A polyp 1 mm. long (VI in table) is seen in section in Pl.

25, fig. 8. Its tentacles bear near the tip one pinnule on each side of the axis. The stomodæum is ·74 mm. long, and exhibits a well-marked siphonoglyph, bearing flagella in its lower half. The dorsal mesenterial filaments are well developed, and extend in a sinuous course nearly 1 mm. down the cœlenteron. The cœlenteron extends 1·4 mm. into the stem, curving so as to be parallel to the neighbouring cœlentera. Its connections with the longitudinal and superficial canals may be seen in the figure. The flagella of the endoderm cells attain 40 μ in length. The distribution of spicules, nematocysts, and zooxanthellæ is shown in the figure. In such a polyp all the adult structures are differentiated. In the growth of the polyp, from this point onwards to the full adult size, there are few features which call for comment. The chief of these are the formation of a greater number of pinnules on the tentacles; the elongation of the cœlenteron outwards (as the free portion of the polyp grows in length) and inwards into the mesoglœa, where it curves, so as to lie parallel to the neighbouring cœlentera (Pl. 25, fig. 8); the increase in number and size of the giant flagella on the endoderm cells, and the development of sperm sacs on the mesenteries.

General Summary.

The following is a recapitulation of the new points to which reference is made (paragraphs 1, 2, and 6 have been already published in 'Proc. Roy. Soc.,' 1898):

1. The absence of ventral and lateral mesenterial filaments usually present in the polyps of the Alcyonaria. The only previously recorded examples of the absence of these filaments are the siphonozooids which occur in some other Alcyonacea and in Pennatulids.

2. The absence of these filaments is correlated with the presence of gland cells in the stomodæum, which occur especially in the ventro-lateral walls which abut on the siphonoglyph. Their position is suggestive of the digestive function of the cells, as their secretion can be readily poured out on to the ingoing food particles.

3. The non-retractile nature of the bodies of the polyps and of the stems is accounted for by the absence of muscle-fibres from their ectoderm cells, and by the presence of numerous spicules in these parts. The presence of ectodermic muscles in the tentacles, pinnules, and distal millimetre of the bodies of the polyps, together with the absence of spicules from these parts, confers the power of contractility, slight though it is, upon these parts. The muscle processes of the ectoderm cells (where present) are longitudinal in direction, while those of the endoderm cells are circular (except the protractor and retractor muscles on the mesenteries).

4. Nematocysts were found in Sarcophyton.

5. An extraordinary number of spicules is present in the basal part of the colony, and much of the mesoglœa is converted into a dense horny substance. Thus a firm base of attachment is provided which would afford a rigid support for the branches which arise from it.

6. Many of the endoderm cells lining the cœlentera and tentacles bear giant flagella, which may attain 120 μ in length.

7. In adult polyps the primitive genital cells are formed by differentiation of some of the endoderm cells which cover the mesenteries. These genital cells migrate into the mesoglœa of the mesenteries, and then move outwards, one at a time, each cell pushing the endoderm and a thin film of mesoglœa before it, and so forming a small tubercle on the side or end of the mesentery. By division of the genital cell the spermatozoa are produced. They remain until ripe, surrounded by a thin film of mesoglœa, and by a layer of endoderm cells, many of which contain from two to five nuclei.

8. The longitudinal canals which traverse the mesoglœa of the stem are very well developed, and are physiologically, and possibly morphologically, equivalent to the cœnenchymal tubes of Heliopora cœrulea.

9. The nervous system is similar to that of Alcyonium; the stellate cells immediately outside the endodermic muscle-fibres are very clearly seen.

10. A complete series of developing polyps is described, beginning with very young specimens, in which the tentacles are devoid of pinnules, and ending with the adult polyps with multi-pinnuled tentacles.

11. A similar series of polyps of Heteroxenia Elizabethæ is described and compared with the siphonozooids of the same colony. It is concluded that Heteroxenia is undoubtedly dimorphic, as stated by Kölliker and Bourne.

12. The origin of the buds of Xenia Hicksoni from the superficial canal system, their subsequent growth, and the appearance in them of the mouth, stomodæum, siphonoglyph, gland cells in stomodæum, mesenteries, dorsal mesenterial filaments, and flagella of the endoderm cells, are traced. It is remarkable that the sexual cells are already differentiated in a young polyp ·95 mm. long.

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EXPLANATION OF PLATES 23—27,

Illustrating Dr. J. H. Ashworth's paper on "The Structure of Xenia Hicksoni, nov. sp., with some Observations on Heteroxenia Elizabethæ, Kölliker."

List of Reference Letters.

A, A 3, A 5. Autozooids of Heteroxenia (the numbers refer to the table on p. 287). *Cg.* Coagulum in central cavity of sperm sac. *Cn.C.* Cnidoblast cell. *D.M.* Dorsal mesentery. *D.M.F.* Dorsal mesenterial filament. *Ect.* Ectoderm. *Ect.Ch.* Chain or cylinder of ectoderm cells round cylinder of denser mesoglœa. *Ect.Str.* Strands of ectoderm cells. *End.* Endoderm. *End.Can.* Endodermic canals. *F.* Flagella of siphonoglyph. *G.C.* Gland-cells in stomodæum. *G.F.* Giant flagella of endoderm cells. *Gen.C.* Genital cells in various stages of development. *Long.Can.* Longitudinal endodermic canals. *L.M.* Lateral mesentery. *M.* Mesentery. *Mg.* Mesoglœa. *Mg.D.* Denser cylinder of mesoglœa around each cœlenteron in stem. *Mo.* Mouth. *Muc.C.* Mucus cells of ectoderm. *M.P.* Muscle processes of endoderm cells. *N.* Nucleus. *Nem.* Nematocysts. *N.C.* Nerve cells. *N.F.* Nerve fibrils. *R.M.* Retractor muscles in mesenteries. *S.S 7.* Siphonozooids of Heteroxenia (the number refers to the table on p. 288). *Sec.* Secretion of gland-cells of stomodæum. *Si.* Siphonoglyph. *Sp.* Spicules. *Spz.* Spermatozoa. *St.* Stomodæum. *Sup.Can.* Superficial canal. *S.S.* Sperm sacs. *V.M.* Ventral mesentery. *Z.* Zooxanthellæ.

PLATE 23.

Fig. 1.—View of the colony of Xenia Hicksoni. From one of the stems on the left side all the larger polyps have been removed, so that the umbellate summit of the stem, with the young polyps growing round its edge, may be

more clearly seen. Note also that the polyps are smaller and closer together near the edge than nearer the middle of the summit. The stem immediately to the right of this also shows that the young and small polyps are on the edge, while the large polyps are near the middle of the summit. The colour of the drawing is, as nearly as possible, the colour of the specimen in spirit. × 3.

PLATE 24.

Fig. 2.—View of the polyp XXI in the table, p. 284. This is one of the largest polyps of the colony. The tentacles show from the outer aspect a single row of pinnules on each side, but from the inner aspect three rows on each side. Note that at the tip of the tentacles the pinnules are not arranged in pairs. × 12.

Fig. 3.—Views of the tentacle of a large polyp. *A* and *B* are views of the tip, *C* and *D* of the base of a tentacle. *A* and *C* show the outer or aboral side, *B* and *D* the inner or oral side. The narrow area free from pinnules, which extends along the middle line of the oral face of the tentacle, is well seen in *D*. At the tip of the tentacle, *B*, the pinnules are in two rows only on each side of the middle line. × 24.

Fig. 4.—Lateral view of the youngest polyp (·32 mm. long) in the colony (I in table). × 40.

Fig. 4a.—Oral view of the same polyp. The tentacles are eight rounded lobes. The depression (*Mo.*) in the centre of the oral disc indicates the position of the future mouth, and the darker area below it is the ingrowing plug of ectoderm which forms the stomodæum. × 40.

Fig. 5.—An older polyp ·95 mm. long (V in table). The tentacles are trilobed at the end, i. e. the first pinnules are being formed. × 24.

Fig. 6.—A polyp 1·42 mm. long (VIII in table). × 24.

Fig. 6a.—View of the oral face of a tentacle of the polyp shown in fig. 6. Two rows of pinnules on each side of the middle line are now present. × 24.

Fig. 7.—A polyp 2·27 mm. long (XII in table). × 24.

Fig. 7a.—View of the oral face of a tentacle of the polyp shown in fig. 7. In the middle part of the tentacle, three rows of pinnules on each side of the middle line are now distinguishable. × 24.

PLATE 25.

Fig. 8.—A thick longitudinal section through the upper part of one of the stems. The section passes along the dorso-ventral axis of a young polyp (VI in table, p. 284) growing out just under the arched summit of the stem. On the dorsal or upper side of the polyp one of the dorsal mesenteries (*D. M.*) is cut through obliquely for a short distance. The stomodæum (*St.*), siphonoglyph (*Si.*), the course of the dorsal mesenterial filaments (*D. M. F.*), the thin edge

of the ventral mesentery (*V. M.*), and the connection of the cœlenteron with the canal system are shown. Three cœlentera of older polyps are also shown. Two of them are crowded with sperm sacs (*S. S.*), many of which, owing to mutual pressure, have lost their original spherical shape and are pentagonal or hexagonal in section. The other cœlenteron contained a similar number of sperm sacs, but they have been omitted in order to more clearly show the dorsal mesenterial filament (*D. M. F.*) and the thin edge of the ventral mesentery (*V. M.*). The superficial (*Sup. Can.*) and longitudinal (*Long. Can.*) canal systems, and their relation to each other and to the cœlentera; the denser cylinder of mesoglœa (*Mg. D.*) with its surrounding ectoderm cells enclosing each cœlenteron; and the distribution of spicules, nematocysts, and zooxanthellæ are also shown. × 35.

Fig. 9.—A thinner transverse section through the upper part of one of the stems. The cœlentera in the peripheral part are smaller than those nearer the centre. The cœlenteron of a very young polyp is seen on the right. The cells in the mesoglœa of the mesenteries and sperm sacs in various stages of development, attached to the mesenteries of the older cœlentera, are also shown. The number of flagella cut across in one section is seen; thus an idea may be formed of their abundance and of their size relative to the endoderm and to the cœlentera. Many of the features to which attention was drawn in the description of the previous figure are also shown here. × 50.

Fig. 10.—Transverse section through a polyp at the level of the lower third of the stomodæum. The chief features shown are the gland-cells and siphonoglyph of the stomodæum, the somewhat feebly developed retractor muscles on the mesenteries, and the distribution of the spicules, nematocysts, and zooxanthellæ. × 50.

Fig. 11.—Transverse section of the outer wall of a pinnule showing the ectoderm containing nematocysts, the mesoglœa, and the endoderm cells with their reticulate protoplasm. × 800.

Fig. 12.—A nematocyst in its cnidoblast cell, from a section. One half of each coil of the thread in the upper part of the nematocyst was cut away in sectioning. × 2000.

Fig. 13.—Eight large spicules selected from those in the base of the colony. Each spicule is situated in a small cavity in the mesoglœa. The nucleus and remains of the protoplasm of the spicule-forming cell are seen. From sections. × 800.

Fig. 14.—A portion of the outer part of the mesoglœa from a transverse section of a polyp. Four of the spicules present their edge and two their flat face to the observer. × 800.

Fig. 15.—Two very young spicules in their respective cells. The spicule in *A* is $3\frac{1}{2}$ μ long and $2\frac{1}{2}$ μ broad. The spicule in *B* presents its edge to the observer. It is 7 μ long and $1\frac{1}{2}$ μ thick. × 1000.

PLATE 26.

Fig. 16.—Section of the wall of a polyp which has passed very obliquely through the mesoglœa, to show the nervous system. Fine nerve fibrils in the mesoglœa are connected on the outer side with cells in the outer part of the mesoglœa, some of which send processes into the ectoderm. On the inner side, the fibrils pass into small stellate nerve-cells situated just outside the muscle processes of the endoderm cells. × 300.

Fig. 17.—Longitudinal section through the body-wall of a polyp, to show the general character of the ectoderm and endoderm cells, and also the processes of ectoderm cells which penetrate the mesoglœa and establish connection with the endoderm. × 400.

Fig. 18.—Longitudinal section of the ventro-lateral portion of the lower third of the stomodæum. The chief feature shown is the large gland-cells, some of which have discharged their contents and appear empty, while others are in the act of discharging their secretion. × 600.

Fig. 19.—Transverse section of the end of a dorsal mesentery showing the V-shaped mesenterial filament bearing cilia. × 600.

Fig. 20.—Transverse section of the end of a ventro-lateral mesentery to show the general character of the endoderm cells, the giant flagellum of one of them, and the cells in the mesoglœa. × 600.

Fig. 21.—Transverse section of the end of the same mesentery taken ·25 mm. higher, showing three flagella occurring close together. × 600.

Figs. 22, 23, 24.—Three flagella from sections of polyps to show their various degrees of flexion. × 600.

Fig. 25.—Two abnormal forms of flagella from sections. *A* from the tentacle of a polyp, *B* from the portion of a cœlenteron in a stem. × 600.

Fig. 26.—Endodermic myo-epithelial cells from teased preparations. In connection with the one on the right there is a small stellate cell with three long processes. This is probably one of the nerve-cells of the endodermic portion of the nerve plexus. × 500.

Fig. 27.—Isolated cells bearing flagella, from teased preparations. × 500.

PLATE 27.

Fig. 28.—Section of a stem at the edge of the umbellate summit, to show the formation of a young polyp ·43 mm. long (II in table, p. 284). Note that the endoderm of the free portion of the polyp is thick, while that of the inner part of the cœlenteron is thin. The neighbouring cœlentera are cut very obliquely. × 50.

Fig. 29.—The superficial canal indicated by the asterisk in Fig. 9 enlarged. The cells of the outer wall are longer and more columnar than those of the inner wall. × 500.

Fig. 30.—Thin transverse section (3 μ thick) of the end of a ventro-lateral mesentery, to which three very young sperm sacs are attached. In the one to the right the primitive genital cell has divided into four, three only of which are visible. × 500.

Fig. 31.—Section of a sperm sac a little older than the largest one shown in the preceding figure. The central cavity containing a coagulum has now appeared. × 150.

Fig. 32.—Section of an older sperm sac. × 150.

Fig. 33.—Section of a mesentery, on the end of which is a sperm sac in which the central cavity, having reached its greatest size, is now being encroached upon by the heads of ripening spermatozoa. Note that the spermatozoa are surrounded by a thin film of mesoglœa (represented in the distal part of the sac by the line inside the endoderm), and by an endodermic follicle, some of the cells of which contain from two to five nuclei. × 150.

Fig. 34.—Ripe spermatozoa.

Fig. 34A.—From a section of the stomodæum, through the dorsal portion of which spermatozoa are escaping. Only the head and point of attachment of the tail are seen. × 800.

Fig. 34B.—An entire ripe spermatozoon from a teased preparation of a large sperm sac. × 800.

Fig. 35.—Cells from thin sections (4 μ thick) of large sperm sacs, to show the two to five nuclei which may be present in each. × 800.

Fig. 36.—Transverse section of a ventral mesentery of a young polyp ·95 mm. long (V in table, p. 284; see also Pl. 24, fig. 5). The section is taken about ·5 mm. below the lower end of the stomodæum. Note the already differentiated primitive genital cells migrating from the endoderm into the mesoglœa, and the short finger-shaped flagella of three of the endoderm cells. × 500.

Fig. 37.—View of a colony of Heteroxenia Elizabethæ. The colony was split in two longitudinally and the proximal half drawn, hence only half the summit of the stem is seen. Note the autozooids with pinnate tentacles, and the very numerous short siphonozooids with short rounded tentacles, and also the young polyps growing near the edge of the summit. × 3.

Fig. 38.—View of the youngest autozooid on the colony, ·64 mm. long (A 1 in table, p. 287). The specimen has been flattened out by pressure against the side of the bottle which contained the colony. Note the long finger-shaped tentacles; the right proximal one is slightly indented near the tip, but the others have simple rounded ends. The stomodæum is indicated. × 40.

Fig. 39.—The largest siphonozooid on the colony (S 7, fig. 38, and table, p. 288). The short simple tentacles, the stomodæum, and the two dorsal mesenteries with their filaments are shown. × 15.

Notes on the Batrachians of the Paraguayan Chaco, with Observations upon their Breeding Habits and Development, especially with regard to Phyllomedusa hypochondrialis, Cope. Also a Description of a New Genus.

By

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With Plates 28—32.

List of Batrachia collected by J. S. Budgett in the Paraguayan Chaco, 1897.

	Length.
I. Leptodactylus ocellatus, L.	120 mm.
II. Leptodactylus typhonius, Daud.	45 mm.
III. Leptodactylus bufonius, Boul.	55 mm.
IV. Leptodactylus pœcilochilus (Cope)	50 mm.
V. Phryniscus nigricans, Wiegm.	33 mm.
VI. Paludicola fuscomaculata, Steindachn.	40 mm.
VII. Paludicola signifera, Boul.	25 mm.
VIII. Paludicola falcipes (Hensel)	15 mm.
IX. Eugystoma ovale, Schn.	♀ 40 mm., ♂ 25 mm.
X. Eugystoma albopunctatum, Boul.	18 mm.
XI. Pseudis paradoxa, L.	50 mm.
XII. Pseudis limellum (Cope)	20 mm.
XIII. Bufo marinus, L.	150 mm.
XIV. Bufo granulosus, Spix.	50 mm.
XV. Phyllomedusa hypochondrialis, Cope	40 mm.
XVI. Phyllomedusa Sauvagii, Boul.	70 mm.
XVII. Hyla spegazinii, Boul.	80 mm.
XVIII. Hyla venulosa, Laur.	70 mm.
XIX. Hyla nana, Boul.	22 mm.
XX. Hyla phrynoderma, Boul.	43 mm.
XXI. Hyla nasica, Cope	28 mm.
XXII. Ceratophrys ornata, Bell.	120 mm.
XXIII. Lepidobatrachus asper, n. sp.	80 mm.
XXIV. Lepidobatrachus lævis, n. sp.	80 mm.

I. Leptodactylus ocellatus, L.

An extremely common frog, frequently found in the streets of Concepcion at sunset and on both sides of the river Paraguay.

At Concepcion, black markings on a greyish-green ground. At Waikthlatingmayalwa the ground is usually of a brighter green.

A triangular black spot at the back of the eyes is very constant.

The natives, who are Lengua Indians, name this frog Nukkmikkting, and use it largely for baiting their hooks. The largest measure 50 mm. from snout to vent.

The call is regularly repeated, beginning on a low note and ending on a high one, and is constantly heard in wet weather.

There is, however, another call, which is heard immediately after rain ; this is a drumming like that of a snipe.

A large variety found in the Chaco is called by the Lenguas Yattnukkmikkting; these measure up to 120 mm., and are only found down in the swamps. I think this may be L. bolivianus.

II. Leptodactylus typhonius, Daud.

Not nearly so common as L. ocellatus; I procured only two specimens, though I saw a few others. These were all seen at Caraya Vuelta on the river bank. The general colour is lighter than ocellatus, the spots are more numerous and smaller, and there is a bright gold band on either side running from the eye to the hips.

It appears to be about the same size as ocellatus.

No Lengua name was obtained for it.

III. Leptodactylus bufonius, Boul.

Small brown frog with blackish spots above, beneath pale

yellow. Most inconspicuous on a background of earth. It is very agile and extremely shy.

In damp waste places on the outskirts of Concepcion I found it in great numbers, but very difficult to capture.

The call is a shrill sharp "ping" kept up constantly until approached, when it immediately ceases. The croaking of so many of them at a time produces an almost continuous sound.

Though only one specimen was secured, it was frequently heard on both sides of the river. This is probably the young form of L. bufonius, which grows to about the same size as L. ocellatus. I never detected large individuals of this form calling, and I am convinced that during the continuous calling described above the individuals about were of the small form almost entirely.

It would appear, then, that either young forms have the habit of calling to one another, or that there is a small and a large variety. Lengua name Ukksaliapertikk. In Lengua Uksaelia means a coin or disc. The name refers to the spots.

IV. Leptodactylus pœcilochilus (Cope).

This frog is much less common than L. ocellatus. It is of a more slender build; the toes are thin and long, especially the second toe. The markings are all in the form of stripes rather than spots. These are dark brown on a greyish-brown ground. At the side yellowish. One broad dark stripe runs down the back on either side at the edges of the transverse processes of the vertebræ. One specimen was found at Concepcion and one at Waikthlatingmayalwa.

I do not know if it has a native name.

V. Phryniscus nigricans, Wiegm.

This is a brilliantly coloured frog of toad-like appearance. The ground colour is black, and is irregularly spotted with

yellow, or sometimes with large yellow blotches on the upper surface. Beneath it is black, with scarlet blotches; the palms of the hands and the soles of the feet are scarlet.

The variety found at Concepcion had on the under surface scarlet blotches extending to the throat, while the variety found at Waikthlatingmayalwa had the scarlet confined to the lower part of the abdomen.

This form, too, had yellow blotches irregularly arranged on the back, while the Paraguayan form had small yellow spots more regularly arranged.

On the journey between these two regions I twice met with large numbers of small black frogs which seem to be of this species. They were characterised by their smallness, and by the absence of either yellow or red markings.

At the breeding season the males and females have a call which consists of two clear musical "pings," followed by a long descending "trill" like that of our British greenfinch. The eggs are laid in separate globules of jelly, which float freely on the surface of the water, and are heavily pigmented.

This frog, which at ordinary times is the slowest and most bold of frogs, is now active and excessively shy. Swimming rapidly between the blades of grass it climbs a tuft, and, dilating its throat, repeats its call, but if in the least disturbed it is suddenly gone. This change of habit is very remarkable.

The spawn is found in quite temporary pools in grassy ground; the development is excessively rapid. Segmentation beginning at 10 a.m., they were hatched and wriggling about by 7 a.m. the following day. They probably are washed down into deeper pools by the retreating waters, and for this purpose the manner in which the eggs are laid, i.e. in separate globules of jelly, seems especially suited.

The native Lengua name is "Pithpaya."

The eggs and larvæ do not seem to differ in any great degree from those of Rana. There is, however, a very large yolk-plug, which remains evident after the closure of the neural groove.

VI. Paludicola fuscomaculata, Steindachner.

This is the largest of the genus that I found in the Chaco. It is a short-limbed frog, with spreading slender toes and small head.

The upper surface is marked with characteristic marblings, which vary, however, greatly in colour. The metatarsal tubercles are large, horny, shovel-shaped, and black.

The peculiar cry which is so constantly heard in the neighbourhood of shallow pools, and resembles that of a kitten, is produced by the alternate inflation of throat and abdomen.

When fully inflated the frog appears to be the size of a golf ball, but, if startled, instantaneously shrinks to one fifth of that size, so that it seems to have vanished. It has also the power of ventriloquising.

In the spawning time it was found at night floating on the surface of pools in the distended condition, and crying to the females in a most mournful way. On coming to the surface it fills its lungs with a few gasps, greatly distending the walls of the abdomen, and then drives the air into the throat diverticula of the pharynx, causing them to become distended as the stomach collapses, and giving rise to a kitten-like cry.

The eggs are chiefly laid in January, and are found embedded in a frothy mass floating upon the surface of the water. The eggs themselves measure 1 mm. in diameter, and are without pigment and with extremely little yolk. They become free-swimming within from eighteen to twenty-four hours of the time of the first segmentation. When ready for hatching they wriggle their way through the froth to the water below, and hang into it from the floating froth.

In this rapidly hatching, free-swimming larva many of the processes of development are blurred, and as it were hurried over. The external gills never reach a high state of development. The cell layers are many cells deep and diffuse, and the involutions and evolutions are difficult to follow.

The natives call this frog "Zing Ye," which of course applies to the genus generally, for the species differ very slightly.

In this species the testes are much pigmented and lobulated.

Its food consists largely of water-beetles.

VII. Paludicola signifera, Boul.

This is considerably smaller than fuscomaculata, and is usually an olive-green on the back without conspicuous markings.

Its general habits seem to be the same as those of P. fuscomaculata, as also its cry. It is most agile. I put this species into a cage in which were many brightly coloured frogs, including Phryniscus nigricans and also Phyllomedusa hypochondrialis. In this cage was also a small grass snake. Hitherto it had taken no interest at all in the gaudy frogs in its cage; but as soon as the little Paludicola made its first spring, it was caught in mid-air by the snake.

VIII. Paludicola falcipes (Hensel).

Only one specimen found at Concepcion by the river side. Its toes are even, long, and slender. Many of the specimens in the British Museum are marked with one broad light band running from nose to vent. But by no means all have this, neither does it depend on sex. In the specimen which I procured this stripe is very much marked.

IX. Engystoma ovale, Schn.

This frog has a small head and pointed nose. The eyes are set far forward, and there is an encircling fold just behind the eyes. The fore-limbs are very small, and the general shape of this frog proclaims it at once to be a burrower. The skin is perfectly smooth. It is greenish brown above, yellow beneath, and a bright yellow band passes up the thighs and over the

vent. In the male this band is bright red. The male is somewhat smaller. The natives call it "Po it," being convinced that the cry which sounds to them thus proceeds from this frog. However, in each case that I tracked down, the frog calling with this cry I found a Leptodactylus ocellatus. The cry was heard everywhere, but I only found one male and one female.

I think the native boys were here mistaken again; they pointed out to me holes in the ground beneath fallen tree trunks, of the size of a cricket ball and lined with a froth containing white eggs and also tailed larvæ. The entrance to the whole was about a centimetre in diameter. This they said was the nest of the "Po it."

I reared some of the eggs, and one as far as the four-legged stage, when the young frog bore a very strong resemblance to a Paludicola, but unfortunately escaped from my tank before it had lost its tail.

Though the information obtained from the natives generally turned out to be fairly accurate, yet I feel sure that in some instances they were quite wrong.

To whatever frog these nests belonged, it is certain that they were a most ingenious contrivance for collecting water and keeping the eggs and larvæ at least moist, between the storms of the wet season. They were always found within the forest belts which lay on the highest ground.

I found with these larvæ that they would exist for a very long time in a small quantity of water without increasing in size, but that when removed to a tank they grew enormously, and very soon left the water.

These eggs were somewhat larger than the minute eggs of Paludicola, 1¼ mm., and pigmentless. As far as my investigations have gone these eggs develop much as Paludicola, though they are rather more heavily yolked.

X. Engystoma albopunctatum, Boul.

About half the size of E. ovale, and found under a heap of

decaying vegetation in the forest. Plum-coloured and very glossy above, and greyish with white spots below. One specimen found. Native name unknown.

(The specimens collected by Bohls, in Paraguay, are all brightly spotted above.)

XI. Pseudis paradoxa, L.

A water-frog never seen on land, and extremely shy. Though often seen floating in a shallow pool, it was caught with great difficulty.

In life most beautifully coloured with bronze, bright green, and black markings above; underneath a satiny sulphur-yellow, with brown spots on the trunk and brown stripes on the thighs. On killing, all the brilliant colours of the back turned to a dull uniform brown in a few minutes.

Though there were a pair of these in a pool all through the early part of the wet season, yet the pool did not contain any of the well-known gigantic larvæ with reference to which the frog is named.

No native name known to me.

XII. Pseudis limellum (Cope).

Small green frog abundant on the camelota leaves at Concepcion. Capable of changing its colour greatly from bright green to dull brown, underneath silvery. Two white streaks run backwards from the eyes. The call is a succession of sharp croaks or vibrations resembling the sound made by castanets. The throat is inflated for each series.

They hop quickly over the surface of the water, and perch on the camelota leaves and stems. They are enabled thus to hop on the surface of the water by reason of the very large webs of the hind feet. The tips of the toes also have dilated discs. Their food consists mainly of small fresh-water Gasteropods. Females larger than the males.

No native name known to me.

XIII. Bufo marinus, L.

The common toad of South America, up to 150 mm. in length from snout to vent. It feeds on all kinds of insects, and is very useful in helping to keep down the mosquitoes. One half-grown toad, sitting by one man's foot, picked off fifty-two mosquitoes in the space of one minute, flicking them up with his tongue as they settled.

This toad, which may be found in every shed or outhouse, is called by the natives "Pinnikk." Its call consists of three bell-like notes, the middle one being the highest. The parotid glands are enormously developed, and, if the toad is roughly handled, are discharged like squirts. When wet weather comes it hops out from its hiding-place, and proceeds to sit in a puddle, with its head out.

XIV. Bufo granulosus, Spix.

A very common small toad, found in great numbers near water after rain. Dark above, with black, brown, and greenish blotches, and a light vertebral line. Skin much tuberculated. Calls with a continuous bell-like tinkle, the vocal sac being greatly distended. A great deal of variety in colour.

Native name "Kelaelik."

This species forms the chief food of the two newly described species of Lepidobatrachus.

XV. Phyllomedusa hypochondrialis, Cope.

A brilliantly coloured grass frog, which I found breeding freely in the Paraguayan Chaco, about 120 miles due west of Concepcion (fig. 34). Above it is brilliant green, which may become brown, grey, or bluish at will; below granular white. The flanks are scarlet with black transverse bars, and the plantar surfaces are a deep purplish black.

The "Wollunnkukk," as it is called by the Lengua Indians, from the call of both male and female at pairing time, is extremely slow in its movements, and is active only at night. At this time, if it is seen by the aid of lantern as it slowly climbs over the low bushes and grass, it is very conspicuous, as shown in the figure. In the daytime, however, nothing is seen but the upper surfaces of the body as it lies on the green leaf or caraguata plant, and here it is most inconspicuous.

This small Hylid has a remarkable power of changing the colour of its skin to harmonise with its surroundings, and can effect a change from brightest green to a light chocolate in a few minutes. The skin is also directly sensitive to light; for if the frog is exposed to the sun while in a tuft of grass in such a way that shadows of blades of grass fall across it, on removal it will be found that dark shadows of the grasses remain on the skin, while the general colour has been raised to a lighter shade. Its food consists largely of young locusts. The ovaries on each side are divided into five distinct clusters. The rectum has a large saccular diverticulum, which is very heavily pigmented.

In the breeding season—December to February—this beautiful grass frog collects in considerable numbers in the neighbourhood of pools. During the night-time they call incessantly to one another, and produce a sound as of a dozen men breaking stones, well imitated by the native name "Wollunnkukk."

As regards the native names for frogs, most species had their separate names; for instance, two species so closely like one another as Leptodactylus ocellatus and L. bufonius had their names respectively "Nukkmikkting" and "Ukseliapertic," but with the Tree frogs it was not so. I could get no name for any frog with dilated discs but "Wollunnkukk," whether they had a call resembling this name or not, and whatever their form, colour, and size. I may mention also that they had no general name for frog, though they had a general name for bird and fish.

Breeding Habits.—On November 30th, 1896, I caught six of these frogs at the edge of a shallow pool late at night, and put them with some leaves in a tin until the morning. Next morning I discovered batches of white eggs, in masses of firm jelly, lying about at the bottom of the tin. I put some of these in water, and some I kept damp. Those which I put in water died immediately; those which I kept merely moist I watched segmenting and developing until December 5th, and preserved several eggs of each stage, but on this day the last of the embryos died, and I tried hard to get some more, and to find out how they were laid in nature.

On December 31st I discovered a small leaf overhanging a pool of water, and containing a batch of the Wollunnkukk eggs. At this same pool I found within the next three weeks about twenty leaves enclosing batches of eggs, in no case more than two feet from the water.

On January 15th I had an opportunity of watching the process of egg-laying. About 11 p.m. I found a female carrying a male upon her back, wandering about apparently in search of a suitable leaf. At last the female, climbing up the stem of a plant near the water's edge, reached out and caught hold of the tip of an overhanging leaf, and climbed into it. With their hind legs both male and female held the edges of the leaf, near the tip, together, while the female poured her eggs into the funnel thus formed, the male fertilising them as they passed (fig. 35). The jelly in which the eggs were laid was of sufficient firmness to hold the edges of the leaf together. Then moving up a little further more eggs were laid in the same manner, the edges of the leaf being sealed together by the hind legs, and so on up the leaf until it was full.

As a rule two briar leaves were filled in this way, each containing about 100 eggs.

The male hurried away immediately the laying was over, and he did not embrace the female except during the act of laying eggs. The time occupied in filling one leaf was three quarters of an hour.

Life History.—Development proceeds very rapidly; within

six days the embryo increases from 2 mm. (the diameter of the egg) to 9 or 10 mm., when it leaves the leaf as a transparent glass-like tadpole whose only conspicuous part is its eyes (fig. 30). These are very large and of a bright metallic green colour, so that when swimming in the water all that is seen are pairs of jewel-like eyes.

The newly hatched tadpole has also a bright metallic spot between the nostrils somewhat in front of the pineal spot. This is the point which touches the surface of the water when the tadpole is in its favourite position. Whether it is a protective coloration, or some mechanical arrangement for holding the surface, I cannot say.

The leaves containing the eggs are not always directly over water, and the newly hatched tadpole has often to make his way many inches to the water.

This migration to the water usually takes place during a shower of rain, when the larvæ tend to be washed into the pool, but they also assist themselves by jumping several inches into the air. They are intensely sensitive to light and shock.

During the embryonic development the jelly surrounding the embryo becomes more and more dilated by the growth of the embryo, and also by the accumulation of fluid within. Towards the close of embryonic life the embryo comes to lie quite freely within a membranous capsule.

The eggs are very heavily yolked, and some yolk persists until the tadpoles are ready to leave the capsule.

On the third day external gills are well developed, and the red blood-corpuscles may be seen coursing through them, and the heart beating rapidly. These external gills reach their highest state of development about the fifth day, when they extend beyond the vent, and are of course bright red (fig. 27).

The tadpole is hatched without a trace of yolk, the external gills have completely disappeared, there is a median spiracle, and the lungs are already clearly visible shining through the transparent body-wall (fig. 30).

The day after the tadpoles are set free, pigment begins to be developed about the head and upper surface of the body.

There is a conspicuous absence of pigment for some time over the pineal body (fig. 26).

Black pigment appears first, then green. At the end of about five more weeks the tadpole has begun to develop its hind limbs. During this period it has grown to a length of 8 cm. The upper surfaces are now a glossy green, beneath silver and rosy; the tail is still transparent, and the red blood-vessels give it a bright red colour (fig. 31).

At the time of the development of the hind limbs there is a very great accumulation of black pigment at the middle of the tail, especially below (fig. 31).

The tail is absorbed very rapidly up to this point; the final absorption of the proximal part of the tail is postponed for some days.

The young frog, having now grown both pairs of legs, leaves the water and betakes itself to the blades of grass close by (fig. 32).

Here it sits during the time of absorption of the remainder of the tail. When lying in the blade of grass, only the brilliant green upper surfaces are visible, and the tail helps to make the young frog still less noticeable by shading off the body, and causing it to become merged in the green of the grass blade.

The young frog at the close of its metamorphosis is two thirds the length of the adult frog, and at this time acquires the red flanks barred with black (fig. 33).

There is a certain stage in the life of this larva when it will not bear transferring from the pool to aquaria. If the larvæ are transferred at the time when pigment in the tail is just beginning to accumulate, that is when they are 3 cm. in length, they invariably die, though both younger and older larvæ stand the change quite well.

Development.

External Characters. — Segmentation is holoblastic, though not so regular as in Rana and most Batrachia (figs.

1—6). The blastopore is formed by a more general overgrowth of epiblast, and is from the first circular; it is only just before closure that it is possible to tell from an external view which is the anterior and which the posterior side of the pore (figs. 6, 8). It is quite during the last stages of gastrulation that the closing mouth of the gastrula swings up to the posterior edge of the egg. When the blastopore has reached this position it becomes pointed at the anterior end, and there can now be seen running forward from this point a groove showing unmistakably the line of fusion of the edges of the mouth of the gastrula (fig. 9).

Finally the yolk retreats, and a slit-like open blastopore remains at the posterior end of the pear-shaped neural plate (fig. 10).

While this fusion has been taking place the centre of gravity has been continually shifting; for along the line of fusion there is a greater accumulation of yolkless protoplasm, i.e. epiblast and mesoblast, than elsewhere. Yolk is heavy, therefore the fused edges of the mouth of the gastrula come to the upper side.

Finally the entire egg has rotated through 180°. The anterior end of the archenteron is now in the position that the posterior end occupied at the beginning of gastrulation. The area occupied by the neural plate has been formed chiefly by the downgrowth of the lateral and anterior edges of the blastoporic rim; however, the posterior edge here takes a greater share in gastrulation than in Rana, and in consequence the blastopore comes to lie not at the extreme posterior end of the archenteron, but further towards the middle, while the neural plate extends beyond the blastopore. The anus, however, makes its appearance at the extreme posterior end of the archenteron, far from the position now occupied by the blastopore (Sections II, III, *an.*).

The centre of the neural plate becomes slightly depressed, and here the blastoporic scar is seen running forwards from the edge of the blastopore along the whole plate as the "primitive streak" (fig. 11).

The neural folds now begin to approach one another at the anterior and posterior ends of the groove, but there is no well-marked anterior transverse fold (fig. 12).

Posteriorly the folds enclose the remains of the blastopore, which then opens only into the neural canal formed by the complete fusion of the edges of the two folds. A tail fold develops of a crescentic form encircling the posterior end of the neural plate, on the posterior convex side of which the anus is formed (Section III, *an.*).

Between the blastoderm and the egg membrane there is now present a considerable space, filled with a milky fluid (fig. 12, *sp.*).

When the neural folds have completely met, i. e. fifty hours after laying, then the anterior end of the neural plate expands to form the optic vesicles, and an elevation extends forwards from them, homologous with the so-called "Sense-plate" of Morgan. Behind the optic vesicles extending laterally and anteriorly on either side is seen the gill-plate or branchial fold.

Later this grows to completely encircle the sense-plate, which now shows a depression at the anterior end, the rudiment of the stomodæum (fig. 20, *Stom.*).

The right and left halves of the "sense-plate" thus divided are very conspicuous features at this stage in development, and for some time later. A little later they become formed into a regular pair of mandibular bars, which only just meet below the stomodæum (fig. 21, *Mnd.*).

In section they appear quite like the succeeding hyoid arches, which are very slightly developed, and also the larger first and second pair of branchial arches.

There is a total absence of suckers such as are borne behind the mouth in most Batrachian larvæ, and the embryo has now more the appearance of a young larva of Acipenser than of Rana.

The gill-plate in life appears as a single elevation on either side, but after fixing with appropriate reagents it may be seen almost from the first to consist of three branchial pouches of the pharynx; the two anterior of these alone persist. The

first pouch is between the hyoid and the first branchial arch. The second pouch is between the first and second branchial arch. The third pouch is between the second and third branchial arch (fig. 14, *3rd br. f.*).

The optic bulbs early begin to bud out from the fore-brain, and now just behind the gill-plate is seen the first rudiment of the pronephros, a slight but defined elevation tapering posteriorly; mediad to this are seen four or five mesoblastic somites (fig. 14, *mes. som.*).

The auditory vesicles are not easily visible until after the appearance of the external gills.

Up to this time the embryo has lain almost flat upon the surface of the yolk, preserving in all a spherical form; now, however, it begins to rise from the surface of the yolk, both anteriorly and posteriorly, but the yolk is still nearly spherical (fig. 15).

The eye-bulbs increase greatly in size, and are exceedingly large in comparison with what is found in Rana at a corresponding stage. The ocular muscles are developed very early, and the eye may be seen to be rotated by them on the fourth day of development. A very conspicuous feature of this stage is the dilated condition of the double gill-pouch (fig. 15). Viewed from the dorsal surface, the head region has now in outline the form of a trefoil.

The tail now begins to grow back from the surface of the yolk, the dilation of the branchial folds ceases, and in proportion to the latter the head portion increases greatly (fig. 16).

The first pair of external gills now may be seen budding out from the first branchial arch. Below the cleft post-oral region, formed from the sense-plates, the rudiment of the heart is clearly visible (figs. 21, 24, *ht.*).

In a side view, more or less transparent as in life, there are to be seen the heart, first pair of external gills, well-formed eye with conspicuous choroid fissure, auditory vesicles, somites, caudal notochord, and extended cloaca. The yolk-sac still retains its spherical form (fig. 24).

The changes in external form which now take place mainly

consist in the appearance of the second pair of external gills, which do not reach nearly so high a state of development as the first pair; also in the rapid growth of the first pair of gills, so as to extend beyond the vent as blood-red filaments through which the corpuscles stream along, propelled by the now rapidly pulsating heart (figs. 25, 27, 17—19).

A dense plexus of vitelline veins ramifies over the surface of the yolk, while the dorsal aorta and cutaneous veins give to the elongated tail a copious supply of blood (fig. 29). Indeed, so noticeable is this, that I am quite inclined to agree with Mr. Kerr's suggestion that the tail of this larva is an important organ of respiration. This view is further strengthened by my observation that in hatched larvæ the tail often remains motionless as a whole, while the extremity of the tail is kept rapidly vibrating. As the larvæ are not propelled by this motion through the water, I am tempted to think that the object of it is that a stream of water may be kept constantly running along the surface of the proximal part of the tail.

The operculum now grows down from the hyoid arch and encloses the gill arches. The external gills become rapidly absorbed (but I think that a study of the origin of the filaments of the internal gills shows them to be really of the same nature as the external gills), the stomatodæal aperture breaks through, and the young frog has reached the end of its embryonic life (fig. 26).

Internal Characters.—On account of the short space of time at my disposal it seems advisable not to attempt a continuous account of the internal phenomena of development, but merely to figure and describe sections illustrating some of the more important points of interest, leaving the fuller account for a future occasion.

Section I[1] passes transversely through the blastopore before the formation of the neural groove. The main points to be noted are the smallness of the archenteron, the absence of a

[1] The numbers here given correspond with those of the figures of Plates 31 and 32.

yolk-plug, the abundance of yolk, and the mesoderm extending only as far as the equatorial region of the egg.

Section II passes longitudinally through the blastopore (*bl.*), the walls of the neural groove being closed anteriorly, but not yet posteriorly. The points to be noted are the anterior position of the blastopore, the fusion of the embryonic layers before and behind the blastopore, and anteriorly the beginning of the first branchial pouch (*br. f.*).

Section III, a sagittal section of an embryo after the closure of the neural groove, showing the comparatively anterior position of the neurenteric canal (*n. cn.*), the brain vesicles, the notochord not yet differentiated posteriorly, the archenteron, and anteriorly the branchial fold (*br. f.*) of the pharyngeal wall, which is continuous across the middle line; also posteriorly the depression which will later become the anus (*an.*).

Section IV is of the same series as the last, but further from the middle line, showing the large lateral cavity of the archenteron caused by the upraising of the branchial pouch (*br. f.*). The section also passes through the optic vesicle (*op. ves.*).

Section V is a transverse vertical section passing through the head region of an embryo in which the body of the embryo is just beginning to rise up off the yolk. To be noted are the regularly formed optic vesicles (*op. ves.*) and stalks, there being as yet no trace of the lens. Below is seen the pharyngeal region of the archenteron (*ph.*).

Section VI is of the same series as the last, passing between the optic and auditory region; it shows the single branchial pouch (*br. f.*) and the accompanying epidermal thickening.

Section VII of the same series, passing through the auditory region. There are seen in section the front end of the notochord (*N. ch.*), the auditory thickening of the epidermis (*Aud.*) and the rudiment of the pronephros being differentiated off from the general mesoderm (*Pn.*). There is seen also a faint indication of a neural crest (*N. cr.*).

Section VIII of the same series, passing through the posterior end of the archenteron, where it is seen to be constricted

into two portions, the upper being the opening of the neurenteric canal (*N. en.*), the lower the rudiment of the rectum (*Rect.*).

Section IX shows the fusion of the layers in the region of the neurenteric canal, and the separation of the latter from the rectum.

Section X of the same series, through the tail and vent, shows the opening of the neurenteric canal into the neural tube; also the fusion of the epidermis with the hypoblast at the anus (*An.*).

Section XI, a transverse vertical section quite at the anterior end of the head of an embryo, in which the first pair of external gills are beginning to bud. The section passes through the bottom of the stomodæum (*Stom.*), and obliquely through the mandibular arches at the point where they meet (*Mnd.*).

Section XII is of the same series, but further back, and on the right side in the figure passes through the centre of one of the eyes, showing the attenuation of the posterior wall of the optic cup (*Op. w. p.*) and the thickening of the anterior wall (*Op. w. an.*), the rudiment of the retina. The lens is also seen arising as a regular involution of the nervous layer of the epiblast, the epidermal layer (*l.*) remaining stretched across as a very thin membrane. The section also passes through the middle portion of the mandibular arches (*Mnd.*). The pharynx and pericardium are also cut through (*Ph.*, *P.c.*).

Section XIII of the same series passes through the centre of the opposite eye. The proximal parts of the mandibular arch are here cut through (*Mnd.*). The formation of the pericardium and heart, with its mesodermal membranous lining, is well seen (*P.c.*, *ht.*).

Section XIV of the same series passes a very little further back through the infundibulum, pharynx, and the two lateral extensions of the pericardium overlying the sinus venosus (*S. v.*).

Section XV is of a slightly older embryo, passing transversely through the eyes. The lens is now nearly completely

constricted off, and the back wall of the lens has begun to thicken and fill up the hollow of the lens (*l.*).

Section XVI is a sagittal section through the pineal eye of an embryo about two days before hatching. It shows the pineal stalk, still allowing free communication between the pineal body and the brain-cavity; this passage is now distinctly ciliated (*cil. st.*). Blood-sinuses are seen in front and behind.

Section XVII is a transverse section of an embryo just before hatching. It passes through the root of the first external gill (*Ext. G.*), and shows the developing first and second internal gills (*Int. G.*).

From this and similar sections there certainly does not seem to me to be any very marked difference in the nature of the external and internal gills.

As regards the development of the Pronephros and its duct, my sections indicate that there is considerable variation. Though by the time the external gills are developed there are invariably three nephrostomes, as in Rana, the first and third being lateral, the second dorsal, yet previously to this stage I find often only two nephrostomes, and in some instances two on one side with three on the other, and in one case but one. This seems to me to indicate that the pronephric tubules do not arise in the way usually described for Rana, namely, by the primitive pronephric groove becoming a closed tube and remaining in open communication with the cœlom at three points, but rather as a solid rod of mesoderm (Section VII), which later becomes hollow and acquires perforations into the cœlom, at first one, later three points—the nephrostomes.

In comparing the development of Phyllomedusa hypochondrialis with that of Rana, Bombinator, Pelobates, and other Batrachians with free-swimming larvæ, the first thing that strikes one as regards external characters is that, throughout, this embryo maintains a greater similarity to ichthyic forms, especially Ganoids, on the one hand, and to the Urodela on the other, than do the free-swimming larvæ of other Batrachians.

Again, we find this difference in general development of the young larva intensified in such forms as undergo a still more abbreviated embryonic development; for instance, in Paludicola fuscomaculata, where the embryonic development is shortened to something between twelve and twenty-four hours. All the points in which Rana appears to be a more modified form of development than Phyllomedusa are intensified, and the external characters are ill-defined. However, a minute comparison cannot yet be made until I shall have had time to study more carefully the details of development in Paludicola. The study of the internal anatomy leads to the same conclusion, namely, that in this protracted development we do not find the course of development distorted and blurred, but on the contrary every organ, so far as I can find, develops as in the ordinary frog, only more clearly and more definitely, and at the same time more as we see it develop in other great groups, Elasmobranchs, Ganoids, and the higher Vertebrates.

Take for instance the eye of a free-swimming batrachian larva, and compare it with the eye of Phyllomedusa. The evolution of the optic cup and lens is hurried over and blurred in the former, so that they are often difficult to trace, while here in Phyllomedusa it is as regular and diagrammatic as in any Vertebrate there is. Contrary to what we find in most Batrachia, the lens develops as an involution of a single layer of nervous epiblast rather than a mere thickening of that layer. In the free-swimming form the eye has been required to become functional as rapidly as possible, while here it has been suffered to go through its normal course of development in peace.

Take again the suckers of the free-swimming forms. They are evidently new adaptations without phylogenetic significance. Through the presence of these structures the form of the mandibular arches has become quite obliterated, while here in Phyllomedusa these would compare favourably with those of an Elasmobranch, reptile, or bird.

The peculiarly symmetrical gastrulation that this egg exhibits must be supposed, I think, to be the effect of a large

amount of food-yolk, as it can hardly be supposed that, at a stage previous to hatching in either mode of development, Phyllomedusa should be more primitive than the free-swimming forms.

I think the median spiracle may also be looked upon as a primitive feature.

The manner in which the branchial fold encircles the head reminds one strongly of Salensky's figure of Acipenser at a similar stage.

From the study of the development of Phyllomedusa, of which I have described the points of more general interest, I am distinctly inclined to think that we are not always warranted in attributing to alecithal free-swimming larvæ a greater biological importance, as far as retaining ancestral characters is concerned, than to heavily yolked embryos.

I think, moreover, that this is what we should expect, for from the time that the larva is hatched onwards it is subjected to the influence of natural selection.

Indeed, in this particular case of Batrachian development it would seem rather that the shortening of the embryonic period may be a specialised and not a primitive condition.

The fact that the majority of frogs have a shorter embryonic life does not seem to me to prove that the minority are the specialised forms in this respect. This particular mode of development is not confined to this species.

Von Jhering has described the oviposition of Phyllomedusa Jheringii, which agrees very closely with that here described. The eggs were laid between two or more leaves instead of being rolled in one, as with Phyllomedusa hypochondrialis. Von Jhering did not, however, work out the development of this species; in all probability it would not differ from this one.

This year S. Ikeda, of Tokio, has published an account of the oviposition in a species of Rhacophorus; from what he mentions of the appearance of the embryos which develop in a froth, much as is the case with Paludicola, I think the development of this form will be found to be quite like that of

Phyllomedusa; indeed, Professor Mitsukuri, who has seen them both, assures me that this is so.

To a paper by Gasser published in the 'Sitz. d. Kön. Ak. Marburg,' 1882, upon the development of the midwife toad, Alytes obstetricans, I have not yet been able to get access, but I feel quite prepared to find that it exhibits the same features that characterise the development of Phyllomedusa hypochondrialis.

XVI. Phyllomedusa Sauvagii, Boul.

This handsome tree-frog was brought to me in the Chaco, but I am not able to state anything about its habits.

XVII. Hyla spegazinii, Boul.

This fine Hyla was fairly common; I often caught or saw young specimens swimming from stem to stem of the Papyrus grass as we travelled through the reed-choked swamps. The full-grown specimens, however, were always taken either from palm tops just felled or from the trees overhead.

When caught in the water by daylight they were a bright light yellow, but at night they turned to a darker shade, and became marbled on the upper surface with brown markings. The full-grown specimens did not in this way become dark at night.

The largest specimens taken measured 80 mm. The eggs in the cloaca appear to be quite like those of Rana in size and colour, and are probably laid and reared in the same way.

One full-grown specimen I obtained in Central Paraguay on the Tibicuari, the rest in the Paraguayan Chaco.

XVIII. Hyla venulosa, Laur.

In life the markings are olive-green or grey upon a whitish ground. When taken from amongst foliage the whitish ground colour is suffused with green. It is a powerful and energetic frog, the large toe-discs having a tenacious sucking power.

The skin glands are strongly developed, emitting a very sticky white slime.

XIX. Hyla nana, Boul.

This small frog was abundant in the swamps, usually found by moonlight sitting on the broad-leaved plants of the swamp, and calling with a rather highly pitched scraping note.

The upper surfaces, as in H. spegazini, are light straw-colour by day, but brown by night. Flanks and underneath pigmentless.

XX. Hyla phrynoderma, Boul.

A light golden colour, shaded with darker above. The discs fairly strongly dilated. The skin is warty and extremely delicate, and it is not easy to catch one uninjured.

They are not common, but make themselves known by their constant call, which is just like the quacking of a duck. All the specimens I obtained were about the palm fencing and sheds.

XXI. Hyla nasica, Cope.

This is the most common Hyla in the Chaco. It is found everywhere, usually upon palm or palm fencing, where it is most inconspicuous.

Its call and habits are much like H. phrynoderma; the note is, however, somewhat lower. The colours are chiefly olive-green and brown, but the markings are variable. It is of more slender build than H. phrynoderma, the body being longer in proportion to the width of the head.

XXII. Ceratophrys ornata, Bell.

I obtained some half a dozen specimens of this curious and well-known South American frog, commonly known as the "Escuerso."

Its ferociousness is its most striking characteristic. If it is

approached to within two feet, it will make a vicious spring at one with its gigantic mouth wide open. If it succeeds in seizing any part of its tormentor, it holds on like a bulldog. The habit it has of distending its lungs to their fullest when teased has given rise to the idea amongst the Argentine people that if teased sufficiently it will burst.

It is perhaps needless to say that I was disappointed in my efforts to obtain this end. The Ceratophrys lives chiefly off other frogs and toads, but it is said also that it will seize and devour young chickens. The largest I saw was 120 mm. in length.

Lepidobatrachus, J. S. B. N. g.

Pupil horizontal. No vomerine teeth; transverse processes of sacral vertebræ not dilated. Large teeth in upper jaw; also two large teeth in dentaries of lower jaw. Tongue circular and free behind. Nostrils the most elevated portion of the head. Eyes close together, not more than the diameter of the eye apart. Fontanelles in the parietal region. Outer metatarsal tubercle very large. Great development of membrane bones in the head; width of jaw very great. Tympanum fairly distinct.

XXIII. Lepidobatrachus asper, J. S. B. N. sp.

Hind legs carried forward, toes reach barely to the eyes. Tips of toes horny. Skin of dorsal surface a dull leaden colour, much tuberculated and tough.

This frog lives continually in muddy pools. Its habit is to float with just the eyes and nostrils above the surface. If disturbed it slowly sinks to the bottom, leaving no ripple on the surface of the water. It feeds largely on Bufo granulosus.

XXIV. Lepidobatrachus lævis, J. S. B. N. sp.

This may possibly be the same species as the last, but

differs from it in the greater width of the skull, greater length of the hind legs, which carried forwards reach tip of snout, and in the skin being smooth, thin, and slimy, with the organs of the lateral line showing clearly upon it. Also the tympanum is larger and more evident. The tips of the toes do not bear horny caps as in the preceding species.

Below is a comparative list of measurements in millimetres in two specimens of XXIII and one specimen of XXIV.

		Total Length.	Hind Legs.	Width of Jaw.	Eye to Eye.	Eye to Ear.	Ear to Ear.
XXIII.	*a.*	80	62	34	4	8	24
	b.	70	60	33	3½	7½	23
XXIV.	*c.*	80	70	38	5	9	28

It is a source of great regret to me that I am obliged to abandon for the present my work in this direction. I have a considerable amount of material at my disposal of the developmental stages of several of the species of frogs, concerning which I have here merely stated the observations which I made a note of while yet in the Paraguayan Chaco. I sincerely hope that I may be able to return to this work at a future date.

Concerning the species Phyllomedusa hypochondrialis I should state that, although I have gone more fully into its development than others of my collection, here also my work has been cut short.

In concluding, I should like to say that I am very greatly indebted to Mr. Graham Kerr for the opportunity he afforded me of obtaining my material, and also for much help and advice in my work.

EXPLANATION OF PLATES 28—32,

Illustrating Mr. Budgett's "Notes on the Batrachians of the Paraguayan Chaco."

All the figures relate to the same species—Phyllomedusa hypochondrialis, Cope.

Plate 28.

(See next page.)

PLATE 29.

The figures on this plate are all drawn under a magnification of eighteen diameters.

Figs. 1—6.—Illustrating the character of segmentation. Figs. 1—4 are views from above; Figs. 5 and 6 from the side.

Figs. 7 and 8.—Views of egg from below, showing diminution in size of the blastopore.

Fig. 9.—Egg seen from below, at a time when the blastopore is much reduced in size, and has nearly reached the level of a horizontal plane passing through the centre of the egg. From the pointed anterior end of the blastopore there passes forwards a distinct groove, indicating the line of fusion of the gastrula lips.

Fig. 10.—View of egg from above and behind, showing the continuation forwards of the slit-like blastopore as a faint groove along the axis of the medullary plate.

Fig. 11.—View of egg from above, showing neural plate and early condition of neural folds.

PLATE 30.

The figures on this plate are all drawn under a magnification of eighteen diameters.

Fig. 12.—View of anterior end of embryo, with well-formed neural folds. *Sp.* Space between embryo and egg membrane.

Fig. 13.—Similar view where the neural folds are arching over towards one another.

Fig. 14.—View of middle of trunk region of an embryo in which the pronephros has appeared on each side (*p.n.*). *Mes. som.* Mesoblastic somites. *3rd Br. f.* Position of third branchial pouch.

Figs. 15—19.—Figures illustrating the further development in general form of the embryo.

Fig. 20.—View of anterior end of embryo, showing the first trace of stomodæum (*Stom.*).

Fig. 21.—Oblique view of embryo, showing the mandibular bars (*Mnd.*) and rudiment of heart (*ht.*).

Figs. 22 and 23.—Views of anterior end of head, showing the fusion of the mandibular bars (*mnd.*) in the mid-ventral line.

Figs. 24—26.—Side views of larvæ, showing the further changes in form up till the time of hatching. *Ht.* in Fig. 24, rudiment of heart. In Fig. 25 the external gills are at about their maximum.

PLATE 28.

(The explanation is given here as the figures run on from Plates 29 and 30.)

Figs. 27—30 are drawn under a magnification of eight diameters.

Figs. 31—35.—Natural size. It will be noticed that Fig. 35 has been wrongly orientated by the lithographer; the plant stem should be vertical.

Figs. 27—29.—Side view of larvæ during the last day of intra-oval development.

Fig. 30.—Larva just after hatching.

Fig. 31.—Side view of larva at time of development of hind limbs, showing accumulation of pigment in the tail.

Fig. 32.—Young frog after leaving water.

Fig. 33.—Young frog after completed metamorphosis.

Fig. 34.—Pair of adults during the process of oviposition.

Fig. 35.—Adult specimen. This figure by comparison with Fig. 8 illustrates the extent of reflex colour change.

PLATE 31.

Fig. 1.—Transverse section through blastopore before formation of neural groove. *bl.* Blastopore. *ep.* Epiblast. *mes.* Mesoblast. *hyp.* Hypoblast.

Fig. 2.—Longitudinal vertical section of embryo with neural groove closed in anteriorly. *bl.* Blastopore. *An.* Depression marking position where anus will appear. *Arch.* Archenteron. *Mes.* Mesoblast. *Br.f.* Branchial outgrowth of archenteron.

Fig. 3.—Longitudinal vertical section of an embryo after the closure of the neural groove. *N.en.* Neurenteric canal. *an.* Anal depression. *Notoch.* Notochord. *hyp.* Hypoblast. *Br.f.* Branchial outgrowth of archenteric wall. *Ves* I, *Ves* II, *Ves* III. Brain vesicles.

Fig. 4.—Section parallel to the last figured, but more lateral in position. *Op. ves.* Optic vesicle. *Br.f.* Branchial outgrowth from archenteron.

Fig. 5.—Transverse section through head of an embryo which was just beginning to be folded off the yolk. *Op. ves.* Optic vesicle. *Ph.* Pharynx. *mes.* Mesoblast.

Fig. 6.—Section of same series as that shown in Fig. 5, through the single branchial pouch (*Br.f.*) and the ectodermal thickening accompanying it.

Fig. 7.—Section of same series through auditory region. *Aud.* Commencing auditory invagination of ectoderm. *N. ch.* Notochord. *Neur. cr.* Neural crest. *P.n.* Rudiment of pronephros.

Fig. 8.—Section of same series through posterior end of archenteron. *Rect.* Rectum. *N. en.* Neurenteric canal opening into this. *hyp.* Hypoblast. *N.ch.* Notochord.

Fig. 9.—Section of same series further back. *N.en.* Neurenteric canal. *Rect.* Rectum.

Fig. 10.—Section of same series showing opening of neurenteric canal (*N. en.*) into neural canal; also anus (*An.*).

Fig. 11.—Transverse section through anterior end of an embryo, in which the first pair of external gills were beginning to develop. *Stom.* Cavity of stomodæum. *Mnd.* Mandibular arch close to its junction with its fellow.

Fig. 12.—Section of same series passing through the rudiment of the eye. *Op.W.an.* Anterior layer of optic cup. *Op.W.p.* Posterior wall of ditto. *l.* Outer layer of epiblast passing continued over mouth of lens invagination. *Ph.* Pharynx. *P.c.* Pericardium. *mnd.* Mandibular arch.

PLATE 32.

Fig. 13.—Section of the same series as that shown in Plate 31, Fig. 12. *mnd.* Mandibular arch. *ht.* Heart. *P.c.* Pericardium.

Fig. 14.—Section from same series through infundibulum (*Inf.*). *P.c.* Pericardium. *S.v.* Sinus venosus.

Fig. 15.—Transverse section through head of slightly older embryo, showing later stage in the formation of the lens (*l.*).

Fig. 16.—Sagittal section through pineal body (*Pin.*) of embryo about two days before hatching. *Cil. St.* Pineal stalk with ciliated lining. *Sin.* Blood-sinus.

Fig. 17.—Portion of transverse section of embryo just before hatching, passing through the origin of the first external gill (*Ext. G.*). *Int. G.* Internal gill.

The Development of Echinoids.

Part I.—The Larvæ of Echinus miliaris and Echinus esculentus.

By

E. W. MacBride, M.A.,

Professor of Zoology in McGill University, Montreal.

With Plate 33.

The development of Echinoids is up till the present very imperfectly known, our information on the subject being in a most unsatisfactory condition. The foundation was laid by Johannes Müller (3), but although he observed and described the external features of the metamorphoses, he was unable to refer with any certainty any one of the larval forms which he described to the adult species from which it was derived. So far as I am aware, Prof. Théel (4) and H. Bury (1) are the only persons who have hitherto reared any species from the egg until the conclusion of the metamorphosis. Through the researches of the former we have obtained an accurate knowledge of the characters of the larva of Echinocyamus pusillus at all stages of its growth. The youngest larval forms of many species have been determined by rearing, nevertheless Mortensen (2), in his most valuable summary of all descriptions yet published of Echinoid larvæ, points out that our knowledge of the later pluteus stages is most imperfect, and that it is not possible to assign the older plutei which have been described to the species from which they have been derived.

Some three years ago I formed the project of making an exhaustive study of the development of one of the British species of Echinus on similar lines to my work on the development of Asterina gibbosa. After several unsuccessful attempts I succeeded this spring in rearing the larvæ of Echinus esculentus and Echinus miliaris from the egg to the latest pluteus form, when the processes (arms) are all fully developed, and the first pedicellariæ of the adult have already appeared. In every stage of their development these two larvæ are easily distinguishable from one another, and, in view of the interest attaching to the question as to how far allied species are distinct at all stages of growth, it seemed worth while to publish an account of these larvæ before dealing with the more general questions of Echinoid development. My best thanks are due to Mr. Allen, the Director of the Plymouth Biological Laboratory, and to his staff, for the assistance and advice they rendered me. To the mechanical arrangement for continuously agitating the water, invented by Mr. Allen, such success as I have hitherto obtained is in large measure due.

The eggs of Echinus miliaris are smaller and more transparent than those of E. esculentus, and as a consequence the stage to which it is possible to rear them without special precautions is much less advanced than is the case with E. esculentus. Larvæ of E. miliaris have lived for a month without showing unhealthiness, but also without developing a trace of the oral disc (the first trace of adult structure to appear), or even the full number of larval processes. The stage at which larvæ of E. esculentus under similar circumstances cease to progress, is that at which there is an unmistakable rudiment of the oral disc of the adult. This curious difference throws light on the extent to which the larvæ depend for support on material stored up in the ovum.

The blastulæ of E. esculentus are nearly spherical; those of E. miliaris, on the contrary, distinctly ellipsoidal in form. When the invagination which forms the gut has taken place, it is seen that the oval outline of the latter larvæ is due to their possession of an enlarged præoral lobe. This enlarged

forehead, as it may be called, long remains a feature of the E. miliaris larvæ; it is seen as a lip overhanging the mouth in plutei with four processes completely developed (see fig. 1, *fr.*).

In addition to this feature the four-armed pluteus of E. miliaris is distinguished from that of E. esculentus by its more pointed posterior portion, and by the smaller length of the processes in comparison to the body (comp. figs. 2 and 3).

In later stages the posterior pole of the E. miliaris becomes rounded, but then the pluteus is broader in proportion to its depth than is the case with E. esculentus, approximating more to the shape of an Ophiurid pluteus, or ophiopluteus, as Mortensen terms it.

The ciliated epaulettes develop about the middle of the third week of larval life. A new distinctive feature is now added to those already possessed by E. miliaris. Just anterior to each epaulette there is a large mass of bright green pigment. The epaulettes of E. esculentus are, on the other hand (*pig.*, fig. 4), loaded with reddish-yellow pigment.

So far as I could observe, the four primary epaulettes at no time form part of the general ciliated band, but are of independent origin. In the pluteus of Echinus esculentus, in addition to these, however, in the fourth week two posterior ciliated epaulettes are formed, one on each side by abstriction from the ciliated band just where it bends from the postero-dorsal to the post-oral process (Mortensen's notation). I did not succeed in bringing the larvæ of E. miliaris quite as far. These posterior epaulettes are mentioned for the first time in Mortensen's work cited above; they are there described in two undetermined forms of larvæ. The drawings are very poor, and it is not possible to be sure whether these larvæ are identical with one or other of the forms I have described, but it is worthy of notice that E. esculentus is placed by Mortensen amongst forms distinguished by not possessing these epaulettes. It is therefore quite possible that here, again, the old error has been made of mistaking two stages in the same life-history for two different species.

The oldest stage of E. esculentus which I succeeded in rear-

ing is represented in fig. 6. In this stage of E. esculentus there are three pedicellariæ, one at the posterior pole and two on the right side posterior to the ciliated band; the oral disc of the adult has encroached very much upon the stomach, and both the anterior and the posterior ciliated epaulettes are well developed: the posterior by coalescence have formed a continuous ring. The cilia covering these epaulettes are much more powerful than those on the ciliated band, and the epaulettes form in later larval life the main organs of locomotion; their appearance in the living animal when expanded recalls the trochal disc of Rotifera.

The "echinoplutei" larvæ (to use Mortensen's term) are distinguished from the more primitive bipinnariæ by an immense reduction of the præoral lobe (Mortensen's frontal area). Under these circumstances it is interesting to find a remnant of this primitive structure surviving in the larvæ of E. miliaris, which develop from small eggs, and it is further interesting to note that this primitive feature is most strongly marked in the early stages of development.

Montreal; Oct. 15th, 1898.

Works referred to in this Paper.

1. Bury, H.—"The Metamorphosis of Echinoderms," 'Quart. Journ. Micr. Sci.,' 1895.

2. Mortensen, Th.—'Die Echinodermen-Larven der Plankton Expedition,' Kiel and Leipzig, 1898.

3. Müller, Joh.—"Die Larven der Echinodermen," several papers, 'Abhandlungen der Kgl. Akademie der Wiss. zu Berlin,' 1848—1855.

4. Théel, H.—"The Development of Echinocyamus pusillus," 'Trans. Roy. Soc. Upsala,' 1892.

EXPLANATION OF PLATE 33,

Illustrating Mr. E. W. MacBride's paper on "The Development of Echinoids."

List of Abbreviations employed.

a. ep. Anterior ciliated epaulette. *an.* Anus. *cœ.* cœlomic sac. *Ech.* Rudiment of oral disc of young Echinus. *fr.* Frontal area. *m.p.* Madreporic pore. *ped.* Pedicellariæ. *p. ep.* Posterior ciliated epaulette. *pig.* Mass of green pigment. *pr. præ. o.* Præoral process of ciliated band. *pr. ant. lat.* Antero-lateral process. *pr. post. dors.* Postero-dorsal process of the ciliated band. *pr. post. o.* Post-oral process of the ciliated band. [These last four names are Mortensen's notation for the pluteus arms.] *rect.* Intestine.

The magnification of all the figures is about that obtained by a Zeiss, obj. A, oc. 2.

Fig. 1.—Larva of Echinus miliaris six years old, side view. Note the præoral lobe (*fr.*) overhanging the mouth.

Fig. 2.—Larva of Echinus miliaris in the second week, dorsal view. *rect.* Intestine. *an.* Anus, seen through; third pair of arms beginning to appear.

Fig. 3.—Larva of Echinus esculentus seven days old, dorsal view. Commencement of third pair of processes.

Fig. 4.—Larva of Echinus miliaris three weeks old, dorsal view. *ped.* First rudiment of a pedicellaria. *pig.* Characteristic green pigment.

Fig. 5.—Larva of Echinus esculentus three weeks old, dorsal view. *Ech.* First trace of oral disc of adult.

Fig. 6.—Larva of Echinus esculentus four weeks old, dorsal view. *p. ep.* Posterior ciliated epaulettes.

Hydroids from Wood's Holl, Mass.

Hypolytus peregrinus, a New Unattached Marine Hydroid: Corynitis Agassizii and its Medusa.

By

L. Murbach.

With Plate 34.

Hypolytus peregrinus, a New Marine Hydroid.

Introductory.

During the last part of the summer of 1895, while searching for the larvæ of Gonionemus[1] in tow and dredgings from the eel pond at Wood's Holl, a curious little hydroid polyp was found. At the time it was sketched, and a few notes made on the supposition that it was the larval form of some other hydroid, perhaps a Tubularian. In the following summer forms of the same kind with gonophores were found. This left no doubt that it was after all a mature form, but the limited number of specimens warranted no further conclusion than that the animal would probably prove interesting on account of its unattached condition, a character which would not have been remarkable in a larval form. Nothing like a perisarc was at first thought to be present, for, as was after-

[1] Although this medusa breeds regularly every summer in the eel pond, yet after three summers of careful searching I have found only few metamorphosing larval stages, but have not been able to get the stages intermediate; nor have I been able to raise any beyond the stage of the polyp with four tentacles.

ward learned, there was simply a tubular secretion, only shreds of which remained on the captured polyps.

Last summer (1897) more specimens were obtained by allowing the tow-net to scrape gently over the eel-grass. These seemed to warrant my previous conjectures, and although specimens were sometimes taken in clear water, I now concluded that they usually are temporarily attached to submerged refuse, eel-grass, &c., by means of their perisarcal secretion.

The attached condition of marine hydroids is so universal, and those able to move from place to place are so few, that up to the present time not much stress has been laid on this characteristic. More cases of this kind would establish such character as very primitive or as reversion to an ancestral type, a free polyp, perhaps Actinula-like.

To the writer's knowledge there are only two marine forms so far known which may be considered free. They are Protohydra Leuckartii and Halermita cumulans.

The former, long ago discovered by Greeff,[1] was considered, as its name indicates, to be an ancestral form of hydroids, but its foot is adapted for fixation, and is therefore of permanent character. Furthermore, no sexual reproduction has up to the present time been observed, and partly for this reason Schaudinn[2] suggests that it may be the larval form of a more highly organised polyp.

Halermita, discovered by Schaudinn[2] in the Berlin Aquaria, is certainly a remarkable form, and as his report of the finding may not be accessible to all, I shall recount the principal features of the polyp. Its name indicates its solitary mode of life. In form it is short and conical (stumpf kegelförmig). There are no divisions into hydranth and hydrocaulus; the tentacles (the figures show only one circlet) are usually four in number, but never more than five, and these

[1] Greeff, R., "Protohydra Leuckartii," 'Zeitschr. f. Zool.,' Bd. xx, 1870.

[2] Schaudinn, F., "Halermita cumulans," 'Sitzber. d. Gesellsch. Nat. Freunde,' Berlin, 1894.

are not knobbed at the end. The pear-shaped nettling organs are of only one kind, and they are evenly distributed, i. e. not in groups. The longest tentacles are 8 mm.; the endoderm of the tentacles is a solid axis. From the paper I infer that the foot end is in no way modified for fixation, but simply sticks in the accumulated débris, hence its species name cumulans.

Schaudinn places Halermita between Hydridæ and all known hydroids, but this is only tentative, since he has not been able to observe the sexual reproduction; he admits that it may also be the larval form of a more specialised hydroid.

Up to the present time, then, all marine hydroids known to be adults are permanently fixed; and even if we consider Protohydra and Halermita to be mature forms, then the latter would be the only one so far recorded which does not seem to be specially modified at any point of its foot for fixation. Naturally the case of Corymorpha suggests itself as a form that might be an exception, but may be dismissed on account of the processes at its foot end, which are undoubtedly remnants of a Hydrorhiza.[1] To the new polyp found at Wood's Holl I have given the name Hypolytus peregrinus.[2]

General.

The polyps of Hypolytus peregrinus are found temporarily attached by the secretion of their ectoderm to some foreign object several feet below the surface of the water, or having become detached, probably by withdrawing from the perisarcal tube (in which case a new one is quickly secreted), they may be found floating at the surface of the water. This no doubt accounts for their being occasionally taken with the tow-net in clear water. Their temporary attachment is again

[1] Korschelt and Heider ('Comp. Embryology') suggest that these may indicate a previous colonial condition of Corymorpha.

[2] From ὑπό, under, below; and λύω, loosen; peregrinus, travelling. Should the name here proposed for this new genus be preoccupied, I propose instead Gonohypolytus.

easily effected anywhere along the tapering foot end. No other part of the hydrocaulus seems to be used to "make fast."

The predominant colour of the polyp is pale to bright pink, resembling many Tubularians in this respect. As commonly occurs elsewhere, the colour is localised mostly in the endoderm cells of the body, showing through the more transparent ectoderm of the periphery. In another part of this paper it will appear that greater activity in any part of the body is marked by greater depth of colour. In a general way this is evident in the more active digestive region of the hydranth. An apparent exception are the intense pigment spots at the free ends of the gonophores.

The regions of the body are well marked into hydranth, bearing besides the mouth two circlets of tentacles and the gonophores, and into hydrocaulus (cf. fig. 1). At the union of these two main divisions of the body there is a thickened collar-like portion studded with nettling organs. From this structure to the free rounded foot end the hydrocaulus is covered with a kind of rudimentary perisarc. The hydrocaulus is never branched.

In size the average adult animal is from 1 to 1½ cm. long, and 1 to 1½ mm. thick. Of course the size varies with the degree of expansion or contraction; the measurements were therefore made from a moderately expanded animal.

Locomotion is slow though definite, and not very extensive. The animal seems to progress by leaving its tubular secretion behind, stepping on it, as it were, so that a relatively long piece of the tube, plainly marked by adhering foreign matter, indicates its progress. The movements of parts of the body are slow,—its tentacles, for example, swaying to and fro in search of prey. When disturbed the hydranth and tentacles contract first, and if the irritation is continued the whole animal contracts into a small mass.

General Anatomy.

The hydranth is terminated at its free end by the usually conical hypostome, containing considerable pigment at its highest point (fig. 1, *h.*). It is pierced by a small mouth-opening leading directly into the cœlenteron. Immediately below the hypostome is the set of oral tentacles, ten in number, and placed at regular intervals like radii (fig. 1, *o. t.*). Ten being the largest number commonly present, I take it to be the normal. These tentacles are one third to one half shorter than those of the lower circle, but are otherwise of the same shape and structure. There are no scattered tentacles on the hydranth, and the lower or aboral set occurs nearly two thirds the length of the whole hydranth from the oral one. They are in no way different from those of the oral circle except that they are longer. There are usually fourteen, though frequently a smaller number has been observed (fig. 1, *a. t.*).

In general the tentacles are stouter in appearance than is usually the case in such small polyps. They are slightly enlarged at the end, though there is no knob present except in the young animal, where they are somewhat knobbed. The larger appearance of the tentacles is no doubt due to the prominent ridges of nettling organs which run in circles and short spirals, pushing their cnidocils considerably above the surface. The ectoderm of the tentacles is very transparent, and not easily separated from the mesoglœa. The endoderm forms a solid axis through the centre of the tentacle, and in polyps somewhat reduced by fasting, much black pigment collects in these cells, giving the tentacles the appearance of being hollow; even in ordinary specimens some pigment may be present.

The gonophores (fig. 1, *g.*) spring from the hydranth just above the aboral circle of tentacles, and number in adult polyps from one to three, never more than three having been observed. They present some peculiar features, which will be more fully described under reproduction.

The predominant colour of the hydranth is located in its

endoderm, the ectoderm being quite clear. Both these layers as well as the mesoglœa have the typical cœlenterate character, and will not need further notice.

The hydranth does not terminate immediately below the aboral tentacles, but, as is evident from its internal and its external character, it extends over one third its length farther down to where it unites with the body. Just below the point where the aboral tentacles are attached there is an enlargement in the digestive cavity, looking like a deeply pigmented band running across the cœlenteron, for which I have so far found no adequate explanation. Where the hydranth joins the hydrocaulus there is a ring-like expansion, which gives the appearance of the former being stuck on to or slipped over the end of the latter, like a collar or flange (fig. 1, *c.*). In an expanded condition of the body it is nearly obliterated, becoming more prominent again after contraction has returned the body to its normal. It marks the upper limit of the perisarcal tube, and this being a rather tightly fitting structure may in part account for the changeable character of the collar.

Large numbers of nettling organs are present in the lower edge of the collar, and although they are apparently complete for use are nevertheless not destined to be used here, for there are no cnidocils present. They migrate from the collar toward the tentacles, and are of no service until they reach these and become erect. Of the nettling organs in general it may be here added that there are at least two kinds, similar in shape but differing in size and structure. They are very short ovals.

The hydrocaulus is somewhat more slender than the adjacent portion of the hydranth, and gradually narrows down to the taper-pointed foot end, which is generally curved and forms a better rest for the polyp (fig. 1, *h. c.*). The character of its layers is practically the same as that of the hydranth, added only to this that the ectoderm cells differ physiologically in that they secrete the perisarc-like tube. A portion of the foot end is frequently of a deeper pink hue, indicating greater activity here; but as this has to do with reproduction it will

be again referred to under that head. There is no special differentiation at the end for attaching the animal. The cœlenteric cavity extends to the tip of the foot end, and in it constant circulation may be seen, due to the flagella on the endoderm cells.

A very delicate perisarcal envelope covers the whole hydrocaulus from the collar to the foot end, or it may even extend farther beyond in a collapsed condition, adhering to foreign objects, showing the distance the animal has travelled. It invests the body so closely and is so thin that it can scarcely be distinguished from the transparent ectoderm which secretes it, except when favourable conditions of illumination show it thrown into folds on the concave side of the body as the latter bends in any direction. When polyps are roughly handled with the pipette it is torn into shreds; in such specimens it first came to my notice. Of course indisputable evidence of its presence and its tubular nature is found in the remains left behind on which foreign matter has collected. Frequently several of these may be found radiating from near the same point, usually a mass of débris, which then marks the place where several polyps from one parent leaving these tubes originally stood (cf. fig. 10).

The temporary nature of the perisarcal tube, which is easily lost or even left, and quickly replaced, indicates that it simply is a somewhat hardened mucous secretion serving for support and protection, and not a true chitinous perisarc, such as other hydroids usually possess.

Sexual Reproduction.

In Hypolytus the sexes are separate, and the males seem to preponderate. Sexual reproduction probably takes place in the latter part of summer, for by the middle of August sperm and ova were just beginning to mature in some individuals. Only one specimen was found with what appeared to be nearly mature ova, and an attempt was made to fertilise them, but was not successful (fig. 1 *a*, *g'''*).

The gonophores (fig. 1, *g'*, *g''*) are limited to a narrow zone

just above the aboral set of tentacles, standing at unequal distances apart, and were not more than three in number on any of the individuals observed. The first sign of a budding gonophore is a slight elevation with a deep pink pigment-spot on the hydranth. Both older and younger stages have a spindle or elongated oval form, which in the mature ones becomes distorted by the growth and aggregation of the sexual products in the ectoderm of the outer wall (fig. 1, *g'*). The general hue of the gonophores is bright pink. In length the older ones equal the part of the hydranth between the two circles of tentacles, but being less contractile may appear longer.

A narrow neck connects the gonophore with the hydranth, and just at the junction there is a small curved process directed aborally (fig. 1, *p.*). It is hollow, and appears to belong to the gonophore, its cavity being connected with that leading from the gonophore into the cœlenteron of the hydranth. In small specimens these processes are not yet present. Their nature and significance have remained an enigma to me. I do not know of a homologue anywhere among the hydroid polyps. The cœlenteron is continued through the gonophore to its tip, where a bit of bright pigment is visible. Active circulation may be observed in gonophores as well as in processes at their proximal ends.

Asexual Reproduction.

My attention was first attracted to the remarkable mode of asexual reproduction by the peculiar appearance of the foot end of a few specimens in a lot of about twenty, taken July 26th. One or two constrictions (cf. fig. 2) marked off deeper pink portions of greater diameter. When these segments were freed from the body of the adult they looked not unlike the large planulæ of Pennaria, obtained at the time in considerable numbers. Indeed, the same day such a planula-like body was found in the tow. It was isolated and watched, to determine if it were the detached foot end of Hypolytus or a planula. It moved about for some time, and then slowly erecting itself, attached by its narrow end (making it at once

evident that it was not the planula of a hydroid), and developed into a young Hypolytus, thus settling the question beyond a doubt.

As the segments freed from the foot end of Hypolytus are destined to form new polyps directly, and differ from any kind of bud heretofore described, as will be shown later, I shall call them blastolytes.

From a large number of cases of asexual reproduction observed, the following record of the typical course of events is made.

The first signs of the process are seen in the deeper hue taken on by the free end of the polyp, which is no doubt due to the concentration of material for future use. A slight thickening also takes place at this time, and both these phenomena may be due to a very slow mass-contraction at the foot end, or they may be due to constructive metabolism. This point is an important one, but must be deferred until microscopic examination of tissues is made. Next a constriction is seen about two and a half times its diameter from the foot end (fig. 2, *b*). The fact that it forms very gradually and without any marked contraction of the body at this point warrants the conclusion that this and the subsequent process of complete fission are purely cellular activities. Frequently before the first blastolyte is entirely constricted off a second circular groove marks off another blastolyte (fig. 2, *a*); and even a third has been seen in close succession to the other two, but not more than two have been observed at one time.

Just as soon as one blastolyte is freed, its oral end (its polarity, judging from all my observations, remains the same as that of the parent) becomes rounded and somewhat thicker, while the aboral is drawn out to be more slender,—probably a shifting of material to a point where it will be soon needed for the rapid development of the two sets of tentacles, the first necessary organs for securing food.

From the usually curved position of the foot, as indicated in the anatomical portion, the blastolyte lies almost horizontal or at most somewhat inclined. From this position it rises up as

soon as free, and apparently dissolves the portion of the perisarcal tube immediately above. In specimens kept in glass dishes the whole process of fission took place in about six hours. The blastolytes given off by specimens in confinement did not show much disposition to move about, as did one found free in some tow. After rising up on the pointed end the tentacles begin to bud out on the enlarged upper end as minute knobs (figs. 5, 6), generally two oral ones first, then two aboral, and almost simultaneously with the two aboral ones the second pair of oral tentacles develops.

When about 2 mm. in length the mouth opening is present and the nematocyst collar begins to show. There are five oral and nine aboral tentacles, all somewhat knobbed. The odd number of tentacles shows that after the first two they do not continue to develop in pairs. At this stage the foot end rests curved like in adults, a character which is also evinced by the larvæ of other hydroids. The perisarcal tube is present, being fully developed up to the collar. The tentacles are solid at a very early stage.

When several blastolytes are given off in succession, a group of polyps may arise, and remain close together for some time. So situated, débris collects on the remnants of the perisarc, and the individuals seem to stick in the accumulated mass. The parent meanwhile has moved a considerable distance from its offspring.

This is a brief account of the normal process of asexual reproduction as it takes place in the larger number of cases; but in some, such a pronounced modification was observed as to warrant a separate description.

In the first case noticed (fig. 4) the constricting mass, the second of two starting out apparently normally, began to show a decided lateral thickening, evidently an accumulation of material for some future use. The first blastolyte continued its normal development, while the enlargement on the second one increased, evidently at the expense of the two ends, for which their attenuation speaks (fig. 4, *a*). At this point my notes read:—After 9.20 a.m., or about two and a half hours after the

first blastolyte was seen free, the second one came off from the polyp and, shortening somewhat, became arched (fig. 5, *a*), and as it was before thickened in the middle, the two ends approached more and more, and formed the foot end of the polyp. Even the next day the forked foot of this little animal could be plainly seen (fig. 5, *b*). Here, then, the anterior or oral end had formed from the side of the parent. On the next day I saw the same result accomplished in another way (figs. 7—9). A constricting segment was found at the end of the nearly severed parent, and it had a large hump on one side. This lateral protuberance became larger as the constriction proceeded, then it grew still more at the expense of the foot end, being now severed from the parent; it became the greater bulk, and the former foot-mass became a narrow process (fig. 9, *b'*). The constricted end was also gradually drawn in until the whole assumed the shape of the ordinary blastolyte (9, *b''*). Here again the oral end of the blastolyte was formed from the side of the parent polyp, and as a lateral outgrowth.[1]

The normal process of asexual reproduction of Hypolytus is different from any of the cases of fission described among hydroids. Comparison with strobilation as it occurs among the Scyphozoa, as furnishing a parallel case among the Hydrozoa, seems too strained, especially since it is at the wrong pole, and the resulting products are different.[2] It is different from the frustulation of Schizocladium ramosum described by Allman,[3] since there fission takes place at what would be the oral end.

The sacculæ of Schaudinn's Halermita represent freed lateral buds, and resemble the blastolytes of Hypolytus only in that

[1] Not thinking of the possible significance of these phenomena, no attention was paid to the relative time it took such a blastolyte to produce tentacles, and it is reserved for future observation to see if the explanation given on another page will be borne out.

[2] The well-known phenomenon of "decapitation" among hydroids does not come into consideration here, since it is not a process of reproduction.

[3] Allman, G. J., 'A Monograph of Gymnoblastic or Tubularian Hydroids,' 1871-2.

they may develop directly into polyps. The processes constricted off at the basal end of Corymorpha, which, according to Allman,[1] develop into new polyps, if not the remnants of a Hydrorhiza might correctly be compared with the blastolytes.

Nearest of all, perhaps, comes the asexual reproduction of Protohydra, L., recently more fully investigated by Chun.[2] But this polyp is more primitive, and fission takes place at almost any point on the body, the fission zone not being constant as it is in Hypolytus.

Normal asexual reproduction in Hypolytus by spontaneous fission of a definite portion of the free foot end is unlike any reproductive process heretofore described among Hydrozoa, and since it precludes an attached condition it probably represents an ancestral mode retained by this form. It may have gone through some such stage as Protohydra now does.

The modification of the normal process described under asexual reproduction remains unexplained, and I offer the following as a possible one. It would be of great advantage to the young polyp to have the organs for obtaining a livelihood developed as early as possible. If, now, the material from which the hydranth and the tentacles are to be developed can be accumulated and differentiated (the lateral enlargement) while constriction and fission are going on, the young polyp at the close of this operation could the sooner be ready for the activities of life. This involves formation of a hydranth from a lateral portion of the hydrocaulus instead of from the axial; in fact, just what does take place in Hypolytus. It furthermore suggests a possible explanation of the origin of lateral budding among marine hydroids, by assuming that the precocious development of a hydranth made the separation from the parent unnecessary.

To account for the unattached condition of Hypolytus we may assume that it is secondary, or, on the other hand, that it

[1] 'A Monograph of Gymnoblastic or Tubularian Hydroids,' 1871–2.

[2] Chun, Carl, 'Bronn's Klassen u. Ordnungen d. Thierreichs,' Bd. ii, p. 115.

is primitive. In the first case it would have been preceded by a fixed condition of the polyp. Then the polyp by some process of fission managed to sever itself from a part of its foot end, and attaching itself again went through the same process until fixation was entirely dispensed with, and thus reverted to the ancestral free form.[1] This is, however, without parallel, unless the case of Corymorpha furnish one. One other consideration seems to outweigh the above, viz. that the peculiar mode of asexual reproduction in Hypolytus involves fission of the free end of the parent. It seems to me, then, that it is a phylogenetic character.

Summing up the characters of Hypolytus peregrinus, we have—a single unbranched polyp of the Tubularian type, with two circles of tentacles, ten in the upper and fourteen in the lower; a primitive perisarc enveloping the hydrocaulus, at whose free end buds are given off by spontaneous fission, and these in turn develop into polyps like the parent directly; sexual reproduction by means of ova and spermatozoa, developed in gonophores situated just above the aboral circle of tentacles; on account of its unattached condition it is free to move from place to place, which it does slowly. These and some minor ones are characters that will have bearing on the ultimate classification of our animal, which is not attempted in this report. It is intended to bring out only those characters that have to do with phylogeny and some other problems, such as fission and budding.

DETROIT, MICHIGAN; *March*, 1898.

[1] In this case we might expect the progeny to form at least a temporary hydrorhiza, which, however, does not take place here as it does in Corymorpha. It may be that the embryology of Hypolytus may furnish some further evidence on this point. Another way of looking at the same question is, that Hypolytus was an attached colonial form, in which spontaneous fission took place first just below and then above a lateral bud, and this becoming permanent the lateral thickenings on the blastolytes are to be interpreted as the last remains of budding.

Corynitis Agassizii, McCrady, and its Medusa, Gemmaria.

Last summer, while examining some sargassum driven into Vineyard Sound from the Gulf Stream, I found a small polyp which proved to be Corynitis Agassizii, McCrady. In his description of Halocharis (Corynitis), Agassiz[1] says the medusoid stage was not found, but later he found his Halocharis identical with Corynitis of McCrady, who had observed the medusæ. But according to Allman[2] the medusa ascribed to Corynitis by both McCrady and Agassiz has four marginal tentacles, each with a clavate extremity beset by nodulated pads of thread-cells, and "four overarched spaces between the roots of the radiating canals," while the immature medusa possesses only two tentacles and no "overarched spaces." Allman accepts the general correctness of McCrady's observations with some reservation, pointing out that McCrady captured his four-tentacled medusa in the open sea. He, therefore, has inferred its relationship to Corynitis by intermediate stages.

As my polyps possessed numerous medusa buds, they were kept under observation to determine the question raised by Allman, and finding no previous record of the occurrence of Corynitis in the vicinity of Wood's Holl, I append a short description of the polyps to better establish their identity, and to add a few new points.

They are found most abundant on Membranipora incrustations below the low water mark, probably because on the reddish calcareous deposit they have very good colour protection, as is evident from the difficulty of readily seeing them.

The hydrorhiza is deep pink, while the tiny hydranths have a delicate, translucent, white shade enveloping body and tentacles, with pink between the lighter edges. The hydrorhiza is slender and thread-like, and anastomoses frequently, form-

[1] Agassiz, L., 'Contr. Nat. Hist. U.S.,' vol. iv, pp. 239, 240, 1862.

[2] Allman, G. J., 'A Monograph of the Gymnoblastic or Tubularian Hydroids,' p. 286, 1870–72.

ing a coarse network. The polyps generally arise singly from the hydrorhiza, and do not branch. While the hydrorhiza is covered with a delicate perisarc, I could not demonstrate it with certainty on any part of the polyps, and so cannot verify Allman's prediction that a rudimentary hydrocaulus will be found in Corynitis.[1]

As a rule the polyps are slender club-shaped bodies, from $1\frac{1}{2}$ to 2 mm. in length (fig. 12). There is no marked division into hydranth and hydrocaulus, as Agassiz has pointed out, except that the proximal third is free from tentacles. The oral end is quite blunt, but the hypostome as well as the body is flexible and contractile. Distally the polyp is beset by from thirty to forty-five short knobbed tentacles, which are not arranged in regular circles, but in somewhat oblique rows, giving rise to the spiral arrangement described by Agassiz. The longest tentacles are not more than $\frac{1}{10}$ mm. in length, being nearest the oral end, while the aboral ones are represented by mere elevations on the body. The upper tentacles do not form a circlet around the hypostome, there being a single one higher than the rest. The longer tentacles bear definite large nettling knobs at their ends; a solid row of endoderm cells forms their axes. Nettling organs are also found in the ectoderm of the body migrating[2] toward the tentacles from the base of the polyp where they are developed.

Medusa buds appear most numerous in a zone where the rudimentary tentacles are, though scattered ones may also be

[1] This summer I have found what appears to be a second species of Corynitis. It differs from C. Agassizii in the presence of a well-developed perisarc on the hydrorhiza and the short hydrocaulus, forming imperfectly annulated cups about one fourth the length of the polyp, and in the fact that the medusa-buds are on branched stalks. The colony was not in good enough condition to be sketched, and no medusæ were freed, so it must be left for future observation to determine its relationship.

[2] In a recent article, 'Biol. Centralblatt,' Bd. xvii, No. 13, 1897, v. Lendenfeld has thrown doubt on the fact of the migration of nettling organs. In this connection it is sufficient to state that for several summers in succession this phenomenon has been observed on fresh Pennaria by our students in the laboratory here.

found higher up on the polyp. The largest, and therefore the oldest buds are borne below the middle of the length of the body on single short stalks. From one to ten buds may be found on one polyp.

Scattered everywhere among the polyps bearing medusa buds are others that appear to be sterile individuals. When there are only a few of them they are not conspicuous, and at a time when none of the polyps of a group had any medusa buds they might not at all be noticed. They resemble the others in all respects except that they are more slender and taller, being often 2 to 3 mm. in height, and that they lack any traces of medusa buds, while those around them are very prolific. I cannot understand this sterility of individuals so nearly like the reproductive ones, unless it be a functional one, and is to be interpreted as the beginning of a division of labour in these simple polyps, which in time will lead to a more striking polymorphism. Allman[1] in his Gemmaria implexa has observed a similar difference, of which he says, "In no case can it be regarded as reducing the hydranth to the condition of a blastostyle."

Shortly before the Corynitis polyps were found here I had been taking in the tow a small medusa bearing on its two tentacles some peculiar stalked organs, not unlike stalked Protozoa. When I found them to be an integral part of the tentacles it became evident that the medusa before me was Gemmaria, especially since other characters agreed. The explanation of the presence of this form in our harbour was soon apparent when the medusæ, freed from the Corynitis polyps, were found to have exactly the same characters, and so proved to be Gemmaria. To leave no doubt whatever, one medusa was observed continuously while freeing itself from its polyp nurse by repeated contractions, and until it had sufficiently expanded to recognise its distinctive characteristics. Its umbrella was more spherical, and its tentacles more contracted —one shorter than the other,—as were also its stalked organs

[1] 'A Monograph of Gymnoblastic or Tubularian Hydroids,' 1871–2.

on the tentacles, than is usually the case in older medusæ. Ova were present on the manubrium.

Older medusæ (cf. fig. 11) measure from 1 to 2 mm. in diameter; the umbrella is obovate, deeper than broad, the walls rather thin. Just opposite the four radial canals on the outside of the bell, and extending up about one fifth its meridian, are swellings (fusiform sacs of Allman, p. 224), filled with large nettling organs. The manubrium, extending through over half the length of the bell, is cylindrical, becoming conical when its walls are distended with sexual products. The velum is well developed, with opening rather small. The two tentacles on opposite perradii are quite long and slender when fully expanded, and are provided with long slender filaments, bearing thick-walled oval capsules, each of which contains from three to five oval glistening bodies, and is beset by stiff hair-like processes. At least the proximal portion of each tentacle is hollow, as is evident from the circulation of food particles, while farther out there appear to be separate vacuoles, each containing minute granules exhibiting active Brownian movement. The bases of the two tentacles are enlarged, and bear irregular pigment masses. At the two remaining perradii are slight prominences (below the swellings before mentioned) filled with small nettling organs and some pigment, representing, no doubt, two rudimentary tentacles.

The very unique feature of the genus Gemmaria, as Allman[1] has pointed out, is the stalked organs on the tentacles. These organs one is tempted to compare with the nettling batteries of some of the Siphonophora, not only on account of their containing a number of nematocysts[2] in one receptacle,

[1] Ibid., p. 225.

[2] Although each nematocyst showed a central body looking like folded barbs, I was at first inclined to doubt their nettling function; for while all other nematocysts of the medusa responded to mechanical or chemical stimuli, these were most obdurate. But finally I succeeded in causing threads to be discharged, and now the evaginated thread showed that the appearance in the intact capsule was due to a number of small folded barbs occurring just below a vesicular enlargement of the thread (cf. fig. 13). Allman (ibid., pl. viii) has also figured such a nematocyst from his Gemmaria polyp.

but because the stalks themselves resemble very much the elastic filaments found in nettling batteries.

In the contracted condition the stalked organs seem to beset the tentacles on all sides, but during expansion they are all directed more or less aborally. The nematocysts in the stalked organs are developed in the bases of the tentacles, and migrate outward to where the capsules of the stalked organs arise as evaginations of the ectoderm (fig. 14). At such points the ectoderm is already supplied with the hair-like processes which later stand on the capsules.

When somewhat expanded the stalks are thick and have a wavy outline, while, expanding still more, they look not unlike an unfolding zigzag line or spiral. This gives rise to the granular appearance described by Allman (p. 225), and is in all probability due to optical sections of the joints or spirals of the unfolding stalks.

Finally, there is a very fine smooth filament, not much thicker than a nettling thread, and about as long as the diameter of the medusa, bearing the quivering capsule on its end. During contraction these several appearances occur in reversed order.

The capsules (fig. 13) are thick-walled and somewhat wavy in outline, as if they were made up of segments, and are pierced by a number of openings for the emission of the threads from the contained nematocysts. Covering at least two thirds of the outer portion of each capsule are stiff hairs capable of vibrating so as to impart a peculiar quivering motion to the capsule. They do not wave as cilia usually do, and so can hardly be compared with them. Allman has called them vibratile cilia, and Agassiz [1] does not mention or figure them. The function of the vibratile cilia may be to move the capsules through more space, or may also be tactile.

As to the identity of our medusa with Gemmaria gemmosa, McCrady, there can be no doubt, and that it agrees as nearly with the European form as with the one figured and described by Agassiz [1] may be attributed to age, sex, and condition of expansion of parts.

[1] Agassiz, A., 'Ill. Cat. N. A. Acalephæ,' p. 184, 1865.

The polyps of the European and American Gemmaria, however, exhibit differences enough to remain generically separate; such are the form of the polyp, the degree of differentiation into hydranth and hydrocaulus, the relative development of perisarc, and the arrangement of tentacles. Here, again, as has been pointed out by others, we are confronted by the anomalous condition of two medusæ, almost identical, being produced from polyps generically separated.

MARINE BIOLOGICAL LABORATORY,
WOOD'S HOLL, MASS.; August, 1898.

EXPLANATION OF PLATE 34,

Illustrating Mr. L. Murbach's paper on "Hydroids from Wood's Holl, Mass."

HYPOLYTUS.

Figures 1—10 are eight times enlarged.

FIG. 1.—Adult male polyp. *a. t.* Aboral tentacles. *c.* Nettle collar. *g''*. An immature gonophore. *g'*. Gonophore with sperm nearly ripe. *hc.* Hydrocaulus. *h.* Hypostome. *hy.* Hydranth. *o. t.* Oral tentacles. *p.* Processes at the bases of the gonophores.

FIG. 1 *a*—*g'''*. A female gonophore. *o.* Ova. *p.* The process at the base.

FIG. 2.—Foot end of a polyp showing constrictions (*a*, *b*) preceding fission.

FIG. 3.—The same with one blastolyte (*b*) free, and the other (*a*) showing a lateral thickening.

FIG. 4.—The same with both blastolytes free.

FIG. 5.—The same showing the hydranth of the young polyp (*b*) formed from the side of the adult. The forked foot was formed by approximation of tapering ends of the blastolyte (*a*).

FIG. 6.—Young polyp normally formed from the blastolyte (*b*).

FIG. 7.—Foot end of another polyp (*a*) showing lateral thickening on blastolyte (*b*).

Fig. 8.—The same (*a*) with freed blastolyte (*b*) showing increasing lateral thickening.

Fig. 9.—*b'*. The same blastolyte as *b*, Fig. 8, showing the increased growth of the lateral thickening. *b''*. Final result of *b*, Fig. 7.

Fig. 10.—Portion of hydrocaulus showing perisarcal secretion.

Corynitis and Gemmaria.

Fig. 11.—Gemmaria gemmosa, representing one tentacle fully expanded.

Fig. 12.—Polyp of Corynitis Agassizii without medusa buds.

Fig. 13.—(*a*) Capsule of one of the stalked organs, from the tentacle of medusa, showing one nematocyst discharged; (*b*) large nematocyst discharged from one of the swellings on the bell.

Fig. 14.—Portion of tentacle, showing origin of stalked capsules and vacuoles in the axis.

NOTE.

Arhynchus hemignathi.

IN the 39th Volume (New Series) of this Journal, p. 207, I described a new Acanthocephalon taken from inside the skin in the neighbourhood of the anus of a Sandwich Island bird, Hemignathus procerus. I suggested the name of Arhynchus for this form, since its chief characteristic is the absence of a proboscis. Recently both Professor C. Wardell Styles and Professor A. Hassall have pointed out that this name is preoccupied, having been used by Dujean in 1834 for a beetle. I therefore now suggest the name of Apororhynchus for the new genus which I described in 1896.

ARTHUR E. SHIPLEY.

ZOOLOGICAL LABORATORY,
CAMBRIDGE;
July, 1899.

The Structure and Metamorphosis of the Larva of Spongilla lacustris.

By

Richard Evans, B.A.,
Of Jesus College, Oxford.

With Plates 35—41.

[This memoir was awarded the "Rolleston Memorial Prize" of the University of Oxford for the year 1898.—E. R. L.]

Contents.

I. Introduction.

This piece of work was begun about eighteen months ago on the recommendation and under the superintendence of my teachers, Professor E. Ray Lankester, M.A., F.R.S., and Mr. E. A. Minchin, M.A., Fellow of Merton College, Oxford. The development of Spongilla was suggested to me as a subject worthy of study, on account of the extreme confusion and startling contradictions contained in the published accounts, rendering it almost impossible to make out what was true or what was false. It was with the hope of determining the true history and eliminating incorrect statements that I embarked upon this study.

In order to show what was the state of our knowledge, I begin with a short historical account, which will be followed by an abridged account of my own results.

A. Historical Review.

There is no need to go further back than the account given by Ganin (4) in the year 1879.

Ganin distinguished three layers of cells in the free-swimming larva, which he called "ectoderm," "mesoderm," and "entoderm" respectively. The "ectoderm" consisted of the layer of flagellated cells at the surface, and the "entoderm"

was made up of the lining of the larval cavity and its diverticula extending into the "mesoderm," by which term he included the remaining cells of the larva. He described the larval cavity as being produced by liquefaction and breaking down of some of the central cells.

When the larva became fixed the "ectoderm" was said to flatten out and to become the flat epithelium. The "mesoderm" was supposed to produce the connective tissue and the wandering cells, while the ends of the diverticula of the "entoderm" gave rise to the flagellated chambers, the diverticula themselves producing the exhalant canals, and the larval cavity becoming the gastral cavity.

The next observer who studied the development and metamorphosis of Spongilla was Götte (5), who wrote in the year 1886. Götte divided the cells of the larva into two classes, which he called "ectoderm" and "entoderm." The "ectoderm" consisted of the flagellated cells of the surface layer, while the term "entoderm" included all the cells enclosed within this layer. He derived the larval cavity by mere separation of the cells at the centre, and held that the flagellated cells were thrown away when the larva became fixed, and consequently that the whole sponge was built up from the "entoderm." He was of opinion that the granules contained in the large cells in the interior of the larva were yolk bodies, which became filled with chromatin, and developed into nuclei, the future nuclei of the cells of the flagellated chambers. Therefore, according to Götte, the flagellated chambers arose from the large cells of the interior,—that is, the cells with vesicular nuclei; the remaining cells of the "entoderm" giving rise to the rest of the sponge.

The third observer to study the development of Spongilla was Maas (7), who wrote in the year 1890.

Maas describes the larva as consisting of "ectoderm," "mesoderm," and "entoderm," terms which had the same meaning as was given them by Ganin. The results of his investigations agree to such an extent with those of Ganin as to need no further statement of them than to say that he considered

the granules in the cells with vesicular nuclei to be purely vitelline.

The fourth observer to study the structure of the larva of Spongilla and its metamorphosis was Yves Delage (1), who wrote in the year 1892.

Delage wisely discarded the terms which had been used by all previous observers, and described the larva as consisting of four kinds of cells, which he called flagellated or ciliated cells, epidermal cells, amœboid cells, and intermediate cells.

The flagellated cells consist of the layer of cells which cover the surface, and which pass into the interior, and are taken in and ejected again by the amœboid cells, finally becoming the collar-cells of the chambers.

The epidermal cells were described as forming a complete layer of cells underlying the flagellated cells, which during metamorphosis travel to the exterior, and give rise to the cells of the flattened epithelium.

The intermediate cells he described as being embedded in the inner mass, and as forming a lining to the larval cavity. They become the lining of the canals in the interior of the sponge, in addition to giving rise to connective-tissue cells.

The amœboid cells are those which possess vesicular nuclei, and which during the metamorphosis take in the flagellated cells by a kind of phagocytic action, ejecting them again later on. On being set free the flagellated cells become the collar-cells of the chambers.

Maas, who in the meantime had, from his studies upon other siliceous sponges, arrived independently at embryological conclusions similar to those put forward by Delage, retracted in 1893 (8) his former statements with reference to Spongilla, and recognised in the larva three kinds of cells, viz. the flagellated cells, the amœboid cells, and all the remaining cells. He denied that the flagellated cells are taken in by the amœboid cells, and held that they become the collar-cells without passing through the peculiar changes described by Delage.

The last observer to study the development of Spongilla was Nöldeke (13), who wrote in the year 1895.

Nöldeke described the larva as consisting of "ectoderm" (flagellated layer) and "entoderm" (all the inner mass). He agreed with Delage that the flagellated cells pass into the interior and are taken in by the amœboid cells, but he differs from him in stating that they are completely digested, and not set free again to form collar-cells. He therefore follows Götte in describing the whole sponge as being developed from the cells of the "entoderm," but he derives the chambers either from a single cell or by the coming together of cells.

B. Summary of the Author's Results.

This brief account is introduced here with the object of making it easier for the general reader to grasp the results, and in order to emphasise the main facts brought out by my investigations; in the first place as regards the histology of the larva, and in the second place as regards its metamorphosis and subsequent development.

(*a*) Histology of the Larva.—The free-swimming larva is egg-shaped, with a broader anterior and a narrower posterior pole. The surface of the body is covered all over with a uniform layer of flagella. At the anterior pole is lodged the larval cavity, while the posterior pole is a solid mass of cells.

In the histological composition of the larva we can distinguish—

(1) A layer of flagellated cells at the surface.

(2) The inner mass.

(1) The flagellated cells are arranged in a complete layer over the whole surface of the larva, and are uniform in character. Each cell carries a flagellum, which can be traced down to the nucleus. The cell body is elongated and somewhat constricted in the vicinity of the nucleus, which is onion-shaped, and situated at the base of the cell (Pl. 35; fig. 29 *a*, Pl. 40, &c.).

(2) The inner mass may consist of as many as three kinds of cell elements, of which two at least are always present.

(*a*) Cells with granular nuclei, always present as an irregular

layer under the flagellated cells as well as lining the larval cavity, and it may be in other parts of the inner mass. They are irregular in shape, but may be very flattened when they border a cavity.

The nucleus is spherical or subspherical, with small irregularly shaped chromatin granules, more or less equal in size, situated at the nodes of an even reticular framework.

The cytoplasm may contain a few yolk bodies (figs. 1*a*, 5, and 6; *c. g. n.*).

(β) Cells with vesicular nuclei aggregated chiefly towards the centre of the solid posterior region of the larva. They are massive cells, spherical or oval in form, especially in the younger larvæ, but sometimes in later stages quite irregular in shape. The cells with vesicular nuclei represent a class of unmodified cells derived from the blastomeres from which the other cell elements arise, and which therefore diminish in number during the progress of the development. Their nucleus possesses a large central corpuscle suspended in a delicate nuclear reticulum which always contains granules, varying in number, size, and distribution. The cytoplasm contains from one to four "nutritive vacuoles" and several or numerous "yolk bodies" (figs. 1, 2, 5 *a* and *b*, 6 *a*, and 7; *c. v. n.*).

(γ) Small cells arranged in groups, which may be termed briefly "cell groups." They are always situated in the interior of the solid posterior part of the inner mass. In many cases the component cells of a group are not completely separated from one another, but present the appearance of nuclei arranged near the surface of an incompletely divided mass of cytoplasm. The nuclei are small, and at a certain stage in their development resemble those of the flagellated cells, but they undergo a process of change which causes them to have a slightly different appearance in different larvæ (figs. 5 *a*, *b*, and *c*, 7 and 7 *a*, 11, 11 *a*, 12, 13, 13 *a* and *b*; *g. c.*).

The composition of the inner mass may be very different in different larvæ as regards the relative quantities of the cell elements above enumerated. Four main types of larvæ may be distinguished, which are connected by transitions, but which

may, nevertheless, be conveniently described apart from one another.

Type A.—In these larvæ the inner mass consists of only two kinds of cells; namely, the cells with granular nuclei (α) and with vesicular nuclei (β), the latter alone making up the central part of the solid posterior region (fig. 1).

Type B.—The inner mass consists of three kinds of cell elements above enumerated. The cells with granular nuclei (α) are smaller and more numerous towards the interior. In the cells with vesicular nuclei (β) the "yolk bodies" are somewhat smaller. The "cell groups" are almost always in the incompletely divided stage (figs. 5, 5 *a*, *b*, and *c*, and 9, 9 *a* and *b*).

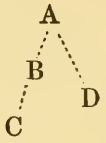
Type C.—The composition of the inner mass is the same as in type B, but with the following differences as regards the development of the cells. The cells with vesicular nuclei (β) are much smaller than in types A and B, and contain fewer "yolk bodies," and as a rule not more than one "nutritive vacuole." The "cell groups" have the cells completely divided, forming in many cases distinct flagellated chambers, and even going so far as to develop collars and flagella (figs. 7, 7 *a*, 11, 11 *a*, 12, 13, and 13 *a* and *b*).

Type D.—The inner mass contains the same three elements as in type B, but in very different proportions, and the cells with granular nuclei (α) are reduced in size and much more numerous. The "cell groups" (γ) are exceedingly few in number, though always present. The cells with vesicular nuclei (β) are much as in type C (figs. 6 and 6 *a*).

The relationship of the four larval types is probably as follows:

Type A represents an early form, from which type B may be derived directly by further differentiation of the cells of the inner mass. Type C may be considered as a further development of type B. Type D, on the other hand, must be regarded as having arisen directly from type A. Two divergent lines of development can therefore be distinguished in the larva, of which the two culminating points are represented by types C

and D, type B being an intermediate stage between types A and C. Type D is not necessarily younger than type C. In the development of type C the vesicular cells (β) give rise to the "cell groups" (γ) rather than to the cells with granular nuclei (α). In the development of type D the exact opposite takes place. The relations can be expressed graphically by the following diagram :



(b) Metamorphosis and Subsequent Development. —The larva fixes itself either by the anterior pole, or by a point not far from that pole, and these differences in the fixation are the causes of transitory variations in the form of the newly fixed stages. In the case of type C the larval cavity becomes obliterated soon after fixation. In the case of type D, however, it seems practically certain that the larval cavity is not obliterated. This may be inferred from the fact that a cavity of considerable size is often found persistent in specimens with fully formed flagellated chambers, with the flagellated layer almost absent from the surface, with the flattened epithelium almost complete all over, and with the developing exhalant canals in some cases already opening into the cavity.

The flagellated cells pass into the interior either individually or in groups, which present a fan-like appearance, and which in some cases consist of a great number of cells (figs. 14, 15, and 15 a and b). They pass in more rapidly on the lower than on the upper surface, and this difference is more accentuated in a larva of type C than in one of type D (comp. figs. 15 and 29). Simultaneously with the passing in of the flagellated cells, the cells with granular nuclei pass out to form the flattened epithelium of the upper and lower surfaces, as well as to form the marginal membrane. The cells with granular

nuclei appear to pass out individually over the whole surface, and when they have got to the exterior they flatten out and become soldered together by their margins to form the layer of flattened epithelium which covers the surface.

The flagellated cells in all cases, after passing into the interior, enter into the formation of peculiar associations in which no cell limit can be seen except just at first. These associations, the "polynuclear groups" of Delage, will be described in this paper as plasmodial aggregations (figs. 16, 16 *a*, 29, 29 *a* and *b*). More than half the mass of the bodies in question must be made up in many cases of the flagellated cells which have entered them by a process of active migration, and which later on separate themselves from them in the same manner.

The nuclei of the flagellated cells undergo peculiar modifications. They contract, and the chromatin becomes rearranged and increases in quantity, so that by the time this change has reached its extreme limit the nuclei of the flagellated cells are very difficult to distinguish from the "yolk bodies." In each plasmodial aggregation the external limit of the cell mass as a whole is at one time sharp and well defined; but this condition is transitory, lasting but a short time, and is therefore not often met with. The plasmodial aggregations become ill-defined and run into one another, thus presenting a syncytium-like arrangement, in which it is most difficult to make out cell boundaries. During this period the nuclei of the flagellated cells change in their characters, and pass by degrees into a condition which represents the definitive state of the nucleus of the collar-cell; their framework becomes looser in texture, owing to the appearing of threads and ultimately of small irregularly shaped granules, and a well-defined nuclear membrane is formed. The nuclei of the flagellated cells, while undergoing these changes, are as a rule arranging themselves in rings in the cytoplasm round cavities of a circular shape, and in each such ring the cytoplasm becomes divided into cell bodies corresponding to the nuclei. The cells so formed are independent of one another,

and develop collars and flagella (figs. 16—22, *fl. c.* = *c. c.*). In the meantime the cells with granular nuclei which are still in the interior are arranging themselves to form a lining to the cavities which have appeared in the syncytium-like parenchyma of the young sponge. The spaces later on become the subdermal cavity, the inhalant and exhalant canal systems, and the gastral cavity; in short, all the cavities of the sponge, other than the chambers, are lined by cells with granular nuclei. This description of the fate of the flagellated cells applies equally to the larval types C and D. The history of the flagellated cells, however, is more easily made out in the type D, owing to the almost complete absence of the small cells which compose the cell groups, than in type C, where the cells in question are numerous.

In the type C, flagellated chambers are in many cases fully developed at the time of fixation, possessing cells which are adorned with collars and flagella (figs. 7 and 7 *a*). These cells are found in groups in type B, and are seen to possess at a certain stage a nucleus so exactly like that of the flagellated cells as to be indistinguishable from it (figs. 5 *c* and 9 *a* and *b*). During the change from type B into type C the nucleus alters in character, and assumes the definitive structure of the nucleus of the collar-cells. Hence in the complete history of the flagellated cells on the one hand, and of the small cells in the "cell groups" on the other, we have two stages in which their nuclei are exactly like one another. First, the nuclei of the small cells of the groups at the time when the cytoplasm is as yet incompletely divided up are exactly like the nuclei of the flagellated cells, so long as the latter retain their position in the superficial layer of the larva. Secondly, they resemble one another so much as to be indistinguishable when both have attained the definitive structure of the nucleus of the collar-cell. Though these two developmental stages correspond exactly, the intermediate conditions through which they pass are very different; the nuclei of the cell groups pass gradually from one stage into the other without any interruption, while those of the flagellated cells pass through a most extraordinary series of

changes in connection with the plasmodial aggregations of cells.

The megascleres are produced in the cells with vesicular nuclei, and arrange themselves in various ways at the metamorphosis. Many of them place themselves almost vertically and raise up the dermal membrane, giving the upper surface of the young sponge an irregular conformation of hills and valleys, as it were. Below the dermal membrane is the subdermal cavity, lined by cells with granular nuclei above and below, while in the centre of the little sponge are the large spaces of the exhalant system opening by the osculum. Along the surface, on the outer aspect, are the inhalant ostia opening into the subdermal cavity. The osculum, which occupies a central position, is at first on a level with the surface, but soon becomes situated at the tip of an erect oscular tube. All the cavities in the interior are lined by flat epithelium cells with granular nuclei, and other cells of this class are also seen in the spaces between the chambers accompanying the cells with vesicular nuclei. The microscleres are secreted by cells with granular nuclei.

Now that a brief historical account and a summary of the results of the work embodied in this paper have been given, the problem to be solved in the more detailed account which is to follow can be stated.

It will be necessary to show that there are several types of larvæ which differ considerably from one another as regards the structure of the inner mass, and that owing to these differences the metamorphosis of Spongilla may take place in more than one way,—that is, the metamorphosis depends on whether the larva at the time of fixation has developed in the direction represented by types B and C, or in that represented by type D.

It will be shown that in the first case there will result two slightly different methods of metamorphosis, the differences between which will depend on the age of the larva. If the larva fixes in the condition of type B, there will be small cells in the interior not yet developed into chambers. If, on the

other hand, it has reached the condition of type C, there will be chambers completely formed which have been derived from the vesicular cells of the larva. In both cases the flagellated cells of the larva develop into collar-cells, passing through the same series of changes as they do in a larva of type D.

In the second case, due to the fixation of a larva of type D, the flagellated chambers arise almost entirely from the flagellated layer of the larva, and scarcely at all from the cell groups, which are very few in number and possibly absent.

The divergences in the metamorphosis will be seen to be differences of degree which merge into one another, but which nevertheless produce strikingly different appearances in the early stages of development. Further, it will be evident that the variations in the metamorphosis are due only to a very slight degree to differences in the age of the larvæ, and depend almost entirely on the divergent courses of evolution which will be described in the account of the larva.

II. Detailed Account of the Histology of the Larva and of the Metamorphosis.

A. Histology of the Larva.

Introductory Remarks.—In describing the histological structure of the free-swimming larva and the changes through which it passes before, during, and after metamorphosis, it will be necessary to bring into line all the phenomena presented by the developing larva and the young sponge, and to avoid having recourse to the easy method of explaining difficulties as abnormalities. Further, it will be convenient to avoid using the words ectoderm, mesoderm, entoderm, and such terms, because they are liable to lead to confusion, and to prejudge the questions of homology which may arise.

By describing the histology of the larva, with a special view to the changes which go on during the free-swimming part of the life history, many of the errors of previous observers will be avoided, and many of their mistakes will be explained; while

several statements, which appear at present to be conflicting, will be brought into line, and a more complete description than has hitherto been given will result.

The larvæ do not appear to hatch, or, at least, to swim out of the mother colony, at the same age. It is quite possible that they pass into the excurrent canals at approximately the same stage of development, but owing to obstructions are unable to swim out. They leave the mother colony by way of the osculum, and are carried along by the current which issues from that opening. They, however, soon gain control over their movements, and swim to the surface of the water, darting down instantaneously should they be disturbed in any way. In all their movements the glistening broad end is directed forward. When they reach the surface they either swim about for a time, or settle almost immediately on the side of the vessel, though they do not at first fix themselves to it. The greater number will, if left undisturbed, fix to the side of the vessel, while others will fix to the film of air at the surface of the water. The interval of time between the actual swimming out of the mother colony and the fixing is not constant. They appear to fix in greater numbers between three and six o'clock in the afternoon than at any other time.

The larva is oval in form, or, perhaps more correctly, egg-shaped, and is covered with a uniform coat of flagella. The broader anterior end has a glistening appearance, owing to its containing a large cavity, the "larval cavity," filled with a kind of clear jelly. The narrower posterior end presents an opaque appearance, owing to its being composed of cells which, in the younger larvæ, are full of food material, which, as development proceeds, becomes used up.

In the following more detailed description of the cellular elements of the larva the same plan will be maintained as was followed in the abridged account given above.

In the histological composition of the larva we can distinguish—

(1) The flagellated layer at the surface (figs. 9 and 11).

(2) The inner mass (figs. 9 and 11).

(*a*) The Flagellated Layer.—In contrast with what occurs in the greater number of sponge larvæ, the flagellated layer completely surrounds the inner mass, each of its constituent cells being provided with a flagellum (figs. 38 *a*, *b*, *c*, and *d*). All the flagellated cells present the same characters, only differing slightly from one another in length; and the nuclei, which in all cases are situated near the bases of the cells, are consequently not on the same level (figs. 1*a*, 5, 6, 7, and 38 *a* and *b*). Their general form is, on the whole, a characteristic one so long as the flagellated layer retains its position at the surface. The cell body, which lies almost altogether externally to the nucleus, is constricted so as to present the appearance of having a waist. The narrower part measures no more than $1\frac{1}{2}\ \mu$ across; while the broader part, which is situated externally, measures from 2 to $2\frac{1}{2}\ \mu$, the length of the cell varying from $5\frac{1}{2}$ to $7\frac{1}{2}\ \mu$.

Owing to the constricted condition of the middle portion of the cells, spaces are often seen between them. The nuclei, on the other hand, are closely wedged against one another, while the external ends fuse with one another to form what might almost be described as a thin membrane in which no cell outlines can be distinguished. The cytoplasm contained in the external position is more opaque than that existing in the waist of the cell. This condition is probably due to the presence of very fine granules, which give it a denser consistency. The cells appear as if they were suspended from this membrane, only touching one another at their inner ends where the bulging nucleus is situated (figs. 1 and 5).

The flagellum with which each cell is provided lies in part outside and in part inside the cell. The portion which lies outside is at least as long as the cell itself, and appears to taper to a point, its base being in some cases surrounded by a cone-like elevation of the cytoplasm. The portion which lies inside the cell passes down to the neighbourhood of the nucleus, and in many cases presents a small swelling which lies on the nuclear membrane, and possibly represents the centrosome. Sometimes the flagellum, instead of ending in a small

body situated close to the nuclear membrane, seems to spread over the membrane in question, which appears to be drawn out like the outer coat of an onion. The internal part of the flagellum can be traced along its whole length in all the larval stages, as well as in some young fixed stages in which the flagellated layer has not completely migrated into the interior (figs. 29 *a* and 38 *a*—*d*).

The nucleus of the flagellated cell, when cut tangentially to the surface of the larva, presents a circular section nearly 2 μ in diameter, but when cut radially it has an oval appearance, or, perhaps more correctly, that of the outer half is almost cone-shaped, while that of the inner half is semicircular. It measures about 2 μ across and $2\frac{1}{2}$ μ in length. By combining these two sections it is evident that the shape of the nucleus is that of a cone with a hemisphere at its base, or, in other words, it is onion-shaped (figs. 1 and 29 *a*).

I must here apologise for going still further into detail concerning the flagellated cells. This is necessary, however, in order to compare the cells here described as "cell groups" with the flagellated cells.

The cytoplasm of the flagellated cells is clear, and contains at most no more than three or four small round granules, which cannot be seen in ordinary preparations[1] (figs. 38 *a*—*d*).

The nucleus has a thicker and better defined nuclear membrane than any other class of nuclei found in the larva. Internally the nucleus contains a few small and irregularly shaped chromatin granules, one of which may exceed the others in size, scattered at the nodes of a somewhat coarse nuclear reticulum. When there is one larger granule, as is often the case, it usually occupies a central position, and the threads pass straight from it to the nuclear membrane; but when there are several granules approximately equal in size, which is the

[1] These small granules could not be seen in sections of larvæ preserved either in Flemming's fluid or in Perenyi's fluid, or in absolute alcohol, and mounted in Canada balsam. However, they were distinctly seen in preparations of larvæ preserved in osmic vapour, stained in picro-carmine, and mounted in glycerine.

usual rule, the granules are evenly distributed throughout the whole nucleus, and are united to one another and to the nuclear membrane by means of threads. These differences, however, are only differences of degree, and not of quality, for there are always a great number of intermediate stages.

To sum up, the flagellated cell may be described as elongated, and constricted at the middle. The nucleus, situated at the base of the cell, has a thick membrane enclosing a network of threads, at the nodes of which from one to five chromatin granules are situated.

(*b*) The Inner Mass.—The inner mass may consist of as many as three kinds of cells, of which two at least are always present. Without any further general remarks I shall proceed to describe these three kinds of cells, leaving certain questions to be dealt with in the appendices, *e. g.* the origin of the "cell groups," the enclosures found chiefly in the cells with vesicular nuclei, and the development of the spicules and changes which take place in the nucleus of the scleroblast.

(*a*) The Cells with Granular Nuclei.—These cells are found to occur in two positions, at least, out of the three which they may occupy. They are always found as a more or less complete layer immediately under the flagellated cells, and as the larva grows older the completeness of the layer becomes more evident. Secondly, they are always present in the immediate vicinity of the larval cavity, especially the anterior moiety of that cavity; and here, again, the layer becomes more complete with age. And thirdly, they may occur in the interior of the solid part of the inner mass, where, however, they do not appear to be present at first, save in exceptional cases, and even then only in very small numbers.

In the first and second of these positions the cells with granular nuclei tend to become flattened, and consequently in a radial section present an oval form; while in the third they present an irregular shape, pushing their processes between the other elements of the inner mass. As the larva becomes older they increase in number at the expense of the cells with vesicular nuclei as well as by their own division. The proportion which

they bear to the number of other cells present varies considerably. The size of the cell, which probably depends on the number of divisions that have taken place, is far from constant, even in the same larva. They become smaller as the larva grows older, measuring from 8 to 10 μ over one of their flattened surfaces, but they are really too irregular in form to admit of proper measurement. Sometimes a spiny microsclere appears in these cells, even in the larva.

The cytoplasm is usually clear, the cell body having at most only a few enclosures. A "nutritive vacuole" is seldom seen in them, but the "yolk bodies" are often present, though few in number.[1] They also contain some of the small refringent granules to which reference was made in describing the flagellated cells (fig. 38 *e*).

The nucleus is either spherical or subspherical. Its usual tendency is to assume the latter form when the cell is in either the first or the second of the above-mentioned positions, and the former when it is in the third. The above facts have no importance other than that the general configuration of the cell influences the shape of the nucleus,—that is, when the cell becomes flattened the nucleus acquires a slightly compressed form. The size of the nucleus, like that of the cell itself, and probably for the same reason, is variable, ranging from $3\frac{1}{2}$ to $5\frac{1}{2}$ μ in diameter.

The nuclear membrane is much thinner than that of the nuclei of the flagellated cells, often to such an extent as to be difficult to distinguish from the surrounding cytoplasm. The interior of the nucleus is occupied by numerous small and irregularly shaped granules, placed at the nodes of a close and fine nuclear reticulum.

There is no reason that I can see to be found in the characters of these nuclei which would justify the division of this class of cells into two, namely, "epidermal cells" and "intermediate cells" of Delage. They appear to be only one class of cells, which are capable of being modified into flat epithelium

[1] For a further discussion of the "nutritive vacuoles" and "yolk bodies" cf. Appendix A, pp. 422—425.

whenever they are situated near a surface. Whether the surface in question is internal or external seems to be immaterial.

To sum up, the characters of the cells with granular nuclei are as follows :—an irregular or flattened form ; clear cytoplasm, which may contain a few enclosures ; and a fairly large nucleus with a thin nuclear membrane, which encloses a great number of small granules placed at the nodes of a fine reticulum.

(β) The Cells with Vesicular Nuclei.—These cells for the most part occupy the posterior moiety of the inner mass. They are seldom found in the anterior region, between the layer of flattened cells which line the larval cavity and the flagellated layer. In the youngest larvæ they are not separated from the larval cavity situated anterior to them, nor from the flagellated layer at the posterior end, but in older ones a layer of cells with granular nuclei is developed in both of these positions. Consequently the cells with vesicular nuclei, mingled with cells of other kinds, become restricted to the internal part of the solid posterior end of the inner mass. These cells are by far the largest in the whole larva, and contrast with the cells which possess granular nuclei in having a perfectly definite outline as well as an oval or circular form, at least in the younger larvæ ; in older specimens they seem to lose to some extent their regularity of outline and compactness of form. Another point of contrast between these two classes of cells is that the cells with granular nuclei contain but a few enclosures, while those with vesicular nuclei are always possessed of one or more "nutritive vacuoles" and several "yolk bodies." The number of these enclosures found in the individual cells depends on the stage of development of the larva. In young larvæ the cells often contain as many as three or four nutritive vacuoles and numerous yolk bodies, while in older larvæ they almost invariably contain only one nutritive vacuole and but a few yolk bodies. These cells, in common with the two classes of cells already described, possess some of the small refringent granules mentioned in describing the flagellated cells.

The nuclei of these cells are the largest in the whole larva, measuring sometimes as much as 7 μ in diameter, but their size, like that of the cell itself, depends on the state of development of the individual. The nuclear membrane, like that of the granular nuclei, is thinner than that of the nuclei of the flagellated cells, but, as a rule, is easily made out. The centre of the nucleus is occupied by the central corpuscle—the so-called nucleolus,—which, again, like the cell and its nucleus, varies considerably in size. The space between the central corpuscle and the nuclear membrane is occupied by a variable number of granules situated at the nodes of the nuclear reticulum. When the number of granules is small they are comparatively large in size, and the threads of the nuclear reticulum are few and coarse. On the other hand, when the number of granules is large they are small in size, and the threads of the reticulum are correspondingly close and fine. The granules in these nuclei vary also as regards position. In some nuclei the granules, whether exceedingly numerous and small, or few and comparatively large, are concentrated in the neighbourhood of the nuclear membrane, leaving a clear zone round the central corpuscle; in others the granules are evenly distributed throughout the whole space, and consequently the clear zone is absent (fig. 41 *a*, and fig. 40 *a*).

These differences in the structure of the vesicular nuclei seem to suggest that the class of cells here described consists of several different kinds of cells. The term "cells with vesicular nuclei" may, in fact, be regarded simply as a convenient one to hide our ignorance of the division of labour that has already come into existence among these cells. The fact that megascleres are being developed in some of these cells may be taken as evidence in favour of this view (figs. 36 *a*, *b*, and *c*).

To sum up, these cells are the largest in the whole larva, occupying the interior of the inner mass, and containing a number of enclosures in the form of "nutritive vacuoles," "yolk bodies," and "refringent granules." They possess a nucleus which contains a large central corpuscle, sometimes surrounded by a clear zone. The nuclear membrane is thin,

and encloses a variable number of small granules situated at the nodes of the nuclear reticulum.

(γ) The Cell Groups.—These groups of cells are situated in the interior of the solid posterior part of the inner mass. The number of cells which constitute a group is highly variable. The groups seem to be present in all the older larvæ, though they are far more numerous in some individuals than in others. They are so few, however, in some older larvæ that they might have been overlooked had it not been that in some individuals they are more numerous. In some cases the groups are well defined and isolated, while in others they run into one another owing to their close proximity. Sometimes they are surrounded by a well-developed membrane, formed, apparently, by the cells with granular nuclei, this being especially true of the groups situated near the centre of the solid posterior end of the inner mass. In the youngest larvæ in which the groups occur the cytoplasmic bodies of the individual cells cannot be distinguished from one another; but in older larvæ the cells are perfectly independent, and may go so far, even in the free-swimming larva, as to form collars and flagella. In the latter case they enclose a cavity, which is that of the flagellated chamber. Just as the cells themselves exhibit a progressive development from an incompletely divided condition to one in which the cells are free from one another, so also the nuclei pass through a series of changes. At one stage they resemble those of the flagellated cells to such an extent that the same description might be made to apply to both, while at a later stage they assume the definitive characters of the nuclei of the collar-cells.[1]

Now that a general description of the cell elements which enter into the histological composition of the larva has been given, it is necessary to discuss the relative quantities, occurring in the different types of larvæ, of the elements above enumerated. Four main types of larvæ may be distinguished, which

[1] For a further discussion of the possible origin of the cell groups cf. Appendix B, pp. 422—425.

are connected by transitional stages, but which may nevertheless be described apart from one another. The flagellated cells are alike in all the types, and need no further discussion. The only difference that exists between their early and late condition is that their nuclei are nearly on the same level, and consequently that they are almost the same length. In addition to this, in the older larvæ the cells with granular nuclei are seen to push their processes between the flagellated cells, indicating that fixation and metamorphosis cannot be long delayed. The differences which exist between the several types of larvæ to be considered concern the inner mass, and the special features of each type will be described in the following pages.

Special Features of Type A.

The inner mass in this type consists of only two kinds of cells, namely, cells with granular nuclei (α) and with vesicular nuclei (β).

The former are almost entirely confined to two positions, occurring first as an incompletely developed layer of cells immediately below the flagellated layer; and secondly, in the vicinity of the larval cavity, where, however, they are almost completely limited to its anterior border. They have not yet assumed the flattened shape which they acquire in later stages in either of these positions.

The latter have the monopoly of the interior of the solid posterior end of the inner mass. They enclose in many cases three or four nutritive vacuoles, together with numerous yolk bodies, and occasionally a developing spicule is found in them.

Microscleres and cell groups are not found in this type, which is evidently the youngest of all free-swimming larvæ (fig. 1). From it all the others are developed, and it is highly probable that the larva never fixes while in this early state of differentiation of the inner mass, but proceeds to develop into one or other of the types which remain to be described.

Special Features of Type B.

In this type the inner mass consists of three kinds of cells, namely, cells with granular nuclei (α), with vesicular nuclei (β), and cell groups (γ).

The cells with granular nuclei are now sufficiently developed to form fairly complete layers in the two positions in which they were recognised in type A, and have also appeared in the interior of the solid posterior end of the inner mass. They have also become flattened save in the interior, and their nuclei are in some cases subspherical in form.

The cells with vesicular nuclei are far less numerous than in type A. They have become slightly smaller in size, and less definite and regular in outline. The nucleus also is smaller, and the central corpuscle is evidently diminishing in size and breaking up. These facts point to the gradual change of some of the cells with vesicular nuclei to such as have granular nuclei.

The cell groups which occur in this type are a new and most important feature which did not exist in type A. The cytoplasmic bodies of the individual cells which make up the groups are as yet incompletely divided from one another, so that the nuclei present the appearance of lying at the periphery of a mass of cytoplasm. It is only when a group is looked at in surface view that anything resembling a dividing line between the nuclei can be seen, a fact which indicates that the division of the multinucleated cytoplasmic mass proceeds slowly from the surface towards the centre (figs. 9 *a* and *b*, *g*. *c*.). The amount of cytoplasm corresponding to each nucleus is not more than that contained in a flagellated cell. The nuclei in a radial section of a group present the same onion-shaped form as those of the flagellated cells. The nuclear membrane is thick, and the granules are small and few. In short, these cells, while differing completely, on the one hand, from the cells with granular nuclei, both as regards the size of the cell and the characters of the nucleus, are almost identical, on the other hand, in both these points with the

flagellated cells. These considerations suggest irresistibly the conclusion that they are developed from cells identical in character with those which gave rise to the flagellated cells, and possibly in the same way (figs. 5, 5 *a*—*c*, 9 *a*, *b*).

I have no reason for thinking that this type of larva often fixes itself, though it may occasionally do so. As a rule, however, it develops further, and gives rise to the larva of type C, the special features of which will be described next.

Special Features of Type C.

In this type of larva the same three kinds of cells are found as in type B, but, owing to the differentiation and development which have taken place, the differences of the cell characteristics are considerable.

The cells with granular nuclei are much more irregular in shape than in the larvæ described above. In this larva they branch extensively, and unite by their processes to form membranes which are still more or less incomplete, both under the flagellated layer and as a lining to the larval cavity. Many of those which are situated in the interior have changed considerably in shape, having in many cases flattened out so as to surround small lacunar spaces or canals (fig. 14 *a*). The lacunæ in question are destined to become the exhalant system, and the cells surrounding them to become the flat epithelium of the same. Simultaneously with the flattening of the cell body the shape of the nucleus is also changed to that of a biconvex disc. In this type some of the cells with granular nuclei may develop a spiny microsclere (fig. 37 *a*).

It is necessary to point out here that the spaces and canals lined by the cells with granular nuclei are not in any way comparable to the spaces surrounded by the cell groups. In the latter case, the spaces are the cavities of future chambers which are either appearing or have already appeared in the larva. The small spaces and short canals here described are lacunar cavities which have the same relation in many cases to the cavities of the cell groups as the exhalant canals have

to the chamber in an adult sponge. In reality the communication between these two kinds of spaces is an already developed exhalant pore or apopyle. It may further be pointed out that these spaces, whether canals or chambers, are structures formed in situ from the cells of the solid posterior end of the inner mass, and not as ingrowths into it from the layer of cells which line the larval cavity. Should some of the short canals appear to open into the larval cavity, the communication is one which has been secondarily acquired during the development.

The cells with vesicular nuclei are less numerous, more irregular in shape and outline, and smaller in size than they are in types A and B. They become less numerous owing, on the one hand, to their conversion into cells with granular nuclei, and, on the other hand, to the formation out of them of the cell groups. They become more irregular in shape owing apparently to the stored-up food material being used up, giving them greater facility for change of form and the exercise of their wandering function. They become smaller in size owing to repeated cell division. In this type it is quite exceptional to find a cell measuring more than 10 μ across, while in type A they often measure from 12 to 15 μ, and in type B from 10 to 13 μ. A nucleus measuring 7 μ across is often seen in types A and B, but in type C the largest vesicular nucleus seldom exceeds $5\frac{1}{2}$ μ in diameter. On the other hand, a vesicular nucleus measuring less than 5 μ across is scarcely found in types A and B, but in type C they constantly occur. The same reduction of size is noticeable in the central corpuscle (or nucleolus) of the vesicular nucleus. In types A and B it often measures 2 μ across, but in the type now under consideration it averages only $1\frac{1}{4}$ μ in diameter. In types A and B these cells contain one or more nutritive vacuoles and several yolk bodies, but in type C they seldom contain more than one of the former, which have also become slightly reduced in size. The number of the yolk bodies is also greatly decreased, though they are by no means completely absent. Moreover, the cells in question often produce spicules

which have a tendency to protrude through the flagellated layer, though they are still completely surrounded by the cells which produced them. The various changes undergone by the cells with vesicular nuclei suffice, apart from any other reason, to prove that the order of the types above adopted is the true one as regards age: i.e. that type A is the youngest of all, because the interior of the solid posterior end of the inner mass is monopolised by the large cells with vesicular nuclei; and that type B must be younger than type C, because the characters of the remaining cells with vesicular nuclei are almost the same as the characters of those cells in type A, though the numerical proportion which they bear to the other cells has changed considerably (figs. 11, 11 *a*, 12, 13, and 13 *a* and *b*).

The individual elements of the cell groups which have been described in type B as being incompletely divided, and as having their nuclei lying near the periphery of a common mass of cytoplasm, are, in this type, completely independent of one another. The cell groups now are as numerous as they were in their incipient condition in type B, a state of things markedly different from what exists in the type of larva still to be described (figs. 7, 7 *a*, 11, and 11 *a*). The cells which constitute the groups are fairly uniform in size. They possess a nucleus which measures about $2\frac{1}{2}\,\mu$ in diameter, showing a slight increase in size as compared with the nuclei found in the incompletely divided groups of type B, as well as a greater number of chromatin granules and a higher degree of complexity in the nuclear reticulum. Sometimes, however, there may be seen in a group one cell larger than the others with a nucleus of corresponding size. The existence of such cells is probably to be explained as being the result of independent growth after the cells have become liberated from the incompletely divided groups of type B (fig. 11 *a*).

The number of cells which constitute a group varies considerably, just as the number of nuclei vary in the multinucleated masses of cytoplasm in type B. There may be no more than four or five cells in a radial section of a group, or there may be a dozen or even more, but it is probable that the

number of cells that can take part in the formation of a group cannot pass a certain limit. Consequently, as the individual elements of the cell groups multiply by division, it seems a well-founded conclusion that the groups themselves multiply in the same way.[1]

The large number of cells found in the bigger groups might be accounted for on the supposition that the individual cells have divided since their liberation from the groups of type B. Though this supposition may be to some extent true, it is more probable that the difference in the number of cells which constitutes a cell group or a flagellated chamber in type C corresponds to a similar difference in the number of nuclei in the multinucleated cytoplasmic masses of type B.

The cytoplasm of the individual elements of the cell groups is usually clear, but may contain a few granules which are either reduced yolk granules or bodies of the same nature as the refringent granules already described as existing in the flagellated cells, and also in all the cells of the inner mass. Another very remarkable fact is that they occasionally contain a small nutritive vacuole, a fact which points to their origin from the cells with vesicular nuclei.

Another feature of some of the cell groups in this type is that their individual cells develop collars and flagella in all respects like those of the collar-cells in the adult sponge. It is true that the greater number of these groups consist of cells which have not as yet developed these organs; and it may be further stated that unless great care is taken in the preservation of the larvæ, all of them, without exception, will be without collars, and will present only a kind of process possessing a more or less conical shape, and pointing towards the cavity inside the group of cells. The collars unite by their margin to form the so-called "membrane of Sollas" (figs. 7 and 7 *a*).

These cells, which from this stage onwards may be called collar-cells, present the same general arrangement and the

[1] For further remarks on the multiplication of the collar-cells and of the flagellated chambers cf. Appendix D.

same relation to the exhalant pore already described as they do in the fully metamorphosed larva or adult sponge. The cells have a horseshoe arrangement when seen in a radial section of a group. The opening, which points towards the lacunar spaces already mentioned, is a true exhalant pore or apopyle (fig. 7 *a*, *B*).

The nucleus of the collar-cells is but very slightly larger than it was in the incompletely divided condition seen in type B. In the interior the chromatin granules have become more numerous, and the nuclear reticulum more complicated. Consequently there is at present no very striking difference between these nuclei and some of the nuclei of the cells which have been described as having granular nuclei. But for the discovery of the origin of these cells in the larvæ of type B, their existence as a separate class would probably have remained undiscovered and even unsuspected, and they might easily have been placed among the cells with granular nuclei—an error which would have almost certainly led the way to another mistake, that, namely, of describing the cavities of the cell groups as incipient exhalant canals.

So important are the main features of this type that they may be briefly summarised. In the first place there are two kinds of cavities, those of the flagellated chambers on the one hand, and the lacunar spaces of the incipient exhalant system on the other hand. In the second place these two kinds of cavities are surrounded by two different kinds of cells,—the former by the individual elements of the cell groups or collar-cells, and the latter by cells with granular nuclei or flat epithelium. The two cavities above described are, in certain cases, already in communication by means of the exhalant pore.

The important point to emphasise is the fact that the collar-cells, in this type of larva, are developed in the interior before any flagellated cells have made their way there; and further, that they have been developed by fragmentation of the nucleus and the subsequent division of the large cells with vesicular nuclei found in type A, and have passed through the condition found to be characteristic of type B.

Special Features of Type D.

In this type of larva the same three kinds of cells take part in the formation of the inner mass as in types B and C, but the numerical proportion is very different. In types B and C the individual elements of the cell groups are as numerous as either the cells with granular nuclei or the cells with vesicular nuclei; but in type D the number of the cell groups is so small that, had they not been discovered in the other types, they would most probably have been overlooked. However, they seem to be present always, though the groups often consist of only four or five cells, and are few in number (fig. 6 *a*, *g. c.*).

The cells with granular nuclei occupy the same position as in type C, and present individually the same characters. A noticeable feature in connection with them in both larvæ is their irregularity of form when they are examined from the flattened surface. In consequence of this, the so-called membranes, one of which lies under the flagellated layer and the other lines the larval cavity, are far from being complete even when they are best developed (fig. 10). Another feature, equally noticeable with the above, is that the cells with granular nuclei situated in the solid part of the inner mass scarcely ever flatten out to surround lacunar spaces or canals, which are almost completely absent from this larva. Their nucleus varies considerably in both shape and size, measuring from 3 to $4\frac{1}{2}\ \mu$ across, but remains uniform in structure, whatever their position. The chromatin granules are always small in size, irregular in shape, and situated at the nodes of a nuclear reticulum which consists of fine threads enclosing small meshes.

The "cell groups" are few, and consist of a small number of cells, as has been mentioned above. Their individual elements average about $5\ \mu$ in diameter, and the nucleus varies from $1\frac{3}{4}$ to $2\frac{1}{2}\ \mu$ in diameter, a size which agrees closely with

that of the nuclei of the flagellated cells. Further, they possess the same structure as the nuclei of the latter cells—that is, a few granules situated at the nodes of a coarse network of threads and a thick nuclear membrane. There is no doubt but that these cells belong to the same class as those of the cell groups found in types B and C, and their presence, however few they may be, accounts for the existence of an occasional chamber in a larva of type D, immediately after fixation.

The cells with vesicular nuclei present much the same characters and general distribution as they do in type C. They are both less numerous and smaller than they are in types A and B. The number of yolk bodies which they contain is much smaller than in types A and B. The nutritive vacuole of course is a constant feature of the cell, with a vesicular nucleus in all stages, but it appears to decrease in magnitude simultaneously with the decrease in size of the cell. The inner mass, therefore, in this larva consists of a fair number of cells both with granular and with vesicular nuclei, together with a few of the elements of the cell groups, in contrast with the large number of them found in types B and C (figs. 6 and 6 *a*).

General Remarks upon the Relationship of the Larvæ.—The author's views with regard to the relationship of the four larval types already described have been stated above (p. 369). It only remains to discuss these relations in greater detail.

There can be no doubt that type A is the youngest larva of the four types described above. The fact that the cells with vesicular nuclei are here relatively more numerous than they are in any other type supports this view, because they are the most primitive cells of the larva. They retain, in fact, in type A almost all their blastomeric characters, which in the other types they gradually lose, so far as the contents of the cells are concerned, though not perhaps in the physiological sense.

Histogenesis seems to advance or to be retarded in separate regions of the larva at different times. At the close of

segmentation, while the embryo is in the maternal follicle, all the cells possess the same characters ; but between the above stage and the time of hatching, the cells at the surface become differentiated into flagellated cells externally, and cells with granular nuclei beneath, while the cells in the interior of the solid posterior part of the inner mass seem to be arrested in their development. As the results of these changes we obtain the larva which has been described above as type A. It has a flagellated layer at the surface, and cells with granular nuclei beneath and in the neighbourhood of the larval cavity. The posterior end, however, is occupied by the large cells with vesicular nuclei, which represent both morphologically and physiologically a number of unmodified blastomeres. After this stage has been attained, further differentiation seems to take place in the interior, while the flagellated cells, situated as a layer completely surrounding the larva at the surface, are, for a while, retarded in their development.

The changes in the interior consist in the further differentiation of the cells with vesicular nuclei which have, as above described, retained the characters of blastomeres, but which no longer give rise to flagellated cells on the exterior as well as to cells with granular nuclei. The place of the flagellated cells is taken now by the individual elements of the cell groups. Thus another argument in favour of the homology of the small incompletely separated cells, seen in type B, with the flagellated cells, is furnished by the fact that, when the cells with vesicular nuclei cease to give rise by differentiation to flagellated cells, they begin to give rise to cell groups. This is an important point when taken, not by itself, but in conjunction with the arguments already put forward, based upon the identity in size of the individual elements of the cell groups with the flagellated cells, and the similarity of their nuclei.

There results, as the outcome of these internal changes, the larva described above as type B, from which type C is derived by further differentiation along the same line. The multinucleated masses of cytoplasm in the former divide into as many corpuscles of cytoplasm as there are nuclei in the whole

mass, and in this way give rise to the chamber-like groups found in the latter. The chief difference between the elements of the cell groups in type B and the flagellated cells at the surface is the lack of a flagellum in the former and its presence in the latter. But the cells which lack the flagellum in type B surpass their rivals, so to speak, in type C, and develop not only a flagellum, but a collar as well.

In order to lay more emphasis on the arguments which favour a homology between the cell groups and the flagellated cells, it seems advisable to summarise them briefly. First, the cells in question are almost exactly of the same size; secondly, the nuclei of the cell groups in type B are identical in characters with those of the flagellated cells; thirdly, when the cells with vesicular nuclei cease to give rise to flagellated cells they begin to give rise to cell groups; and fourthly, though at first devoid of a flagellum, the individual elements of the cell groups develop both a collar and a flagellum, becoming the collar-cells of type C.

The relationship of types A, B, and C to one another seems to be a simple problem to solve, for the passage from A to B and from B to C is very gradual; but when type D is taken into consideration, the problem of the relationship of the larvæ to one another becomes much more difficult to solve. Type D cannot, apparently, be fitted anywhere into the above series of larvæ, either in an intermediate position or at the end, but can only have been produced directly from type A. The almost complete absence of cell groups in type D is conclusive against its origin from types B and C. On the other hand, it cannot possibly have given rise to type B, in which many of the cells with vesicular nuclei still retain the structural characters of blastomeres. It would be equally impossible to imagine type D giving rise to type C, for were the cells with vesicular nuclei to proceed to divide and to produce cell groups, the result would be a larva possessing quite different characters from those of type C. There would be a great number of cells with granular nuclei on the one hand, and of cell groups on the other, but hardly any cells with vesicular nuclei. It may there-

fore be fairly concluded that type D has been produced directly from type A.

From these considerations it seems that two divergent lines of development can be distinguished in the larva of Spongilla, of which the two culminating points are represented by types C and D, type B being an intermediate stage between types A and C. Type C is not necessarily older than type D, or vice versâ, but both of them have attained that stage at which they usually fix themselves. The differences exhibited by these larvæ are in no way more striking than those which will be found in the newly fixed stages, as the results of these variations. In fact, the structure of the larvæ seems to cast its shadow, as it were, over the whole period of metamorphosis, even up to the appearance of the young sponge.

B. The Fixation, Metamorphosis, and Further Development of the Larvæ.

General Remarks on the Fixation of the Larvæ.—There are two facts at least which tend to make the study of the larvæ of Spongilla during fixation and metamorphosis a laborious task, and to render difficult a correct interpretation of the phenomena observed. In the first place there must be taken into account the difference in the structure of the larvæ at the time of fixation, a difference which is the result of divergent variation culminating in the types C and D. In the second place there are found other differences, due to the fact that some of the larvæ fix themselves at an earlier stage than others; such differences are exemplified by the types B and C. In the former case we are confronted with a diversity of the most fundamental nature, one in which the numerical proportion of the several classes of cells which build up the inner mass vary, and which no amount of delay with regard to fixation can rectify. In the latter case the variation is not so far-reaching in its effects, and the fixation need be delayed but a very short time to obliterate them.

The types of larvæ which appear to be ripe for fixation are C and D, but type B may occasionally settle down and undergo

metamorphosis. Type A seems to be incapable of fixation, owing to the fact that the cells with granular nuclei are as yet few in number. The immediate result of the fixation of types B and C is absolutely different from that obtained when type D settles down to its sessile life. When a larva fixes after attaining the stage of development described above in type C, flagellated chambers which have acquired their definitive structure are present as well as the rudiments of the exhalant system. But when a larva possessing the structure described in type D becomes fixed, both the flagellated chambers and the rudiments of the exhalant system are almost completely absent.

The Actual Fixation of the Larva.—The larva fixes either by the anterior pole or by a point on the side not further back than the line of separation of the larval cavity from the solid posterior part of the inner mass. No larva was observed to fix by the posterior pole, or by a point near it. The fixation is brought about by the passing out of the cells with granular nuclei that lie beneath the flagellated cells. The larva just before it settles down turns about much in the same way as a spinning-top does when about to end its spin. The point at which the cells with granular nuclei make their way out corresponds to the peg of the spinning-top in the above comparison. That point in the flagellated layer seems to be, as it were, paralysed. At the time when the cells with granular nuclei have actually penetrated the flagellated layers, and are beginning to spread themselves along the surface of fixation, the motion of the larva as a whole inevitably ceases, though the flagella of the flagellated cells situated elsewhere may continue in motion for some length of time.

The Obliteration of the Larval Cavity after Fixation.—The persistence or obliteration of the larval cavity depends upon the structure of the larva. When type D fixes itself by the anterior end, the solid part of the inner mass, which lies at the posterior end, approaches the surface of fixation, and the larval cavity is thereby reduced to mere slits (fig. 15), and ultimately disappears completely (fig. 16), the fixed larva flattening out fairly symmetrically in all directions. But when

the same larva fixes by the side the solid posterior part of the larva topples over on to one side, and the larval cavity is obliterated by the coming together of the opposite sides. In this case the larva flattens out as before, but owing to the fact that the greater number of cells are situated on one side, it lacks the radial symmetry characteristic of the larva that fixes by the anterior pole, and presents a temporary variation of form, which, however, is soon lost. On the other hand, it is very doubtful if the larval cavity is ever completely obliterated at the fixation of type C, whether the larva settles down by the anterior pole or by the side. In type C either the chambers are already formed—their individual elements being adorned with collars and flagella—or they will be formed soon after fixation. In either case the young sponge can ill spare, so to speak, the time necessary for the destruction of the larval cavity, and the subsequent new formation of the inhalant and exhalant systems of canals. Besides, it has been shown above that in some larvæ the chambers, being fully formed, already open into small spaces and lacunar canals, which represent the beginnings of the exhalant system, and that these canals open in turn into the larval cavity, at least in some cases. Individuals possessing the above characters have been seen soon after fixation to possess so large a cavity, evidently derived from that larva, that it is impossible to believe that the larvæ in question would have lost it had they been allowed to develop to maturity. The fact that the flagellated chambers and the finer portions of the exhalant system have been developed already, and that nearly all the food material stored in the egg cell has been used up, render it absolutely necessary that the canals along which the current carrying food material for the young sponge is to pass should be in working order as soon as possible. Taking these facts into consideration, it may be fairly concluded that the larval cavity is not usually obliterated after the fixation of type C, but becomes a portion of the exhalant canal system and gastral cavity.

The Metamorphosis.—In describing the metamorphosis, which is a process involving the reversion of the layers that

takes place during and after fixation, it will be necessary to consider two extreme cases which will be the result of the settling down to a sessile life of types C and D.

As far as possible the following arrangement will be pursued. First, the features common to both types will be described; secondly, the special features of the two types will be considered—in the first place of type C, and in the second place of type D. The formation of the cavities and canal systems in general will be followed in the subsequent pages.

The larva described above as type B, so far as its changes need be considered, can be taken along with type C, for they both present in common the important feature of possessing cell groups,—either as small cells more or less incompletely divided, or quite independent of one another, but not provided with collars and flagella; or as flagellated chambers in which the cells are adorned with collars and flagella, and have attained their definitive arrangement. There is also another difference between types B and C when they are about to fix themselves, which will be of necessity the cause of a variation occurring in the structure of the young fixed stages. The difference in question is the presence of a great number of yolk bodies in the cells with vesicular nuclei in type B, while in type C they are always few, though invariably present. This is an important feature, the bearing of which is only rightly appreciated when it is recognised that there is a stage during the metamorphosis at which it is almost impossible to distinguish the nuclei of the flagellated cells from the yolk bodies.

The larva described above as type A need not be taken into consideration, as it probably never fixes itself at so early a stage in the development. Type D, on the other hand, presents extremely important features which must be specially described. It agrees with type C to the extent of possessing cells with vesicular nuclei which contain but few yolk granules. It must be admitted, however, that there is nothing impossible in a larva fixing itself which was in an earlier stage than type D, but situated on the line of development from types A to D, and

therefore comparable to type B, intermediate between types A and C. Such a larva when settled down would contain yolk bodies in the same way as type B does soon after fixation. Hence it is to be recognised that, whether we deal with larvæ developed along the line of histological differentiation passing from type A to type C, or from type A to type D, we may come across specimens which possess cells with vesicular nuclei which contain numerous yolk granules instead of a few, as in types C and D.

(*a*) The Features Common to Both Larvæ at the Time of Fixation and Metamorphosis—the Disappearance of the Flagellated Layer from the Surface, the Formation of the Flattened Epithelium and of the Marginal Membrane.

The flagellated cells may pass into the interior either individually or in groups of several cells. They generally tend to pass in groups from the lower surface, that is the surface of fixation, and individually from the upper surface. The usual result of this difference is, that all the flagellated cells which once occupied the lower surface are well within the body of the young individual, while those of the upper surface still form a more or less complete layer, for a time retaining their flagella, though the cells are by no means so closely packed as in the free-swimming larva (fig. 29). However, there is a considerable amount of variety in the mode of flattening out on the part of the different larvæ. Some appear to flatten so quickly that the best way to describe it is to say that the larva appears to tumble into pieces almost instantaneously. These differences are due, probably, to the number of points on the larva through which the cells with granular nuclei make their way out. If these cells break through the flagellated layer in several places at the same time, the result is a quick and rapid metamorphosis. If, on the other hand, they burst out merely at one point on the surface, that point will become the area of fixation, from which the

cells with granular nuclei will only spread gradually. The flagellated layer will in consequence remain for a long time on the upper surface, and immigrate from it slowly, the cells passing in one by one. This is the natural result of the breaking out of the cells with granular nuclei at one point rather than at many points. The internal pressure has thereby been decreased to such an extent that the resistance of the flagellated layer is enough to prevent the cells with granular nuclei spreading over the upper surface until the pupa,[1] by its expansion, has decreased the cohesion of the flagellated layer.

These two processes go on at the same time. While the flagellated cells are passing in, the cells with granular nuclei struggle to the exterior. The process is, apparently, a reciprocal one in which both classes of cells take an active part. The reversion of the layers does not take place so quickly on the upper as it does on the lower surface, and this is especially true of type C. There is also a slight difference between the cells with granular nuclei situated at the upper and lower surfaces respectively, the nuclei of those in the former position being smaller than those of the cells in the latter. The cells with granular nuclei, after passing through the layer of flagellated cells and arriving at the surface, become flattened out, their edges meeting one another. In this way a continuous layer of cells is formed on both upper and lower surfaces, derived from those cells which in the free-swimming larva are situated below the flagellated layer; that is to say, they formed originally a part of the "inner mass."

Owing to the large size of the nuclei of those cells which pass to the lower surface, a most satisfactory and conclusive proof is obtained that the flattened epithelium of the young sponge is formed from cells which once lay in the interior of the larva, and not from the flagellated cells at the surface. The nuclei of the latter are small, and whatever other change they may undergo at this stage, it is certain that

[1] This term is used by Mr. E. A. Minchin to indicate that stage in the development which occurs between actual fixation and the appearance of the dermal pores and osculum.

they do not increase in size, which they would have to do were they to become the nuclei of the surface layer of cells. For these reasons the flagellated cells cannot possibly give rise to the flattened epithelium of the lower surface, where the nuclei of the cells forming it are many times as large as those of the surface layer of the larva (fig. 16 *a*).

The cells with granular nuclei not only give rise to the epithelial layers of the upper and lower surfaces, but also produce the marginal membrane, which differs considerably in thickness and compactness according to the length of time which has elapsed since the fixation of the larva took place. At first only a few cells are seen to creep outside the limits of the body of the larva which is in the act of fixing itself, but the number of cells that wander out seems to increase with a wonderful rapidity. In a short time they present the appearance shown in fig. 23, in which the cells have not as yet arranged themselves so as to form a complete layer, for there are large spaces to be seen between them. In their outward course they seem to struggle on, passing over and across one another. The outer margin of the as yet incomplete membrane is quite irregular, and the cells which form it appear to be absolutely independent of their neighbours, the limit of each cell being well defined (figs. 23, 23 *a*).

The cytoplasmic mass of the cell body is exceptionally clear, and presents the appearance of an alveolar structure in which the meshes are slightly elongated in the direction of motion. Pseudopodia are not always produced by these cells as they move out; in many cases they present at least a complete and uninterrupted margin. Sometimes the cells, as they creep out, carry with them some of the flagellated cells, and in many cases they contain yolk bodies.

As the fixed individual becomes older the marginal membrane becomes thicker and more compact in structure. It now consists of two or three superposed layers of cells, save at the extreme margin, where it is only one cell thick. The outer margin is still quite irregular in places, owing to the continued outward movement of the cells which constitute it; but later on this

irregularity disappears (figs. 24 and 24 *a*). When the marginal membrane has become so thick as to consist of two or three layers of cells, there often appears a space between these layers, and into this space flagellated cells, accompanied either by cells with granular nuclei or by cells with vesicular nuclei, find their way. The nuclei of the lower layer of cells, constituting the marginal membrane, display the same difference of size from those of the upper layer as was described above as existing between those of the lower and upper surfaces in general. The clearly defined limit characteristic of its cells at an earlier stage is now no longer visible, and the extreme margin presents in all cases a regular and unbroken edge. The marginal membrane seems to be nothing more than a continuation of the dermal epithelium of the upper and lower surfaces, into the inner portion of which the internal substance of the sponge enters comparatively late, never passing into the outer margin, which later on is retracted.

The Changes through which the Flagellated Cells pass at Fixation.—The passage of the flagellated cells to the interior has been already described, but it must not be forgotten that there is considerable variation in the rate of change of position, which affects, in its turn, the time necessary for the complete enclosure of the cells in question in the interior of the pupa. It often happens that they have completely disappeared from the lower surface, while they still remain as a fairly complete layer on the upper.

The flagellated cells after passing to the interior undergo a most extraordinary series of changes, during which the nuclei seem to lose their internal structure so completely that at one stage they are almost indistinguishable, except by chemical reactions, from the yolk bodies. The changes in question have already commenced in the larvæ represented in figs. 15 *a* and 29 *b*, where a few cells are seen adherent to the surface of some of the cells of the inner mass, i. e. to cells with vesicular nuclei, and in some cases even to cells with granular nuclei as well. The first sign of the disappearance of the ordinary

structure of the nuclei of the flagellated cells is a certain amount of contraction, which in itself suffices to account to some extent for the density of structure found in them during these stages, though by no means sufficient to explain the whole of it. Another reason is probably to be found in the fact that when the flagellated cells pass to the interior they come within reach of a quantity of food material stored up in the cells with vesicular nuclei, and available for their use. When at the surface they are to all intents and purposes starved, the result being the arrest in the progress of their development that has already been mentioned when describing the larva. In consequence of this partial starvation, the flagellated cells, on passing to the interior, are attracted to the stores of food material which they find there. The nucleus being the main instrument, if not the only instrument of constructive metabolism, is proportionately affected and altered in structure; while the cell body itself, which was never very big and is not destined to grow to a very great extent, becomes plastered to the body of the cell in which the food material is stored up. Hence the changes which go on in the flagellated cells after emigration seem to result from absorption of food material and consequent increase of nucleoplasm, especially of the chromatic portion of the nucleus.

Simultaneously with the contraction of the nucleus described above the nuclear threads become thicker, and the chromatin rearranges itself. Instead of being scattered about in small granules, it appears, as a rule, as irregular patches lying against the nuclear membrane, though at this stage it does not invariably conform to a definite type of arrangement. At the commencement of the above process of adhesion the outlines of the flagellated cells are visible (fig. 29 *b*); but later on they become so closely adherent that they are indistinguishable as separate units in the morphological sense. As a rule they fix themselves to the cells with vesicular nuclei, but it often happens that a number of them become attached to cells with granular nuclei, which in some cases contain several yolk bodies, and which therefore act as

centres of attraction in possessing stores of food material. At this stage it is almost impossible to distinguish the nuclei of the flagellated cells from the yolk bodies always present in greater or less number.

The cell aggregations compounded each of numerous flagellated cells, and one cell with a vesicular nucleus or a granular nucleus, as the case may be, must be carefully distinguished once and for all from the "cell groups" of type B, in which the cells were incompletely divided from one another, and never had a separate existence. The groups here described will be referred to as "plasmodial aggregations," for they follow in their formation the principle involved in the building up of a plasmodium rather than that which has to be considered as connected with phagocytic action. In the former case no cell is subordinated to the other in any way, but in the latter one cell takes in the other, and seeks to destroy it. From the bionomic point of view the action of the flagellated cells in this case is comparable rather with that of the commensal, which feeds, as it were, at the table of another, but does not directly harm the host. Similarly the flagellated cells feed on the food material which has been stored up in the cells with vesicular nuclei, and which they inherited from the egg cell in the course of their development. If the formation of these groups were a case of phagocytic action the large central cell would have to be considered as the phagocyte, taking in all the flagellated cells it could lay hold of, and endeavouring to absorb and destroy them. This view has been put forth, but, as will be seen, it obtains no support from the subsequent development of the plasmodial aggregations.

The outline of the plasmodial aggregations is at a certain stage as well defined as that of the cells with vesicular nuclei in the youngest larva, that is in type A. During the pupal life there is a stage, however transitory, during which the young individual consists of only cells with granular nuclei at the surfaces and plasmodial aggregations inside, provided always that such an individual has been produced from the larva described above as type D (figs. 16, 16 *a*, 26, and 27).

In an individual produced by the metamorphosis of type C, the plasmodial aggregations will of necessity be mixed up with the flagellated chambers derived from the cell groups.

Since individuals occur with neither chambers nor free cells capable of forming chambers, but with the interior full of plasmodial aggregations surrounded by cells with granular nuclei at the surfaces, the important question of the origin of the cells which later on become the collar-cells of the flagellated chambers forces itself upon us. Are they developed anew from the cells with vesicular nuclei, or do the flagellated cells—no longer flagellated, it is true—separate themselves from the plasmodial aggregations into the composition of which they have entered, in order to develop into collar-cells? If the latter be the actual course of the development, it would furnish a further proof of the homology of the flagellated cells with the constituent cells of the "cell groups," which are characteristic of types B and C, and which in the latter have developed into flagellated chambers. To answer this question it is necessary to trace further the morphological changes undergone by that constituent of the plasmodial aggregations which owes its origin to the immigration of the surface layer of cells in the larva.

The small nuclei of the flagellated cells which have already been traced through a series of changes, leading them to acquire a structure almost indistinguishable from that of the yolk bodies, now embark upon a similar series of transformations, but in the reverse order, as the result of which they revert to a condition slightly different from that which they presented as the flagellated locomotor layer at the surface of the larva.

The small nuclei—that is the nuclei of the cells which were once flagellated—in the plasmodial aggregations commence this series of changes by increasing slightly in size, simultaneously with some internal changes in disposition of the chromatin and nuclear reticulum. At one stage they appear as oval masses, uniformly coloured in stained sections, but now the chromatin becomes looser and aggregated into small

granules, and the threads of the nuclear reticulum become visible. The chromatin granules are smaller and stain faintly. They are more numerous than they were in the primitive condition of the cell, and the nuclear reticulum is not so coarse (figs. 17—20).

In the description of the larva given above reference has been made to a slight change in the character of the nuclei of the individual elements of the cell groups, taking place as they pass from the condition of structure found in type B to that which exists in type C. It was pointed out that the nuclei of the cell groups in type B are indistinguishable from those of the flagellated cells at the surface. As a matter of fact, the nuclei of the collar-cells in type C are equally indistinguishable from those of the flagellated cells at the period when the latter—by their own energy, apparently—emancipate themselves from the plasmodial aggregations into which they entered, and again assume their individual form. Here, therefore, is another argument, in addition to those already brought forward, in favour of the view that the flagellated cells at the surface and the cell groups in the interior are homologous, and really belong to the same class of cells.

As it is impossible to observe the above changes in the living pupa, we are forced to the method of studying them in sections, and so drawing our conclusions. In our examination of sections we may follow one of two courses. It happens sometimes that the changes in the structure of the nuclei of the flagellated cells, as they pass from the condition in which they are difficult to distinguish from the yolk bodies to that found in fully developed collar-cells, can be traced while they are still inside the plasmodial aggregations,—in other words, the changes can be followed in one individual, or even in one section of such an individual.

Fig. 17 illustrates the changes which go on in these nuclei as they pass from one condition to the other, and shows a number of transitions in one individual. The nucleus marked *a* has become enlarged, and the chromatin is scattered almost uniformly in it; while in the nuclei marked *b* the nuclear

reticulum begins to appear, and the general structure is looser than in the nucleus *a*. The nuclei labelled *d* show a further change, and, though they are still enclosed in a plasmodial aggregation, they are so like the nuclei marked *e* as to be indistinguishable from them, though the latter are undoubtedly the nuclei of free cells which will later on develop into collar-cells. An unprejudiced examination of the above figure can hardly fail to satisfy the most sceptical person that the plasmodial aggregations contain the nuclei of the flagellated cells in a state which is only an intermediate condition between that which they possessed when the cells were free at the surface, and that which later on they assume as collar-cells in the interior.

While the above proof seems conclusive, it is perhaps advisable, though it may appear superfluous, to confirm our results by the comparison of several individuals preserved at different stages in their development, in order to trace in them the series of changes which have been described above as taking place in the same pupa. In the pupa represented in figs. 16, 16 *a*, and 26 there are scarcely any nuclei or cells save those enclosed in the plasmodial aggregations and those of which the flattened epithelium consists; while in that represented in figs. 18, 18 *a*, and 18 *b*, which is an older pupa, the nuclei of the flagellated cells are emancipating themselves at all points from the plasmodial aggregations, which are losing their sharp and well-defined outline. At this stage and slightly later such a thing as a cell outline or limit can hardly be discerned. The nuclei of the flagellated cells can be seen clearly becoming looser in structure; the linin threads are well developed; and the chromatin in general is rearranging itself preparatory to the subsequent stage which is represented in fig. 19, and especially in fig. 20, in which the nuclei have attained their definitive characters.

It would not be out of place at this juncture to lay special emphasis on a fact which has been already mentioned, namely, that the plasmodial aggregations always contain yolk bodies, besides several nuclei. The former may vary considerably in

number, and are indistinguishable from the latter at that stage at which the nuclei of the flagellated cells have reached the extreme limit of the modifications which they undergo during metamorphosis. When, however, this limit has been passed, and the nuclei in question are gradually assuming the ordinary structure of those of the collar-cells, the difference between them and the yolk bodies becomes more marked stage by stage. Fig. 16 *a* illustrates a condition in which it is impossible to distinguish between "yolk bodies" and nuclei; in fig. 18 this is less difficult, while in fig. 19 and still more so in fig. 20 the difference, as a rule, is well marked.

The similarity at a certain stage between the nuclei and the yolk bodies makes it difficult to say whether all the flagellated cells are set free from the plasmodial aggregations or not. Judging, however, from certain pupæ, such as that represented in figs. 18, 18 *a*, and 18 *b*, there appears to be no reason whatever for the supposition that they are in any way absorbed by the central cell of the plasmodial aggregation. Those bodies which appear to be reduced in size, and not to be gradually acquiring the ordinary nuclear characters, can be more than accounted for from the number of yolk bodies occurring in the larva at the time of fixation. There is no reason, however, why a flagellated cell should not be completely absorbed if by any mishap it was injured during the interchange of position taking place at the time of fixation and subsequent metamorphosis. Any such cell would probably fall an easy prey to the cells with vesicular nuclei, which in the young sponge are amœboid and nutritive in character. Nevertheless it appears almost certain that the vast majority, if not all the flagellated cells emancipate themselves from the plasmodial aggregations.

The results obtained by a comparison, on the one hand, of nuclei in a single individual, and on the other hand, by following the different stages in several pupæ, may, therefore, be summarised as follows. The plasmodial aggregations contain both yolk bodies and a number of nuclei. The former appear to decrease in size as development goes on, and have therefore supplied an argument in favour of the view—evidently incor-

rect—that the nuclei of the flagellated cells became completely absorbed. The latter, on the other hand, become nuclei of the young sponge, and this is true equally of the central vesicular nucleus and of the numerous smaller nuclei belonging to the flagellated cells.

Simultaneously, as a rule, with the above changes in the characters of the yolk bodies, and the small nuclei contained in the plasmodial aggregations, the cytoplasmic bodies of the groups in question lose their sharp outline. They spread out and become irregular in shape and almost indistinguishable from one another. By the time the nuclei have attained the definitive structure of the nuclei of collar-cells, the internal arrangement of the cytoplasm belonging to the various groups of plasmodial aggregations may be described as being syncytial. Spaces begin to make their appearance in the undifferentiated cytoplasm (fig. 18), which soon develop into large cavities (fig. 19), lined by cells possessing granular nuclei. Meanwhile the nuclei of the flagellated cells arrange themselves in the rings of the cytoplasm, which are at first quite irregular and ill-defined (fig. 19 *a*), and do not appear to consist of individual cells; but this syncytial condition soon passes away, and the separate individual collar-cells make their appearance. They develop collars and flagella at the time of separation as free cells, and in this way the plasmodial aggregations give rise to the flagellated chambers. The cell with vesicular or granular nucleus which occupied the central position retires outside the chamber, and takes no part in its formation.

(*b*) Special Features of the Metamorphosis of the Different Types of Larvæ.

The phenomena of the development common to all the types having been described in the previous section, in the following pages only those features which characterise the metamorphosis of the fully developed types, namely, C and D, will be specially considered. Reference must be made also to

type B in cases in which the appearances figured are such as would result from the fixation of individuals of that type. But as type B is only a younger stage of type C, and not a special variation of the fundamental type of larva, so to speak, no special description of the changes taking place in it appear necessary.

(1) Special Features of Type C during Metamorphosis.—It is characteristic of the metamorphosis of all the types that the flagellated cells disappear more quickly from the lower surface of the pupa than from the upper. However, this feature is strongly emphasised in the pupa formed from type C, as compared with that formed from type D. But it is highly probable that this difference is not so great in all the pupæ derived from larvæ of type C as it is in the one actually represented in section in fig. 29. The structure of the pupa in question is such that it is even possible that it could thrive if the flagellated layer at the surface were thrown off altogether, as described by Götte. I have seen, however, no evidence of such a procedure on the part of any fixed larvæ. In the case of the pupa figured, the flagellated layer at the surface is as complete as in a free-swimming larva. Both the nuclei and the flagella present the same appearance as in the larva, and are equally well defined. On the other hand, the flagellated cells which were at one time situated at the lower surface have disappeared completely from that position, and have migrated into the interior. They have become plastered to the surfaces either of the cells with vesicular nuclei, or of those with granular nuclei, as the case may be, to form the plasmodial aggregations, which have already been described in the previous section of this paper. The contrast between the appearance presented by the flagellated cells at the upper surface, and those which have at this stage travelled to the interior from the lower surface, may be seen on comparing figs. 29 *a* and 29 *b*, two figures drawn from the upper and lower surfaces respectively of the same larva as fig. 29.

Another feature characteristic of the pupa which results from the fixation of type C, is the presence of fully developed

flagellated chambers (figs. 29 and 29 *a*), in which the individual cells are provided with a collar and a flagellum (fig. 29 *c*), resembling in every respect those of the collar-cells of the adult sponge. There can be no reasonable doubt, therefore, as to the origin of the chambers in question from the cell groups of types B and C. Inasmuch as the cell groups found in type B are developed from the cells of the inner mass, that is, from the cells with vesicular nuclei; and since, further, these chambers are produced from the cell groups; it follows that the flagellated chambers found in the young pupa derived from type C, and represented in figs. 29 and 29 *a*, are produced by the multiplication of the cells of the inner mass, and not from the flagellated cells which migrated into the interior, and which have as yet, even at the lower surface, only gone so far as to form plasmodial aggregations (fig. 29 *b*). These facts furnish a clear proof that cells of the inner mass are capable of giving rise to collar-cells, and that the flagellated chambers do actually so arise in the development. The cells arranged in groups surrounding spherical cavities in the larva do not flatten out to form the canals of the sponge. On the other hand, it has been shown that the cells which in the larva become flattened to form the lining of the lacunar spaces, belong to an entirely different class of cells, i.e. the class which has been described as consisting of cells with granular nuclei, from which arise the epithelial membranes of the sponge in general.

Having traced the origin of the flagellated chambers from cells of the inner mass, it remains to inquire what happens to the flagellated cells after they have entered the plasmodial aggregations in those pupæ in which the cells of the inner mass do indubitably take part in the formation of the flagellated chambers.

It has already been pointed out that the flagellated cells migrate into the interior and form plasmodial aggregations (figs. 29, 29 *b*, 24 *b*, and 25), though they migrate far more slowly from the upper than from the lower surface. In figs. 30 and 30 *a* the flagellated layer, which has not completely disappeared from the surface even at this stage,

presents an extraordinary change in the arrangement of the cells. Its constituent cells are migrating into the interior individually, and consequently the layer itself becomes less dense, the cells shorten, and at the same time become broader, owing to the superficial expansion of the sponge as a whole. The above changes in the characters of the flagellated cells might seem at first sight to support the view, held by many authors, that the flagellated cells become the flattened epithelium. But a more careful study of the figures is sufficient to refute any such idea, and to force us to the conclusion that whatever happens to the flagellated cells, they do not flatten out to become the constituent cells of the dermal epithelium. In the first place, the nuclei of the flagellated cells are seen to be passing in, though it is almost impossible to make out the cell body, and the portion nearest the margin of the pupa is full of them (figs. 30 and 30 *a*). In the second place, the cells with granular nuclei have come to the surface already in the portion nearest the margin (fig. 30), while nearest the centre they are seen engaged in the struggle, so to speak, of passing to the exterior between the few remaining flagellated cells (fig. 30 *a*). And in the third place, the nuclei of the cells, which before metamorphosis were situated in the interior, retain their large size and granular character, while those of the flagellated cells, which were once at the surface, are undergoing the usual changes through which they pass when they are about to enter into the composition of the plasmodial aggregations.

There is, therefore, no reason whatever to suppose that the flagellated cells flatten out and become indistinguishable from the cells with granular nuclei, which they would have to do were they the cells from which the dermal epithelium is developed.

The flagellated cells have been traced to the interior already, and their nuclei have been shown to be undergoing the changes usual in the formation of plasmodial aggregations. It remains to show what further changes they will pass through. In the pupa which is represented in fig. 31 the flagellated cells are

no longer independent. Fully formed plasmodial aggregations are found to exist, in addition to chamber-like rings of cells derived from the "cell groups" of the larva. The nuclei of the plasmodial aggregations, however, in a short time emancipate themselves, and the cells consequently, becoming free and sharply individualised, give rise to the collar-cells of a new series of flagellated chambers. The collar-cells of the sponge are therefore developed, on the one hand, from the cells of the inner mass, and, on the other hand, from the flagellated cells.

In fig. 28 two flagellated chambers are represented; in one of them the cells are perfectly separate and independent, with the nuclei all alike, and possessing the same structure as the nuclei both of the collar-cells—i. e. chamber cells, for they have not as yet developed collars, represented in figs. 24 *b*, 31, and 31 *a*—and of the cells which have already developed collars and flagella as seen in fig. 29 *a*. In the other chamber in the same figure the cells are not so distinct, and the nuclei are much smaller and more irregular in size. In fact, both the arrangement of the cells and the size and structure of the nuclei resemble those of the flagellated chambers represented in figs. 19, 19 *a*, 20, and 21, which are drawn from specimens developed from type D, in which, however, flagellated chambers derived from "cell groups" are practically absent. It is evident that the cells of the lower chamber in fig. 28 have emancipated themselves from one or more plasmodial aggregations, which they formed in combination with the large cells with vesicular nuclei, situated close to the chamber in question. With regard to these cells with vesicular nuclei, it may be pointed out that those in the immediate neighbourhood contain very few yolk bodies, which at this stage are of necessity much reduced in size, while those further off contain more yolk bodies. This fact points to the view that the yolk bodies in these cells have been almost completely used up by the flagellated cells which grouped themselves round the cells with vesicular nuclei to form the plasmodial aggregation. As has been stated above, the nuclei of the flagellated cells in the plasmodial stage can be distin-

guished from the yolk bodies only by their affinity for a differentiating stain.[1] The nuclei then pass during this stage through a series of changes in rapid succession, and are therefore not constant in structure. The yolk bodies also change from stage to stage, owing to their being used up in the formation of living protoplasm. These changes, both in the nuclei and in the yolk bodies, render interpretation of the phenomena observed a most difficult task. It is evident, however, from the characters of the chambers represented in fig. 28, that the flagellated cells give rise to a series of chambers in the pupa derived from a larva of type C, in the same way as they do in the one of type D. The former statement is far more difficult to prove than the latter, owing to the complication brought about by the presence of a number of chambers derived from the cells of the inner mass in type C, and consequently in the pupa derived from that type of larva. But the presence of yolk bodies in the pupa on the one hand, and of flagellated cells on the other, suffice to explain the conflicting statements which have been made by various observers, and to reconcile them in the following manner. Some of the bodies contained in the plasmodial aggregation, namely, the yolk granules, become used up and disintegrate, whilst others of a different nature emancipate themselves and give rise to the nuclei of the collar-cells.

The question of the formation of the flattened epithelium from the cells with granular nuclei has been incidentally mentioned, but it must be described here at a greater length from the point of view of the structure of the pupa derived from the larva described as type C. The flattened epithelium of the lower surface forms much more quickly than that of the upper surface, in correspondence with the different rate at which the flagellated layer disappears from these surfaces. The difference in question is brought out very sharply in fig. 29, while in

[1] With carmine and bleu de Lyon the nuclei are stained red, while the yolk bodies are stained blue. This reaction is difficult to bring about owing to the thickness of the nuclear membrane, which stains blue in the same way as the yolk bodies.

fig. 30, which represents an older stage, the difference is not so marked, as the cells on the upper aspect have in some cases made their way to the surface, and in fig. 31 the epithelial membrane of the upper surface is as complete as that of the lower.

In the interior of the pupa the cells with granular nuclei continue to line the persistent larval cavity, which in some larvæ may be of great size (figs. 29 and 30). The cells with granular nuclei, situated in the solid part of the inner mass, flatten out to form the lining of the exhalant canal system. The canals, which are short at this stage when they exist, may be seen to communicate on the one hand with the larval cavity, and on the other with the cavity of the flagellated chambers. These connections explain why some observers have made the mistake of describing the flagellated chambers as developed from the cells of the inner mass at the blind ends of outgrowths from the flattened layer of cells lining the larval cavity, i. e. from the so-called "entoderm."

The special features of the pupa derived from the larva described above as type C may be summed up as follows:

1. The flagellated cells pass in at different rates from the lower and upper surfaces, and consequently the flattened epithelium forms much more slowly on the latter than on the former. The difference in question is much more marked in this pupa than in the one derived from the larva described as type D.

2. Flagellated chambers are always present, appearing in section as rings. In many cases the individual cells are provided with a collar and a flagellum even at the time of fixation.

3. The flagellated cells, after passing into the interior, take part in the formation of plasmodial aggregations, from which they later emancipate themselves and give rise to flagellated chambers, the cells of which for a time can be distinguished from the cells of the chambers derived from the "inner mass" by their nuclear and other characters (figs. 28, 31).

4. The canal systems appear as early as the time of fixation,

The larval cavity is not obliterated, but takes part in the formation of the exhalant system and gastral cavity.

(2) Special Features of Type D during Metamorphosis.—There remain but a few statements to make with regard to the metamorphosis of the type of larva now under consideration, owing to the completeness of the description given above of the features common to the metamorphosis of all the larvæ.

The interchange of position between the flagellated cells on the one hand, and the cells with granular nuclei on the other, takes place much in the same way as in the larva described as type C. There are two features, however, which are perhaps worthy of further notice: first, the flagellated cells migrate almost at the same rate from the upper and lower surfaces; and secondly, they tend to form fan-like groups at the lower surface.

In fig. 15 only a few plasmodial aggregations have been formed, but notwithstanding this fact the flagellated cells have disappeared from both surfaces, and the flattened epithelium is complete almost everywhere. The arrangement of the cells is, therefore, quite different from that represented in fig. 29, which has been drawn from a pupa derived from the larva described as type C. In figs. 15 *a* and *b* the second point, namely, the formation of fan-like groups, is illustrated. The groups in question may in some cases consist of so many cells as to make it possible that an actual invagination has taken place, though for other reasons this is not probable (fig. 15 *b*).

The individual cells of the groups in question appear as if they had been drawn inwards by some internal force, the body of the cell being in consequence greatly elongated and attenuated. The dark streak which was described above as being situated externally to the nucleus of the flagellated cells in the free-swimming larva is seen clearly in many of the cells contained in the groups, though there is no sign whatever of the portion of the flagellum that lay outside the cell. The nuclei of the flagellated cells do not appear to change in character so long as the cells remain in these

groups, but immediately after breaking away from them, and coming into contact with the cells with vesicular nuclei situated in the interior, they undergo the structural changes which have been already described.

The peculiar arrangement of the flagellated cells in the groups under consideration appears to be due to the pressure exerted upon them by the cells with granular nuclei, which are struggling towards the exterior at all points on the surface of the individual. The two processes, namely, the passing in of the flagellated cells and the passing out of the cells with granular nuclei, go on at the same time, both classes of cells taking an active part, apparently, in effecting the interchange of position.

The next feature to be considered is one of the greatest importance, and consists in the existence of a stage in which there are no free cells derived either from cell groups or from the flagellated layer of the larva. The pupa at this stage consists of plasmodial aggregations inside and flattened epithelium on the outside (figs. 16, 16 *a*, and 26). There are no signs whatever of the larval cavity. The formation of the plasmodial aggregations has been so fully dealt with already, that it is unnecessary to say anything further with regard to them. However, it must be pointed out that the nuclei of the flagellated cells sometimes—though this is not the usual rule—undergo the changes which have been described while still remaining in close association with the central cell which possesses a vesicular nucleus. The nuclei marked *n. fl. c.* at the left-hand corner of fig. 20 illustrate this point. Further, the nuclei labelled *a*, *b*, and *d* in fig. 17 exemplify the successive stages in the series of changes through which they pass. The nucleus marked *a* in fig. 17 has already increased in size, and contains the chromatin in an evenly distributed condition; while in those labelled *b*, linin threads of considerable thickness, covered with chromatin, are appearing. The nuclei marked *d* present the ultimate structure of those of the collar-cells, though they are still situated inside a plasmodial aggregation. In the face

of such evidence it seems impossible to doubt that a great number of the bodies contained in the plasmodial aggregations give rise to the nuclei of the collar-cells.

Fig. 17 illustrates the commencement of the breaking up of the plasmodial aggregations, and the change which takes place in the characters of the nuclei of the flagellated cells. Both of these changes are seen to have advanced still further in figs. 18, 18 *a*, and 18 *b*, in which, however, there is no sign as yet of a flagellated chamber. In figs. 19 and 19 *a*, which still lack flagellated chambers, the plasmodial aggregations have disappeared as completely as if they had melted away. A stage is therefore produced in which the cells present a syncytial arrangement. In fig. 20 a stage is illustrated in which the nuclei of the flagellated cells are becoming arranged in rings round cavities which in some cases are more or less circular in form, but the general arrangement is still syncytial. Fig. 21 represents a further change. The cytoplasm, surrounding the nuclei arranged in rings in the stage represented in fig. 20, becomes divided into masses corresponding to the nuclei, and the individual collar-cells provided with a collar and flagellum make their appearance.

The changing nuclei described above must not be confused with the yolk bodies which exist inside the cells with vesicular nuclei, and which become smaller stage by stage, while the nuclei of the flagellated cells, on the contrary, become larger. The number of yolk bodies found in the cells with vesicular nuclei varies considerably, according as the larva fixed at an early stage of development or a late one. In fig. 26 the yolk bodies are coloured blue, while the small nuclei are red. In figs. 18, 19, 20, and 21 the distinction between yolk bodies and small nuclei is such that it is impossible to mistake the one for the other. The important point for our purpose, however, is that the nuclei of the flagellated cells and the yolk bodies exist side by side in the plasmodial aggregations, the former giving rise to the nuclei of the collar-cells, while the latter are used up during the development as food material.

To sum up, the special features of the pupa derived from the

larva described in the early part of this paper as type D are briefly as follows:

(1) The flagellated cells pass in at a rate which is but slightly different for the upper and lower surfaces.

(2) There is a stage, however brief, during which the pupa consists only of plasmodial aggregations inside and flattened epithelium outside. During this stage there are no signs either of the larval cavity or of incipient canals. The former has been obliterated, while the latter have not as yet made their appearance.

(3) The flagellated chambers are derived for the most part, if not entirely, from the flagellated cells of the larva.

(4) The canal system only appears after the plasmodial aggregations break up and form the flagellated chambers.

(*c*) Further Remarks on the Formation of the General Canal System.

In specimens derived from the larvæ of type D all the canals and spaces must be formed anew, for the larval cavity is entirely obliterated, and there are no lacunar spaces or canals such as were found in the pupæ derived from type C. The canals appear as spaces in the interior soon after the flagellated cells have begun to emancipate themselves from the plasmodial aggregations, and have passed into the syncytial condition described above. It is needless to say that the canals at their first appearance do not possess a proper lining in the form of flattened independent cells. Later on, however, they become lined with cells possessing granular nuclei, which are derived from two sources. In the region of the subdermal cavity they are derived to a great extent from the cells with granular nuclei which had been already produced at the time of metamorphosis. In the interior, on the other hand, they are produced as in the larva by the gradual conversion of the cells with vesicular nuclei, as can be seen from the presence of small central corpuscles in many of these cells.

Owing to the continued formation of the cells with granular nuclei from those with vesicular nuclei, many of the former

are found in the spaces between the chambers where the latter are always situated. In these positions, especially in the neighbourhood of the developing skeletal fibres, they form a sort of connective tissue, and the spongin occurring in the sponge seems to owe its origin to these cells. Wherever a canal is formed, it is, of course, lined by these cells. The cells with vesicular nuclei also frequently take up a position adjacent to the canals. Whenever they do so the character of their nuclei is doomed to change just as the nuclei of the cells which give rise to the megascleres become modified.

It makes no difference, so far as the various classes of cells are concerned, whether the gastral cavity is a new formation, as in the young sponge developed from a larva of type D, or is derived mainly from the larval cavity, as was shown to be the case in the development of the larva of type C. In each case the cavity is lined by the same class of cells, that is, by those with granular nuclei. This fact does away with any difficulty that might arise from the histological point of view.

The cells with vesicular nuclei, after the flagellated cells have separated themselves from them and have become arranged in chambers, remain in the neighbourhood of these structures and constitute the amœboid elements of the young sponge. They often adhere to the surface of the chambers, being in many cases situated at a point not far from the apopyle as well as elsewhere, and to a certain extent spread over them. It is quite possible that some of them in this position become perforated to form the openings of the inhalant canals into the flagellated chambers. This point is further explained in the descriptions of figs. 32, 33, and 34, in which inhalant pores are figured. It is difficult to prove that this pore is intra-cellular because of the great superficial expansion of the cells in the neighbourhood of the pore, and the distance which, as a consequence, intervenes between the nucleus of the cell and the pore. If these pores are really intra-cellular, then the cells in question would correspond to the porocytes which have been described by Mr. Minchin in the Ascons. The only explanation I can give of the appear-

ances represented in fig. 33 is that the pore is intra-cellular passing through the large cell, the vesicular nucleus of which is drawn in fig. 33 *d*. If some of these cells do become porocytes we would expect the nucleus to become granular. In fig. 24 *b*, which represents a surface view drawn at different levels, there are two extremely large nuclei situated deep in the tissues of the individual among the small cells which are evidently becoming grouped to form chambers. The cells to which these two nuclei belong seem to lie more or less loosely on the cells of the chamber, and it is quite possible that they are cells similar to that containing the large vesicular nucleus drawn at the top of fig. 33 *d*, in which the nuclei have lost their vesicular character. The porocytes in the Ascons are described by Mr. Minchin (11) as large cells, situated near the surface when the sponge is fully expanded, which in the contracted condition of the sponge push their way inwards between the collar-cells, and ultimately come to lie inside the gastral layer as a granular axis to the Ascon tube. The granules, according to Topsent (14), consist of reserve food material. If so, the cells probably collect it when the sponge is expanded, and distribute it during the contracted condition to the other cells. The passing in of the porocytes to the interior would seem, therefore, to have a physiological meaning. In Ascons, contraction or expansion may happen at any time in the history of the sponge. Let us now turn to the pupa of Spongilla. Here the large cells with vesicular nuclei, and containing food material in the larva at the time of fixation, would correspond to the porocytes of the Ascons in the expanded condition of the sponge. During metamorphosis, however, they become surrounded by the flagellated cells, that is by cells which are potentially collar-cells—a condition of things found in the Ascons during the contracted stage, with the difference, in the two cases, that in Ascons the gastral layer is continuous, while in Spongilla it is broken up into groups of cells. Hence during the contracted stage in the Ascons we find a continuous axis surrounded by an uninterrupted gastral layer; but in Spongilla, during the stage with plasmodial aggregations, we find the

individual cells with vesicular nuclei surrounded by groups of flagellated cells. When the plasmodial aggregations break up, the cells with vesicular nuclei pass out of the group of cells and plaster themselves to the outer surface of the flagellated chambers subsequently formed, in the same way as the pore cells of the Ascons pass out of the gastral cavity to the surface when the sponge expands. From the physiological point of view the correspondence between these two classes of cells seems pretty complete. Of course it is quite needless to say that the cells with vesicular nuclei do not all become porocytes, for many of them retain their amœboid character as well as their blastomeric properties, and serve later on for the building up of the gemmule. This discussion regarding the physiological correspondence of these cells strengthens the argument in favour of their morphological homology; in other words, in favour of the view that some of the cells with vesicular nuclei become porocytes in Spongilla.

The Subdermal Cavities.—These spaces are situated below the dermal membrane, and communicate on the one hand with the exterior by way of the inhalant ostia, and on the other with the flagellated chambers by way of the inhalant canals and prosopyles. The cavities in question have a superficial position, and they and their ostia are arranged on the slanting sides of the young sponge, while the osculum takes up a more or less central position. Deeper down, and nearer the centre, the exhalant canal system and gastral cavity are found.

The spaces which have been figured in such profusion in the region between the marginal membrane and the body of the young sponge are by no means all of them true ostia opening into the subdermal cavities. Many of them are merely the nutritive vacuoles found in the cells which have wandered into the space between the two layers of the marginal membrane. The vacuoles in question in many cases present the exact appearance of an intra-cellular opening, the nuclei of the cells lying close to them, but on focussing the microscope more carefully the cells of the flattened epithelium can be seen overlying them. However, this does not do away with the presence of

true ostia, which are small openings measuring about 15 μ across, and which appear to be intercellular, and not intracellular. They occur all over the surface, even close to the osculum (fig. 8).

The osculum is much larger than an ostium, and is situated near the centre of the young sponge. At first it appears as a funnel-shaped opening, the rim of which is on a level with the general surface. Its sloping sides are perforated by one or more smaller openings which communicate with the exhalant system beneath. The thickened rim which surrounds the osculum soon grows up, and forms a tube carrying the opening at its extreme end.

Appendix A.

On the Nutritive Vacuoles and Yolk Bodies.

The enclosures found chiefly in the cells with vesicular nuclei are of two kinds, and must be described separately. The less numerous and larger enclosures may be termed the "nutritive vacuoles," while the smaller and more numerous will be given the name "yolk bodies."

To have described them fully at the same time as the cells which contain them would have burdened with too much detail the description of the more important elements of the larva, and also would have caused a certain amount of the technique to be mixed up with the description. For these reasons I thought it advisable to describe these enclosures, as well as certain other processes which go on during the development, in a number of appendices.

(1) The "Nutritive Vacuoles."—These structures are almost invariably restricted—in the free-swimming larva at least—to the cells with vesicular nuclei, which in the earlier stages of the development may contain as many as three or four of them, though one is the rule. Owing to the large size of these cells it often happens that the nucleus and the vacuole must be looked for in different sections. These structures have not been described by previous authors, save as an "occasional

vacuole," but they appear to be present in all cells with vesicular nuclei, and occasionally in the cells with granular nuclei, and even in the collar-cells in the pupa and young sponge. Their occurrence in the last two kinds of cells shows them to have been developed recently from the cells with vesicular nuclei.

The vacuoles here described behave very differently under the action of various preserving reagents. When the specimen is preserved with absolute alcohol, or a mixture of corrosive sublimate and glacial acetic, or Perenyi's fluid, these enclosures appear as clear vacuoles. When Flemming's fluid is used for a short time some of them are clear while others are black, but when it is used for a long time they are all as black as ink, as they are, also, when the specimens are preserved in Hermann's fluid. Whether as clear vacuoles or as black enclosures they are always circular in section, and equal or even exceed the vesicular nucleus in size (figs. 7, 11, and 11 *a*).

When a section of a specimen preserved in Hermann's fluid is subjected to the bleaching action of chlorine, these bodies appear as clear spaces, just as in sections from specimens preserved in either absolute alcohol, or corrosive sublimate and glacial acetic, or Perenyi's fluid. This proves conclusively that the clear vacuoles observed in specimens preserved in these reagents correspond to the black enclosures found in material preserved in Hermann's fluid. By stopping the bleaching action short of its completion the black enclosures can be seen in different stages of decoloration (fig. 2).

In sections stained according to Heidenhain's iron hæmatoxylin method they become pale, though with other stains they remain black. In sections of larvæ preserved in absolute alcohol, or in bleached sections, the vacuoles were never stained, though a kind of a membrane which surrounded the vacuole always stained with safranin, when followed by gentian violet and iodine, or even with gentian violet alone, as well as with hæmatoxylin followed by fuchsin S (fig. 2). Whatever substance these vacuoles contained in the living condition, it may be concluded that it has been dissolved out of the preserved specimens; and further, the blackening under the action of

Hermann's or Flemming's fluid tends to prove that it was of a fatty nature.

(2) The Yolk Bodies.—These bodies are far more numerous than the nutritive vacuoles, but the same remarks apply to them as to the vacuoles in regard to their occurrence in different classes of cells. They are chiefly found in the cells with vesicular nuclei, which, as a rule, contain only one nutritive vacuole, while they contain several yolk bodies, and in many cases they may be described as being full of them. Their average size in the youngest larvæ is about $2\frac{1}{2}\ \mu$ across, and their shape is either spherical, oval, or plano-convex (figs. 1—4). As the larva becomes older, and cell-differentiation progresses, they become smaller in size, and some of them seem to be used up completely, though this is not true of all of them. No larva, even of types C and D, was observed to be absolutely devoid of yolk bodies; on the other hand, they are often very numerous in the fixed stages (figs. 25 and 26), a fact which points to the early fixation and metamorphosis of such individuals.

They almost invariably stain like the nucleus, but when sections are successfully stained with carmine, and subsequently with bleu de Lyon, the nuclei take the former, whilst the yolk bodies take the latter (figs. 1, 3—6 *a*, 25, and 26). The outer layer stains more deeply than the central part, which, as a rule, is devoid of any stain, the colourless space being almost circular in shape, and slightly eccentric in position. It happens sometimes that this space, instead of being clear, is stained red, while at other times there is a red patch near the border. This space seems to be only a vacuole in certain cases, while in others it is occupied by a substance which stains differently from the bulk of the granule. When sections are stained with Bismarck brown, followed by malachite green, there can be seen in the clear space inside the yolk bodies one or more refringent granules which are devoid of crystalline structure. The granules in question vary considerably in size and shape, having the form of dumb-bells, spheres, or rods, all of which are represented in fig. 4.

As has been stated already, they are present in all the types, though they are far more numerous in types A and B than in types C and D. In types A and B they show great affinity for bleu de Lyon, while in types C and D the substance which displayed the above affinity for the blue colour seems to have been used up almost completely, so that had they not been observed in types A and B they would have been probably overlooked in the other two types, owing to the difficulty with which they are stained. They can be stained, however, with bleu de Lyon in these types as well as in types A and B, provided the specimens have been preserved in absolute alcohol, or in corrosive sublimate and glacial acetic (fig. 6 *a*). But if they have been preserved in Hermann's or Flemming's fluid they often display an affinity for the carmine rather than for the bleu de Lyon, and are consequently stained red (fig. 7).

Appendix B.

On the Origin of the Cell Groups found in Types B and C.

The groups, the origin of which will be described in this appendix, must have originated from one or other of the three kinds of cells described in type A. They may have been derived from the flagellated cells by immigration, or from the cells with vesicular nuclei, either directly by division or fragmentation, or indirectly by passing through the intermediate condition of the cells with granular nuclei.

There are two points in favour of the view that they have been derived from the flagellated cells by immigration. In the first place, the nuclei of the cell groups on the one hand and of the flagellated cells on the other are almost identical both in structure and size, while in both these respects they differ widely from the granular nuclei. In the second place, the size of the cells of the incompletely divided groups agrees with that of the flagellated cells rather than of any others. But, on the other hand, there are several points which go against this

view. In the first place, the peculiar grouping and incompletely divided condition of the cells does not fit in with the view under consideration. In the second place, no cells have been observed in the act of migrating from the flagellated layer in type B. When all these facts have been considered, the balance of evidence seems to be against the view that they have originated from the flagellated cells by immigration.

The second possible view of their origin is that they have been developed indirectly from the cells with vesicular nuclei, which were first of all transformed into cells with granular nuclei, which, in their turn, divided and subdivided to produce the groups in question. There is only one argument in favour of this view, the fact, namely, that the nuclei could be described as being granular, which is of doubtful importance, since the nuclei of the flagellated cells could also be described as being granular. On the other hand, there are several reasons for rejecting this view. In the first place, the cells with vesicular nuclei as well as those with granular nuclei divide by mitosis, but at no time was a karyokinetic figure found in these groups of small nuclei, in spite of the fact that these cells themselves later on divide by mitosis. In the second place, when the cells with granular nuclei divide, the daughter-cells become completely separated from one another, the constricted nuclear spindle being often seen as a fine thread connecting the two cells; but here a great number of nuclei lie in a mass of cytoplasm, which as yet shows hardly any sign of dividing (figs. 5 *a* and *b*, and 9 *a* and *b*). In the third place, these nuclei are remarkably uniform in size, which would not have been the case had they originated from those of the cells with granular nuclei. The nuclei of the latter are much larger, and differ among themselves in size. This uniformity in size is a most remarkable thing, for in later stages, when these cells become the collar-cells, their nuclei are not uniform, since they divide repeatedly, and consequently the size of the nucleus varies. The equality of size alone, apart from any other argument, would incline us towards the opinion that the nuclei of the cell groups in type B have been produced not by

successive division of any kind of nuclei into two, then into four, and so on, but by the breaking up or fragmentation of one nucleus, the fragments becoming the nuclei of the cell group.

The discussion so far has had but a negative tendency, my aim being to show that the cell groups have not originated either by immigration from the flagellated layer or by division of the cells with granular nuclei. It remains, therefore, to bring forward some positive proof of their origin by the third possible method,—that is, directly from the cells with vesicular nuclei by fragmentation of the nucleus.

It has been pointed out that there is a considerable amount of variation among the vesicular nuclei, and it is quite possible that a certain amount of it is connected with the production of the nuclei of the cell groups. There are also in type B a certain number of cells which possess an irregular and blotchy nucleus, as well as certain cells which apparently do not possess a nucleus at all. In the cells just mentioned there are, however, a number of bodies quite distinct from the yolk bodies, and usually exhibiting a certain amount of structure.

It will be my purpose at present to show how these fragmented nuclei give rise to those of the cell groups, the changes involved in their production being represented in fig. 40. The cell marked *a* presents a rather unusual condition of the vesicular nucleus. The granules, instead of being small and numerous, are large and few, while the network of linin fibres, instead of containing small meshes, encloses large ones, and consists of a few threads instead of several. The cell *b* represents the next stage, in which the central corpuscle has lost the sharp outline it had in the previous stage, seen in cell *a*, and the granules are situated nearer the centre. The cell *c* illustrates the next stage, in which there is no sign of a central corpuscle, but the nucleus contains a great number of small granules of about the same size as those found in the cells *a* and *b*. The cell *d* exemplifies the next stage, in which the granules have grown so as to give the nucleus a blotchy appearance, and in some cases the chromatin can be seen

passing out of the nucleus through the nuclear membrane, which may occasionally, so to speak, heal up again. In such cases, though the chromatin has been cast out of the nucleus to form the small nuclei, a kind of skeleton of the old nucleus is often seen to accompany the smaller ones in the incompletely divided mass of cytoplasm (fig. 5 *b*). The cell marked *d'* is a peculiar one, not often seen, and had I not been quite familiar with mitotic figures I might possibly have made the mistake of explaining it as a stage in mitotic division. But the great number of the granules is a sufficient reason for rejecting such a view. It is far more probable that it is a cell in which the chromatin of the nucleus has become aggregated into granules before the nuclear membrane gave way for its extrusion. The stage represented in the cell *d* passes gradually into that illustrated in *e*, in which the chromatin appears in the form of small and spherical bodies which display no structure whatever, and which are distributed throughout the cell, the nuclear membrane having disappeared. By a gradual transition the condition found in the cell *e* passes to that represented in *f*, in which the chromatin bodies show a certain amount of structure. The fig. *g* represents a still later stage, in which the chromatin bodies have grown considerably, and are not very different from the nuclei of the cell groups illustrated in fig. 9 *a*. If the group of small nuclei represented in fig. 40 *g* be compared with that in fig. 9 *a*, and the one represented in fig. 40 *g'* with fig. 9 *b*, the resemblance in all their characters will be found to be a most striking one. The additional fact that the figs. 40 *g* and *g'* represent the same cell in successive sections, and the figures of the "cell group" in 9 *a* and *b* do the same, increases the importance of the resemblance between them, and the only conclusion possible is that the one becomes the other, and that the changes above described prove the nuclei of the cell groups to have originated from the vesicular nuclei by fragmentation.

Since the cell groups are produced in the way described, it follows that they must be considered as a category of cells quite apart from the cells with granular nuclei. Though they

originate from the same cells, they are different in their mode of origin as well as in their destiny.

The above conclusion may be further strengthened by the analogy of the early development of the larva while still within the maternal follicle. At the close of segmentation the young embryo consists only of cells with vesicular nuclei. Subsequently the outer layer of these divides up to produce the flagellated cells, while some of the cells more deeply situated give rise to cells with granular nuclei. In this way the youngest free-swimming larva, described in this paper as type A, originates. In its development, cells which were quite similar in character gave rise to two different classes of cells, namely, flagellated cells at the surface and cells with granular nuclei within. In the later stages of development, that is in the production of types B and C, these two processes seem to be going on, but instead of going on at the surface they go on in the interior of the solid part of the inner mass. The formation of the cell groups in the interior corresponds to the formation, during the earlier stages, of flagellated cells at the surface, and is, in fact, a continuation of the same process, just as the formation of cells with granular nuclei deep in the interior of the inner mass is a continuation of the same process that gave rise to similar cells underneath the flagellated layer.

If this view be correct, a process of differentiation is continually going on, producing, on the one hand, cells with nuclei exactly similar in their character to those of the flagellated cells; and, on the other hand, adding to the number of the cells with granular nuclei. It might be argued that these cells do not develop the flagella characteristic of the flagellated cells, and cannot, therefore, be homologous with them, or even belong to the same class of cells. The reply to this argument is that they do produce flagella, and that later on they develop a collar. As collar-cells their shape is not very different from that of the flagellated cells, the nuclei in both being situated at the base of the cell.

The conclusion adopted, after all these arguments for and against, is that the flagellated cells at the surface, and the cell

groups in the interior, are to be regarded as one and the same class of cells, while the cells with granular nuclei form another class. Both classes are derived directly from the cells with vesicular nuclei which retain their blastomeric characters, and are therefore capable of giving rise to any tissues of the sponge.

This view of the larva enables us to give a rational explanation of the development of the gemmule of Spongilla into the adult sponge. Zykoff has shown that all the cells of the gemmule of Spongilla are alike. Each gemmule cell possesses a vesicular nucleus, and in all its characters, morphological and physiological, may be compared to a blastomere of the ovum. Hence the gemmule cells are capable of giving rise on the one hand to collar-cells, and on the other to cells with granular nuclei. The same is true of the cells with vesicular nuclei in the larva, and the developmental processes are strictly comparable in the two cases.

The gemmule, therefore, is an aggregation of these cells brought together in its formation from the neighbouring parts of the adult sponge. Its constituent cells, having retained their blastomeric characters, are capable of giving rise to the whole sponge. From this it follows that the gemmule of Spongilla cannot in any sense whatever be described as a bud.

Appendix C.

The Development and Structure of the Spicules.

The facts already known of the development of the spicules of the Monaxonida have been so well summarised by Mr. Minchin in his paper (11) published in this Journal at the beginning of the year 1898, that I shall simply refer the reader to that account.

There are two kinds of spicules in the species, the development of which has been the subject of this paper, termed respectively "megascleres" and "microscleres." The former are smooth, either straight or slightly curved, and sharply

pointed. The microscleres, on the other hand, are covered with spines. The megascleres form the main skeleton, and have been for this reason called the skeletal spicules; while the microscleres take no part in the formation of the main skeleton, but are scattered about loosely in the sponge tissues, especially in the epithelial membranes, and have consequently been termed the flesh spicules.

The Megascleres.—These spicules always make their first appearance in the cells with vesicular nuclei. They are found in the very youngest free-swimming larva, and are placed at an angle of less than 90° to the line which joins the two poles of the larva. In the pupa, and subsequently in the young sponge, they become arranged in various ways, but the majority of them are placed almost vertically and slightly tilted towards the periphery of the young individual. As they grow above the general surface of the flattened or cake-like young sponge, they carry with them the dermal membrane, which therefore becomes raised up from the bulk of the tissues which lies underneath. This lifting up of the dermal membrane on the points of the spicules gives rise to the subdermal cavity. In individuals which have been fixed for a number of days the spicules, instead of appearing singly as at first, become aggregated together to form fibres in which the axes of the spicules are parallel to one another. They are surrounded by a layer of cells which secretes a substance, presumably spongin, which stains deeply red with fuchsin S.

Figs. 36 *a*, *b*, *c*, and *d* illustrate successive stages in the development of the megascleres. At their first appearance and for a considerable time afterwards they are enclosed completely by the scleroblast, but what happens to the secreting cell ultimately I have not been able to make out. The cytoplasm of the cell is exceptionally clear, but occasionally contains a few yolk bodies and one or two nutritive vacuoles. So far as my observations go, the nucleus of the cell does not divide, but seems to lose its vesicular character, and to become granular as the spicules increase in size. It is almost certain that when a cell with a vesicular nucleus begins to secrete a

spicule, it is destined to change in character, and to become a cell with a granular nucleus.

The spicules when they have grown somewhat in size show very clearly an axial thread, which traverses their longitudinal axis from end to end, but which cannot be seen in the early stages of their development. The thread in question appears to consist of some form of organic material which lies in the canal of the spicule, and which stains red with fuchsin S near the broken ends of pieces of spicules. The stain seems to penetrate into the canal from the broken end of the spicule and fades away gradually, showing clearly the difference between the stained and the unstained portion of the thread.

The Microscleres.—These spicules are not formed so early in the development as the megascleres. However, they are found soon after fixation,—for example, the spicules represented in fig. 37 *a* have been drawn from a specimen which still retained the flagellated layer almost complete on the upper surface. They are not developed in cells with vesicular nuclei, but in cells with granular nuclei, that is in those cells which give rise to the flat epithelium of the sponge. And, moreover, they are often seen inside the cells of the flat epithelium, two cells of which are represented in fig. 37 *b*, one containing a flesh spicule.

It is seen from the above description of the development of the spicules that cells belonging to two out of the three classes found in the youngest larva, i.e. type A, are capable of secreting spicules.

Appendix D.

On the Division of Collar-cells and the Multiplication of Chambers.

There occurs in Mr. Minchin's able paper on the position of sponges in the animal kingdom the following statement:— "The fact remains, that both the multiplication of collar-cells

and the formation of new ciliated chambers in a growing sponge are scarcely known, and no satisfactory observations have been recorded with reference to this point." In the present appendix it is my purpose to give a brief description both of the multiplication of collar-cells and of the formation of new chambers.

The collar-cells, when they have been fully formed either by the fragmentation of a cell with a vesicular nucleus or by the immigration of the flagellated cells during metamorphosis, divide by mitosis. It is a rather remarkable thing that these cells, after having been developed, in one case at least, from cells with vesicular nuclei by fragmentation, begin to divide by mitosis.

The nuclei of the collar-cells are situated at the base of the cell, but when they are going to divide the nuclei travel to the other end of the cell, and are seen situated close to the collar, which is gradually withdrawn (fig. 33 *b*, the cells *c. c.*). The nucleus soon loses its ordinary structure, and travels into the middle, and there goes through all the changes of mitotic division. The longitudinal axis of the nuclear spindle is placed tangentially to the wall of the flagellated chamber. Consequently the cell must divide radially with respect to the chamber (fig. 35, the cell *c. c.*). The presence of the spindle in the collar-cell marked *c. c.* in fig. 35 proves, beyond any doubt, that the collar-cells divide, and consequently increase in number. It might therefore be argued, on a priori ground alone, that the chambers also must divide, because the number of cells which go to form a chamber has its limits. But there is no need to have recourse to a priori reasoning, for flagellated chambers which are actually dividing have been often observed in sections. One such chamber has been drawn in fig. 29 *a*, and is marked *C*. The chamber in question is actually being constricted into two, the collar-cells having already become arranged in two groups round two different points as centres, and the cells with granular nuclei, especially from the outer side, are making their way in with a view to the formation of the lining of an exhalant canal, which will

be formed by the separation of the daughter chambers. The other chambers situated further in illustrate a slightly more advanced stage in the division of a chamber. The space between these latter chambers is not furnished as yet with its lining cells, or at least they have not flattened out to form flat epithelium. The large cell with a granular nucleus, situated close to the nutritive vacuole (*n. v.*) on the inner side, is about to flatten out to form the lining of the exhalant canal that is being developed between the two chambers.

From the above facts the following conclusions may be formulated :—The cells of the flagellated chambers divide by mitosis. They become too numerous to constitute one chamber, and consequently are separated into two groups or daughter chambers, which have their apopyles facing each other, and at first, at least, open into a common exhalant canal lined by cells with granular nuclei.

Appendix E.

On Mitotic Division in the Cells of the Free-swimming Larvæ of the Young Sponge.

Though it was not the object of this paper to describe the mitotic division of the nuclei, still, owing to the fact that in sponges little has been done on this line of inquiry, and that I have from time to time seen phases in cell division and drawn them, it is deemed advisable to record them. Though they are in no way complete, they may serve as the bases of further research.

The chromatin, immediately after the nucleus has lost its ordinary characters, presents the form of small granules—about a dozen in number—which are by no means easily made out (fig. 42, 1 *a*).

During the next stage the chromatin granules become arranged in the form of an equatorial plate in which the individual chromosomes are difficult to identify. The threads of

the nuclear spindle have not yet appeared, or are indistinctly seen. It seems that they are in the process of development (fig. 42, 2 *a*, 2 *b*). In the next stage the chromatin assumes the form of a well-defined equatorial plate. The individual granules (or chromosomes), which were difficult to identify in the previous stage, are much more distinct, being apparently twelve in number. Each one of these chromatin granules seems to divide, so that a double plate of granules appears in the median plane of the spindle (fig. 42, 3 *a*—3 *e*). In this, the metaphase stage, the spindle threads are distinctly seen, but the poles of the spindle seem to be embedded in a cloudy, ill-defined area of the cytoplasm. The nuclear spindle has evidently arisen inside the nucleus, for the nuclear membrane in many cases still exists. Though I have often suspected the presence of a centrosome in the cloudy area of the cytoplasm, I have not yet been able to demonstrate its existence with certainty. The two small bodies in fig. 42, 4 *a*, have probably no connection of any kind with the centrosome, supposing such a body to exist. The chromosomes after dividing at the centre pass in two groups towards the poles of the spindle (fig. 42, 4 *a*, 4 *b*). When they have reached the poles of the spindle, they are arranged at first more or less in the form of a ring in which the individual granules are distinct (fig. 42, 5 *a* and 5 *b*). During the passage of the chromosomes from the centre to the opposite ends of the spindle the spindle threads are lost sight of, but after the passage they again become distinct. Concurrently with the changes represented in fig. 42, 4 *a*—4 *c*, the nuclear membrane disappears. Whether the threads visible in fig. 42, 5 *a* and 5 *b*, are the same as those seen in 3 *a*—3 *e*, or are developed from the nuclear membrane which has disappeared, I am uncertain, but incline towards the latter view. The chromosomes after reaching the poles of the spindles soon lose their individuality, to all appearance at least, and become united together so as to form a kind of cap of chromatin, which at first gives some indications that it has been formed by the coming together of several bodies. From

this cap of chromatin short processes pass along the interzonal fibres for a short distance, thus giving them the appearance of being thicker at the ends than in the middle. The fibres during this and the previous stage are more distinct than they are at any other time (fig. 42, 6 *a*, 6 *b*). The cap of chromatin becomes gradually more uniform, and shows fewer signs of its multiple origin from a number of chromosomes, but the interzonal fibres still retain their sharp outline (fig. 42, 7 *a*, 7 *b*).

The next change observable is the constriction of the cell. The interzonal fibres, at the same time becoming much elongated and attenuated, are difficult to make out, though lying parallel with one another and still keeping their independence (fig. 42, 8 *a*, 8 *b*).

The chromatin presents, in some cases, a blotchy appearance, while in others it shows signs of preparation for the final breaking up into granules. In the next stage the cell has become completely divided, the daughter cells, however, being connected with each other by means of a fine thread which consists of the constricted remains of the interzonal fibres. The chromatin shows further signs of the final breaking up (fig. 42, 9 *a*, 9 *b*).

Finally the two cells become separated, and all traces of the fibres connecting the two masses of chromatin in the previous stages have disappeared. The chromatin itself presents the appearance of a granular blotch lying in a clear area, and subsequently breaks up so as to produce the small granules characteristic of the resting nucleus (fig. 42, 10 *a*).

Appendix F.

Technique.

The colonies of Spongilla from which the larvæ were obtained were procured from the river Cherwell, in which Spongilla grows in large quantities on the roots of the trees

which line the bank of the river, on logs of wood in the river, and on the pillars which support the bridges. The sponges were taken from the river in the early morning. The roots on which they grew were cut, and the whole of each colony was transferred from the water into a glass jar full of river water in as complete a condition as possible, and taken into the laboratory of the University Museum, where, through the kindness of Professor E. Ray Lankester, this piece of work was done. Great care was exercised so as not to leave the mother sponge for any length of time out of water. The sponges, having been brought into the laboratory, were placed in glass vessels with wider openings than those in which they were carried in. The vessels were then put in the tanks, and a slow current of water was allowed to flow over them. In this way as many larvæ as was desirable could be collected.

Owing to the continual supply of fresh water the sponge was kept in an active and healthy condition. The oscula were always open, and a stream of water could be seen to issue out of them, carrying with it at intervals the larvæ. The larvæ were almost invariably produced on the same day as the mother sponge was taken from the river, often on the same morning. The larvæ appear to be carried helplessly out of the oscula by the current, but in all cases they soon gain complete control over their movements, and swim towards the surface. They dart downwards instantly when disturbed. As they swam at the surface they were easily caught with a pipette and removed into watch-glasses. Some of the larvæ so obtained were preserved at once, while others were reared in watch-glasses, allowed to fix and to undergo metamorphosis, and were preserved at various stages in their development.

The watch-glasses in which the larvæ were placed measured about 18 cm. across, and were about two thirds full of river water. The watch-glasses were first of all carefully cleaned with strong hydrochloric acid and with ether successively. They were then covered over with a thin layer of glycerine, except for about half an inch near the edge, and finally with a thin layer of paraffin of low melting-point. The glycerine

prevented the paraffin sticking to the glass in the centre, while near the margin, where there was no glycerine, it became firmly adherent.

The larvæ, after they were placed in the watch-glasses, used to swim about for a time, as a rule, near the surface, though a few were always found to sink immediately and to move about at the bottom. Those which sank, whether sooner or later, usually became fixed and underwent metamorphosis. Some larvæ, however, never went to the bottom, but continued to swim near the surface, and would fix ultimately to the film of air at the surface of the water if allowed to do so. In order that some use might be made of the larvæ which fixed at the surface, glass cover-slips were floated on the surface that they might settle on them instead of fixing to the film of air. The majority, however, fixed at the bottom of the watch-glasses. After a certain number of larvæ had become fixed, the watch-glasses were sunk in a larger vessel, so that those undergoing metamorphosis might have a greater supply of water, which was always obtained direct from the river.

Various reagents were used for the preservation of both the free-swimming larvæ and the fixed stages. The former were merely dropped into the preserving fluid with a pipette. The specimens which had fixed to the glass cover-slips were similarly treated by dropping the cover-slip with the individual fixed on it into the preserving medium. In no case were these specimens removed from the cover-slips. Those specimens which had fixed to the paraffin were removed from the watch-glass together with a piece of the paraffin to which they had become fixed by running a needle round them. The piece of paraffin along with the specimen settled on it floated to the surface, and was then dropped into the preserving fluid in the same way as the cover-slips to which some larvæ had become fixed.

The reagents used for preservation were the following:

(*a*) Absolute Alcohol.—The specimens were placed in absolute alcohol for five minutes, and were then removed into 90 per cent. alcohol. They were afterwards stained with a

weak solution of borax carmine and subsequently washed with spirit and acid, being left in both of these liquids for about fifteen minutes. They were usually left in the spirit and acid for the same time as they were left in the borax carmine solution, but the specimens on the cover-slips had to be left in longer, as they were to be examined whole. They were afterwards treated with 90 per cent. alcohol, in which they were kept to await further treatment.

The specimens on the glass cover-slips, which were to be examined whole, were further stained with a weak solution of bleu de Lyon in 90 per cent. alcohol. To guard against overstaining they were taken out from time to time and examined, and when sufficiently stained they were passed through absolute alcohol, cleared in pure xylol, and finally mounted between two cover-slips in Canada balsam dissolved in xylol. Being thus mounted they could be examined from both surfaces.

(*b*) Corrosive Sublimate and Glacial Acetic Acid.—This mixture was made up of four parts saturated solution of corrosive sublimate in 90 per cent. alcohol and one part glacial acetic. The specimens were left in this fluid until they became whitish in colour, and were afterwards treated in essentially the same way as those preserved in absolute alcohol.

(*c*) Flemming's Weak Solution.—The specimens were left in this fluid for about ten minutes, and were then washed in a current of water for an hour and finally brought up through the alcohols into 90 per cent., in which they were left to await further treatment.

(*d*) Perenyi's Fluid.—The specimens were left in this fluid for an hour and were then transferred into 70 per cent. alcohol, in which they remained for two hours, at the end of which they were removed into 90 per cent., which was changed several times during the subsequent twenty-four hours.

(*e*) Osmic Vapour and Müller's Fluid.—The specimens were held in the vapour given off from a 2 per cent. solution of osmic acid. The bottle which contained the solution was previously warmed. They were afterwards placed in Müller's

fluid for twenty-four hours, and then washed for an hour in a current of water, and finally brought up through the alcohols into 90 per cent.

Some of the free-swimming larvæ which were held in osmic vapour as above described were afterwards stained in picro-carmine and mounted in glycerine. When transferred into the glycerine the larva tumbled into pieces, and the cells separated owing to the maceration that had gone on in the picro-carmine. In this way free cells were obtained.

(*f*) Hermann's Fluid.—Though I did not use this reagent myself, I obtained from Mr. Minchin a number of valuable specimens both of free-swimming larvæ and of fixed stages.

After the specimens had been preserved in these various ways, and had been brought into 90 per cent. alcohol, they were then mounted on thin sections of liver.

The larvæ were placed directly on the liver, that is without anything intervening between them, and were covered with a small drop of glycerine albumen, which coagulated in the alcohol. The liver was then cut parallel to the longitudinal axis of the larva, and thus enabled the orientation of the specimen to be made out when sections had to be cut from it.

The fixed stages, on the other hand, were placed on the liver sections, with the piece of paraffin on which they had settled intervening between them and the liver. In no case were the specimens removed from the paraffin. In this way the whole specimen was mounted, without danger of any part of it being broken away, and the lower surface being always nearest to the liver the manner of fixation could be easily made out in the early stages. After the specimen and the paraffin to which it had fixed itself had been covered with glycerine albumen, the liver section was cut off on all sides as near as possible to the specimen so as to reduce its size.

When the specimens had been placed on liver they were dehydrated by passing them through three successive changes of absolute alcohol, leaving them for about two hours in each. They were afterwards transferred to tubes containing some chloroform at the bottom and absolute alcohol at the top.

The specimens when first put in floated in the intermediate layer, but gradually sank to the bottom. The liquid above them was being continually drawn away with a pipette, so that by the time they had sunk to the bottom they were in almost pure chloroform, after which they were transferred to the pure liquid.

After the specimens had been brought into pure chloroform they were transferred into small watch-glasses, which contained some chloroform together with a certain amount of high-melting paraffin (135° F.), and were placed on top of the water-bath. The chloroform soon evaporated, and the paraffin simultaneously melted. They were then placed in the water-bath, where the last traces of chloroform were driven away. They were left in the water-bath only for a very short time, in order that they might not be subjected to high temperature for a long interval, which is an important thing if histological details have to be considered.

The paraffin used for embedding was that which melts at 135° F.

The sections were always cut with a Jung microtome, the block being painted over with a mixture of collodion and gum mastic dissolved in ether when exceptionally thin sections were required. The usual thickness was about 3 μ, though sections varying from $2\frac{1}{2}$ to 6 μ were occasionally cut.

So many methods of staining have been tried, that it would be going too far even to enumerate them all. However, the most important and generally useful in connection with the development of Spongilla will be given.

(*a*) Borax Carmine followed by Bleu de Lyon.—This is a most important method, as it has been used by previous observers, and has given different results in different hands. The staining of the specimens with borax carmine has already been described; there is therefore no need but to give the last part of the process. Several ways of staining carmine-coloured sections with bleu de Lyon have been tried, but the following has proved to be the most useful. The sections were overstained with carmine, and could on that account be

washed over with spirit and acid, being subsequently immersed for some time in 90 per cent. alcohol. They were afterwards placed in a weak solution of bleu de Lyon dissolved in alcohol of the same strength, and were left in it for from ten to fifteen minutes. The sections were subsequently washed with 90 per cent. alcohol in order to take away the excess of bleu de Lyon, which sometimes tended to mask the carmine. The differentiation went on slowly, and could be watched, therefore, under the microscope. When this washing process was complete they were washed with absolute alcohol and subsequently with pure xylol, and finally mounted in Canada balsam dissolved in xylol.

(*b*) Carmalum and Bleu de Lyon.—Sections of material preserved in Flemming's fluid and also in Hermann's fluid were stained for twenty-four hours in carmalum, and were then treated in the same way as the sections stained in borax carmine, omitting, however, the washing with acid, and to some extent varying the time as well as using stronger solution of bleu de Lyon.

(*c*) Hæmatoxylin and Fuchsin S.—The sections were first stained by Heidenhain's method in iron alum and hæmatoxylin. After differentiation in the alum solution they were washed in a current of water for twenty minutes, treated with absolute alcohol, and stained for nearly a minute in a saturated solution of fuchsin S in absolute alcohol, and then mounted in the usual way.

(*d*) Safranin and Gentian Violet.—The sections were stained in safranin for twenty-four to thirty-six hours. They were then washed with spirit very slightly acidulated, and subsequently with absolute alcohol. Afterwards they were stained with gentian violet for from three to five minutes, when they were washed with absolute alcohol and placed in a solution of potassium iodide and iodine dissolved in water until they were quite black, the time required for this purpose being usually four or five hours. They were then slowly differentiated in absolute alcohol and mounted in the usual way.

The staining solutions which were used had the following composition :

90 c.c. distilled water saturated with aniline oil.
10 c.c. absolute alcohol.
1 gramme of the stain (safranin or gentian violet).

(*e*) Gentian Violet.—The sections were left in the staining solution for fifteen minutes, and were then subjected to the action of absolute alcohol and clove oil until they were sufficiently differentiated, being then mounted in Canada balsam as usual. The solution used for staining purposes in this case was the same as in the previous method.

Though the above are the chief methods which have been used, several other methods have been tried, both in the way of combining the above stains in a different manner and of using other staining solution not mentioned above.

The following may serve as a few examples of the numerous combinations which have been tried :—first, safranin, followed by gentian violet and orange G; secondly, Bismarck brown, followed by malachite green; thirdly, iodine green, followed by fuchsin S; and lastly, hæmatoxylin, followed by orange G or eosin or picric xylol instead of fuchsin S.

The above enumeration of the staining methods which have been tried suffices to show that no trouble has been spared to test the correctness of the conclusions arrived at in this paper.

III. Comparison of the Above Account with those of Previous Authors.

When I embarked upon the study of the structure and metamorphosis of the larva of Spongilla I hoped to be able to show that one or other of the accounts which had already been published was in the main correct. As I had no theory whatever to uphold, I started on my work with an open mind. However, I never expected that the result of my work would be to show that nearly all the accounts were in the main correct, though they were all incomplete.

In the following discussion of previous works we shall take first each class of cells in the larva separately, and consider the various theories that have been held with regard to it. This method of treatment will result in less repetition than that of dealing with the various accounts in the order in which they were published. We may consider, in the first place, the fate of the flagellated layer; and, in the second place, the differentiation of the inner mass, both during the free-swimming period and the pupal stage.

A. The Fate of the Flagellated Layer.

The views held as to the fate of the cells which constitute the layer in question may be divided at the outset into two classes: first, that of Götte (5), who holds that they are cast off during the metamorphosis and lost altogether; secondly, the views of all other authors, who hold that they become of use to the young sponge in some form or other.

This second class of views may be divided into three sub-classes: first, the view of Ganin (4) and Maas (7), that they become flattened out to form the cells of the flat epithelium; secondly, the view of Delage (1) and Maas (8), who hold that they become the collar-cells of the young sponge, though these two authors differ considerably as to the details of the process; and thirdly, the novel view of Nöldeke (13), who holds that they are devoured by the cells with vesicular nuclei.

In considering the view held by Götte (5), it must be admitted that larvæ such as that represented in section in figs. 29 and 29 *a* could possibly dispense with the flagellated layer, for the inner mass already contains all the elements necessary for the building up of the young sponge. But I have never seen any signs of these cells being thrown off. The only explanation of what seems to be an error on the part of Götte is that he observed specimens which had in some way or other become injured. However, the credit belongs to him of discovering that the flagellated cells do not become the flat epithelium or "ectoderm."

The second view to be discussed is the one which was for a long time almost universally held to be the fate of the flagellated cells in most sponges. Ganin (4) and Maas (7) are the authors who have given expression to this view with regard to the fate of the flagellated cells of the Spongilla larva. Further comment on the view under consideration would be unnecessary, as it is practically obsolete, had not Mr. H. V. Wilson (17) given it his support as late as the year 1894 in his observations on the gemmule and egg development of marine sponges. However, in spite of Wilson's attempt to reinstate this view in its former position, all that is necessary is to point out that he makes some very important admissions: first, that he has never seen the flagellated cells being transformed to the cells of the flat epithelium or so-called "ectoderm;" and secondly, that some of the ectoderm cells (meaning flagellated cells) of the larva migrate into the interior during metamorphosis. These admissions, together with others made in Wilson's account, are fatal to the view which he upholds, not to speak of the fact that his figures in spite of himself tend to support the opposite view. However, with regard to Spongilla the only statement I wish to make is that I consider that such figures as 15 *a* and 15 of the present paper cannot be otherwise explained than as a most convincing and final proof that the cells with granular nuclei pass out to form the flattened epithelium, and that the flagellated cells pass to the interior, which is the view now held by Maas (8), and from the first by Delage (1) and Nöldeke (13).

Though these three authors agree as to the immigration of the flagellated cells, they differ considerably as to their ultimate fate. Maas and Delage, however, support the view that they become the collar-cells of the young sponge, but differ widely as to the details of the process of transformation.

Maas holds that they become the collar-cells directly, and without passing through any such series of changes as have been described by Delage in the formation of his "polynuclear groups." Maas undoubtedly saw the "polynuclear groups" of Delage, the plasmodial aggregations of the present

account, and came to the conclusion that the granules contained in them were purely vitelline. He must, therefore, have missed that peculiar stage of the development in which there are no small cells in the interior, but only plasmodial aggregations. Maas always found small cells in the interior, either loose or forming flagellated chambers, and hence arrived at the conclusion that the granules, which he supposed to be inside the cells with vesicular nuclei, are all vitelline, thus overlooking the distinction between the yolk bodies, which are really inside the amœboid cells, and the small nuclei of the flagellated cells plastered to their surface. Another fact helped to establish him in his error, namely, the continual decrease in size of the yolk bodies contained in the amœboid cells.

Delage (1), on the other hand, goes to the opposite extreme, for, according to him, the granules in his polynuclear groups are all nuclei of flagellated cells, and are situated inside the cells with vesicular nuclei. He is not in any way impressed by the decrease in size and the complete disappearance of some of the granules in his polynuclear groups, facts which alone drew Maas' attention, and which, as we shall see later on, have influenced Nöldeke. The following expression, which occurs on p. 356 of Delage's valuable paper, may throw a certain amount of light on his failure to distinguish between the vitelline constituent and the nuclear one in the granules of his "polynuclear groups:"—"Chez la larve libre, les cellules amœboides ne contiennent rien autre chose que leur noyau propre." I can hardly understand how such an able observer as Prof. Delage made such a statement. But his method of treating the mother sponge and of obtaining the larvæ may account for it. He kept the mother sponges in vessels, and instead of providing them with a continuous current he changed their water every twenty-four hours, and thus obtained his larvæ only at the time he gave them fresh water. In consequence of not providing the sponges with a continuous current they were for nearly twenty-four hours in a contracted condition, and no current passed through them. Therefore no larvæ could be hatched, though they might be ready to emerge.

When the sponge again expanded on being supplied with fresh water the larvæ came out, but owing to their detention inside the mother colony many of them had used almost all their food material, and were so far abnormal. It is highly probable, however, that had they been preserved in some other fixing reagent than absolute alcohol Prof. Delage would have found the granules in question, though less numerous and reduced in size.

Another detail of Prof. Delage's account, with which I cannot agree, is that the flagellated cells are actually taken in by the amœboid cells. After the most careful consideration of the point in question I am of opinion that they are plastered to the surfaces of the amœboid cells rather than enclosed by them. If Delage's view were correct we should not expect to be able to see the outlines of the flagellated cells after they had entered these associations, but they are easily made out for some time, as can be seen from fig. 29 *b*. Delage was led to this view by the presence of the yolk bodies, which he did not distinguish as such, and which are undoubtedly inside the cells with vesicular nuclei which occupy the centre of the groups.

It appears, therefore, that owing to his failure to detect the yolk bodies in the larva Delage concluded that all the small bodies in the "polynuclear groups" were nuclei; and further, owing to the presence of true yolk bodies inside the cells, and his failure to detect the outlines of the flagellated cells soon after they had entered the groups, he came to the additional conclusion that the small nuclei were inside the central cell. The fact is that these aggregations contain both yolk bodies inside the central cells, and the small nuclei of the flagellated cells plastered to their surfaces.

The last view with which we have to deal is that of Nöldeke (13). The peculiarity of this view is that it involves as a consequence, like that of Gotte (5), the formation of the whole sponge from the inner mass, or so-called "endoderm" of certain authors. Nöldeke's observations seem to be far too limited to enable anyone to come to such drastic conclusions. He seems never

to have seen individuals in which the interior is full of plasmodial aggregations alone. He has also failed to distinguish between the yolk bodies and the nuclei of the flagellated cells which have been taken in by the large amœboid cells in the same way as is described by Delage. But, on the other hand, he has seen some of them decreasing in size and disappearing, which Delage did not see, and consequently comes to the conclusion that all the bodies figured in his large amœboid cells, which are the same as the "polynuclear groups" of Delage and the "plasmodial aggregations" of the present memoir, are completely absorbed, a conclusion which is in no way warranted.

Moreover, the magnification to which he has drawn his figures is not sufficiently high to enable him to distinguish between yolk bodies on the one hand, and nuclei on the other. The figures, therefore, which scarcely show any nuclear structure, save what is sufficient to distinguish a vesicular nucleus as such, have no value whatever for deciding such a point. These facts detract considerably from the value of Nöldeke's account, and cast a doubt upon his conclusions.

This brings to a close what we have to say as to the fate of the flagellated cells, which, in our opinion, migrate into the interior and become plastered to the surfaces of the cells with vesicular nuclei. Ultimately, however, they break away from these associations, and become the collar-cells of the young sponge.

B. The Differentiation, &c., of the Inner Mass.

The views held with regard to the constitution and differentiation of the inner mass may be divided into two classes: first, those according to which it consists of both "mesoderm" and "endoderm," held by Ganin (4), and by Maas in his first account (7); secondly, those according to which it contains only an aggregation of cells called by some authors "endoderm," which is divisible, however, into several kinds of cells.

The second class of authors may be further subdivided: by some the inner mass is supposed to give rise to the collar-cells as well as to the rest of the sponge—the view held by Götte (5) and Nöldeke (13); while according to another view, supported by Delage (1) and by Maas (8) in his later paper, the inner mass only gives rise to the "dermal layer," including in that term the amœboid cells.

Since the views of Ganin and Maas (7) are practically the same with regard to the structure of the inner mass and the fate of its constituents, it will suffice to discuss Maas' account alone. Before making any statements with regard to the above account it is necessary to refer to Delage's criticism of it, but for the present I shall reserve what I have to say on Delage's views of the structures described by Maas as flagellated chambers.

Delage says that the larvæ observed by Maas are abnormal, or rather unusual and pathological. I have shown in the account already given that the larvæ which Delage describes as abnormal are capable of developing to the adult sponge. He is, therefore, not justified in describing such a larva as a pathological one incapable of righting itself. The larva in question is not an abnormality, but a variety, which is capable, and also does give rise to the same end result as Delage's normal larva, which is the same as type D of the present account.

The chief peculiarity of both Ganin's and Maas' view of the development of the flagellated chambers is that they derive them from the layer of flattened cells ("endoderm") which lines the larval cavity. The "endoderm" grows out into the layer which intervenes between it and the flagellated "ectoderm," i. e. into the "mesoderm" of the triploblastic larva. The swollen or expanded end of the evaginations in question are held to be the flagellated chambers, while the intervening canals become the exhalant system. What is peculiar about this view is that it is absolutely right as to the fate of the parts in question, but equally wrong as to their origin. It appears that Maas' mind was at that time so dominated by the

idea that the sponge larva must be subjected to the dogmas of the "germ-layer theory," that, having discovered both "endoderm" and "mesoderm" in the inner mass, he could not conceive the canals and chambers as developing in situ, which is their true origin. In many cases the short canals which have been described in the present account do not seem to communicate with the larval cavity at all, save in exceptional cases, and for that reason cannot be produced as evaginations from it.

The swollen or expanded ends described by Maas are really the cell groups of the present memoir, and become the flagellated chambers. They are derived, as has been shown in Appendix B, from the cells with vesicular nuclei, by fragmentation of the nuclei and susequent division of the cell body. The canals which he described as communicating with the chambers on the one hand, and with the larval cavity on the other, have been shown to have their lining formed by cells with granular nuclei developed in situ. They consequently belong to the same class as those which line the larval cavity, as stated by Maas, though for erroneous reasons.

In the next place we have to examine Götte's view, namely, that the cells of the inner mass ("endoderm") give rise to the whole sponge, the "ectoderm" being completely lost. The flagellated chambers are described by him as being produced by groups of cells—each of which has arisen from a single cell—enclosing a cavity, just as has been described at full length in the present account. Götte did not, however, distinguish clearly between yolk bodies and fragmenting nuclei. A somewhat similar view of the origin of the flagellated chambers was put forward by Saville Kent (6), though he did not understand the true nature of sponges, and held that they were Protozoa. Dendy (2) also described a similar origin for the flagellated chambers in some horny sponges (Stelospongus).

With regard to the development of the lining of the canals from cells of the inner mass I agree with Götte. But for several reasons I hold that the cells which line the canals belong to quite a different class from those which become the

collar-cells. It is true that both classes are developed originally from cells with vesicular nuclei, but their mode of origin is absolutely different, as well as their fate. The "cell groups" have been shown to be developed by the breaking up of one cell, while the cells with granular nuclei increase in number by the gradual transformation of the cells with vesicular nuclei.

Delage divides the cells of the inner mass into three classes, which must be compared with the similar classification I have adopted above. Delage's three classes are the following:— "cellules épidermiques, cellules amœboides, et cellules intermédiaires;" while my three classes are termed "cells with granular nuclei," "cells with vesicular nuclei," and "cell groups." The second class in each system is identical. Therefore only the other two remain to be examined and compared. Since Delage considers the larva described by Maas, and in the present memoir referred to as type C, to be an abnormal form, he has probably not taken the class of cells described above as cell groups into account, and has included what there was of them in the larva which he considered normal, which is type D of the present account, among his "cellules intermédiaires." As has been said already in describing the larva type D, the cell groups are so few that probably they would have been missed by me had I followed Delage in considering type D abnormal.

It seems, therefore, that Delage's "cellules épidermiques et cellules intermédiaires" are equivalent to the class here termed cells with granular nuclei, together with a few cell groups which might have existed in his normal larvæ, but which he did not recognise. There is no valid reason for the division of this class of cells into two. The "cellules intermédiaires" are separated from the "cellules épidermiques" for no other reason than that their nuclei are slightly larger and their situation deeper. The former statement, which is true only of the cells lodged in the interior of the solid part of the inner mass, and not of the cells which line the larval cavity, and which are also included in this class, results from their more recent origin from the cells with vesicular nuclei. How-

ever, great credit is due to Delage for showing that the flagellated cells of the larva became the collar-cells of the young sponge, though he is in error in denying that they may also be developed from the cells of the inner mass; and the division of the class of cells with granular nuclei into two is, to say the least, needless.

The last account to be considered is that of Nöldeke (11), who derives the whole sponge, like Götte, from the inner mass. Nöldeke describes the inner mass as "endoderm," and distinguishes between endoderm cavity and endoderm nucleus. The latter varies according to the age of the larva. In the youngest larvæ, which correspond to type A of the present account, the endoderm nucleus consists of large cells which contain several food granules. In older larvæ differentiation has set in, so that the inner mass consists of "Bildungszellen" and "Amoeboidzellen," the former class being equivalent to the two classes described in the present memoir as cells with granular nuclei and cell groups, the latter class being equivalent to the class of cells here described as cells with vesicular nuclei. The most important error in this grouping of the cells is the classifying together of the cells with granular nuclei and the cell groups. We may repeat, in fact, the remark made in reviewing Götte's work; their mode of origin is different as well as their ultimate fate. Consequently they cannot belong to the same class.

Nöldeke says there are two methods by which the flagellated chamber originates: first, from one mother cell by division; secondly, by the coming together or migration of many distinct cells to one spot. These two statements are highly suggestive. If the flagellated chambers were derived from cells of the inner mass alone we would hardly expect to find these two methods of origin occurring side by side. But if the flagellated chambers are derived on the one hand from flagellated cells, and on the other hand from the cell groups, as has been shown in this paper to be the case, we would almost expect to find that they were formed in two different ways. Nöldeke's two methods evidently correspond to the two kinds of cells which give

rise to them. The method by which the cells come together to form chambers corresponds to their origin from the flagellated cells, and that by which the cells are derived from one cell to the formation of the cell groups described above. It is, therefore, highly probable that Nöldeke has seen the formation of flagellated chambers from the flagellated cells of the larvæ, as well as from the cell groups, the peculiar mode of origin of which he did not recognise, and consequently classed them along with the cells with granular nuclei as "Bildungszellen."

This brings to a close our remarks on the accounts already published, and what most of all impresses us is their incompleteness in every case. Ganin and Maas appear to have recognised larvæ belonging to type C alone—perhaps type B as well,—while Götte found types A, C, and D. But he seems to have drawn type C as a larva, and described the metamorphosis of type D. Delage was aware of the existence of types C and D, but considered the former to be an abnormality, and believed the latter to be the only one that pursued the normal course of development; while with regard to the former, so far as he described it, he arrived at a quite erroneous conclusion with regard to the origin and fate of the rings of cells occurring in it. Götte and Nöldeke are the only two who were acquainted with type A as a free-swimming larva. Nöldeke, however, seems to have completely missed the larva described in this memoir as type D. The larva which I have described as type B seems to be an entire stranger to the literature on the question. However, it is one of the best examples possible of the value of intermediate stages. It may be that Maas saw it, for he draws amœboid cells with yolk granules side by side with incompletely divided cell groups.

Now that the accounts already published have been compared with that offered in the present account, it is necessary to make a brief summary of the points which are considered to have been proved.

Conclusions.

(1) That there are different types of free-swimming larvæ, which have been described as A, B, C, and D. Type A is the youngest form of all, type B is an intermediate form between types A and C, while type D is a variation derived along a different line of development from type A.

(2) That the flagellated cells of the larva in all cases become the collar-cells of the young sponge.

(3) That certain cells of the inner mass, distinguished by their vesicular nuclei and blastomeric characters, are capable of giving rise to collar-cells, viâ the cell groups, as well as to the flat epithelium, &c.; i. e. both to the dermal and the gastral layer.

(4) That consequently, during the metamorphosis of type C, flagellated chambers are developed from the "cell groups," derived originally from cells with vesicular nuclei situated in the inner mass, as well as from the flagellated cells.

(5) That in type D hardly any cell groups are formed, and consequently the gastral layer is developed almost completely from the flagellated cells of the surface layer.

(6) That both the cell groups and the flagellated cells of the larva are to be considered as belonging to the same class, the latter being developed on the outside, and consequently producing only flagella, while the former originate inside, and ultimately develop both collars and flagella.

(7) That all the cavities, canals, and surfaces are lined by cells possessing granular nuclei, which are capable of producing microscleres, even when they are situated in the flat epithelium of the surface layers.

(8) That the megascleres are produced at first in cells with vesicular nuclei, which later on become granular.

(9) That some of the cells with vesicular nuclei plaster themselves to the surfaces of the flagellated chambers in the young sponge, and become pore cells, their nuclei subsequently changing in character and becoming granular.

(10) That there is always a residue of cells with vesicular nuclei which retain their blastomeric characters, and which are therefore capable of giving rise to the whole sponge. Some of these or perhaps all of them become wandering cells, and ultimately give rise to the gemmule which is capable of producing both the dermal and the gastral layers of the sponge.

(11) That the collar-cells multiply by karyokinetic division, and that owing to the multiplication of the collar-cells in the flagellated chambers the latter become separated into two groups, and so produce two daughter chambers.

IV. Theoretical.

I do not intend to embark on a complete discussion of the position of the sponges in the animal kingdom. However, I have a few considerations to bring forward in favour of what I consider to be their true relation to the Protozoa on the one hand, and to the Metazoa on the other. My chief reason for not wishing to debate this question at length is the publication, only a year and a half ago, of a very able article by Mr. Minchin. I shall take all he has said for granted, and refer the reader to his article (12). However, I must quote a few expressions from the concluding paragraph of his paper. After giving an account of the views held by different observers, and the arguments for and against such views, he says, "We have two theories to choose between; either to regard sponges as descended from choano-flagellate ancestors independently of the Metazoa, or to regard them as true Metazoa composed of the two primary layers, ectoderm and endoderm, which have become reversed in position in the adults." He then states that the choice "will depend on which of these two assumptions is the most difficult. If sponges are Metazoa, the collar-cell occurring in no other Metazoa must have been independently acquired. If sponges are descended from choano-flagellates, then the sexual reproduction, the segmentation of the ovum, and the formation of two germ layers must be processes analogous and not homologous with the similar

processes in the Metazoa." Mr. Minchin finally concludes that the "theory of the Metazoan nature of sponges offers in the present stage of our knowledge fewer difficulties than the theory of independent descent from Choanoflagellata."

In the course of the few remarks added here I propose to point out the difficulties attending a theory of the Metazoan nature of sponges, and to lay stress on certain points which favour the theory of their independent descent from the Choanoflagellata.

In the above quotation from the last paragraph of Mr. Minchin's article three points are mentioned as the mainstays of the Metazoan theory, namely, (*a*) sexual reproduction; (*b*) segmentation of the ovum; and (*c*) the formation of germ layers.

Those who uphold the Metazoan theory of the nature of sponges have strained every nerve to find some parallelism between the development of sponges and that of the Metazoa, while they have neglected to seek for points of comparison between their development and certain processes which occur in the multiplication or reproduction of the Protozoa, especially in the higher and most differentiated members of the latter sub-kingdom.

We may consider in the first place the arguments derived from sexual reproduction. Nothing more is meant by this term than that two cells, specially developed, unite together to form one cell, which is capable of giving rise to an animal similar to those which produced the original cells, which by their fusion became the cell in question, or fertilised egg-cell.

In the Metazoa the development and maturation of the germ cells are more or less uniform, allowing for certain variations, when the processes are looked at from a theoretical point of view, especially in the mode of reduction of the chromosomes. The only account existing of the maturation of the ovum in sponges is that of Fiedler (3), in which the ovum of Spongilla is described as showing remarkable deviations from the type usually considered as normal for the Metazoa. Perhaps the most important peculiarity is the origin of the polar bodies by a process more akin to direct division than to mitosis.

They are, as it were, budded off, after disappearance of the nuclear membrane, from the central corpuscle of the vesicular nucleus. The importance of such a difference is evident without further discussion. So remarkable is the difference, that we are almost inclined to state that the process witnessed in the preparation of the micronucleus of such Protozoa as Paramœcium presents greater resemblance to what occurs in Metazoa than does that occurring in sponges. In the Protozoon just mentioned the micronucleus divides a number of times before conjugation is brought about, and the interchange of micronuclear substance takes place. Some of the products of division in Paramœcium degenerate and come to nothing, much in the same way as the polar bodies do in the sponges; but the important point is that the micronucleus divides always by mitosis. There is, therefore, greater similarity, as regards the point in question, between what may be termed the maturation of the micronucleus in the Ciliata, and that of the nucleus of the egg-cell in Metazoa, than there is between the maturation of the latter and that of the egg-cell in sponges. It seems, therefore, that the Metazoan theory derives no support from the maturation of the ovum, and the facts appear to cut both ways.

Again, we find among the Protozoa, e. g. some of the Volvocineæ, both male and female cells. The male cell in Volvox and Eudorina is small, active, and motile, being comparable to the spermatozoa of the Metazoa; while the female cell is large, inert, and sought for by the male cell in the same way as is generally the rule among the Metazoa. I do not mean to assert that the fusion of the male and female cells in Volvox is comparable in all its details with what occurs in Metazoa, but the very existence of such a process does away with any difficulty —based on the presence of sexual reproduction in the sponges— of adopting the view of the independent origin of the Porifera from the Protozoa. Since these processes occur in the Protozoa, their transmission during the phylogenetic evolution of the sponges from that group does not after all appear so improbable. In short, the incipient methods of sexual reproduc-

tion found in the Protozoa will serve as a sufficient reason for its full development in the Porifera.

The second argument that will have to be examined is the segmentation of the ovum.

The division of the cell produced by the fusion of a male and female cell is a phenomenon which occurs in both Protozoa and Metazoa. In Eudorina, one of the Volvocineæ, the egg-cell divides regularly into two, four, eight, sixteen, and sometimes thirty-two cells, which lie in a kind of jelly and constitute the colony. In Volvox, again, the fertilised egg-cell divides with a regularity almost unknown among the Metazoa, and develops a colony of numerous individuals. The colony, becoming spherical in shape, might be described as a veritable blastula if it were a young stage of a multicellular animal instead of being an aggregation of unicellular ones, somewhat more closely bound together than Protozoan colonies usually are. Since such regular division of the egg-cell takes place in Protozoa, the strength of the argument based upon the segmentation of the egg in sponges appears to be completely lost. Seeing that such division takes place in Protozoa, we are tempted to ask, what difficulty is there in concluding that it was transmitted along one line of evolution to the Porifera and along another to the Metazoa?

In the third place, the argument based upon the formation of germ layers must be examined. It is perfectly evident that we cannot argue back from the Metazoa and Porifera to the Protozoa when examining the present argument in favour of the Metazoan theory of the nature of sponges, as was done when dealing with the other two arguments. It will be necessary, however, to take some of the Protozoa into consideration even here.

I am kindly informed by Mr. Minchin that the first histogenetic differentiation among the simplest and most primitive of sponges, i. e. the Ascons, is the formation of a ciliated layer on the one hand, and of "posterior granular cells" on the other. In Clathrina the youngest larva consists of a ciliated layer and from one to four posterior granular cells, while in Leucosolenia

there is a group of granular cells placed at the posterior pole in the pseudogastrula stage, and afterwards lodged in the interior of the larva. The granular cells in question are none other than the mother cells of the amœboid wandering cells, i. e. of the cells which later on become, either wholly or in part, the reproductive cells. Clearly, therefore, the first division of labour occurring among the cells of the Ascon larva is the differentiation of locomotor and reproductive cells respectively.

Further, Mr. Minchin believes, and I fully agree with him, that the same differentiation takes place in the Sycons. The few granular cells at the posterior end in the youngest Sycon embryo (pseudogastrula) are the same as those found in the Ascon larvæ, and they are situated, at the time of the invagination of the blastula to form the so-called pseudogastrula, adjacent to the almost obliterated cavity of the blastula,—that is, they are the innermost cells of all. The other non-ciliated cells are developed later from the ciliated layer at the posterior pole, thus producing the characteristic comphiblastula larva, composed of ciliated cells anteriorly, non-ciliated (dermal) cells posteriorly, and a mass of granular cells in the centre. The central cells of the larva probably become the amœboid cells of the adult sponge, i. e. they become the cells which will give rise to the reproductive cells. If this interpretation be true, the first division of labour in the Sycons would be into mother cells of the sexual cells on the one hand, and ciliated cells on the other, the cells which are destined to become the dermal layer developing later from the ciliated cells, in the same way as has been described in Leucosolenia variabilis by Mr. Minchin (8). It is highly probable that the methods of histogenesis which take place in the Ascon and Sycon larvæ are essentially similar. The point I wish to emphasise, however, is that the first cell-differentiation in these larvæ is into ciliated cells on the one hand, and reproductive cells on the other, the latter being represented by the posterior granular cells. Among the Metazoa this does not always occur; the reproductive cells or their immediate precursors appear early in the development only in a few cases. As a rule, epiblast and

hypoblast are formed, and then the reproductive cells become separated from one of these along with the mesoblast where this latter occurs. Again, we may invoke the aid of the Protozoa, and notably of Volvox, a colonial Protozoon, in which a phenomenon occurs which is on a par with what takes place in sponges. In Volvox the reproductive cells become marked out from all the others as male and female cells, but the vegetative cells remain all alike. Clearly the Volvox colony with its reproductive cells is comparable with the larvæ of calcareous sponges at a time when they consist of only a few posterior granular cells and of ciliated cells.

In Volvox no further division of labour takes place among the ciliated or vegetative cells of the colony, but it would not be very difficult to imagine the ciliated individual cells becoming differentiated to two groups in the same way as those of the larvæ of Leucosolenia and Sycandra. I conclude, therefore, that the order of differentiation of the sponge cells from the phylogenetic point of view, as well as from the ontogenetic—in the Ascons at least—is, first, into reproductive and locomotor cells; and secondly, the latter become differentiated into two groups, one without flagella and collars, and another which retains these cell organs.

These considerations seem to cast a doubt upon the whole idea of germ layers in the sponge larva. Given a group of cells such as is found in the sponge embryo at the close of segmentation, two layers, an outer and an inner, are bound to appear sooner or later. Such a thing might easily happen in the case of Volvox, in the same way as it does in the case of the sponge larvæ.

Schulze's (13) great argument against the theory of the independent origin of sponges from Choanoflagellata is that they should have as a necessary consequence the occurrence of choanocytes in the larva. Though I do not agree with Schulze, rather than controvert the statement I am prepared to meet his demand. I venture to point out that such cells do often occur in the larva of Spongilla. They do not occur on the outside, it is true, but in the inner mass in the form of

flagellated chambers; and besides, it has been amply proved that the ciliated cells of the larva are potential collar-cells.

It may be argued, perhaps, that the occurrence of flagellated chambers in the larva of Spongilla is a case of precocious segregation. This term is a highly convenient one, and the principle involved in it is often found to operate in nature; but one cannot help thinking that its assistance is invoked far too often to solve the riddles presented to us by embryology. In the case now under consideration the appearance of choanocytes or collar-cells in the free-swimming larva of Spongilla may be a case of reversion rather than of precocious segregation.

However, the existence of choanocytes or collar-cells in the sponges, as well as their early appearance in the free-swimming larva, together with the ontogenetic method of differentiation found in the Ascons, the most primitive of sponges, inclines me to believe that sponges have been evolved independently from Choanoflagellata. This conclusion is further strengthened by the consideration that collar-cells do not occur in any of the various phyla of the Metazoa.

Conclusion.

As has been stated already, this piece of work has been carried out under the supervision of my teacher, Professor E. R. Lankester, M.A., F.R.S., and Mr. E. A. Minchin, M.A. To the former, whose pupil I have the honour of being, I wish to offer my heartfelt thanks for the free use of his laboratory and all its resources, as well as for many valuable hints and suggestions kindly given, both in connection with the work done and its publication. To the latter I am greatly indebted for his most generous and invaluable assistance, especially in connection with the technique, and am glad of the present opportunity of expressing my thankfulness.

I have also to offer my sincerest thanks to the Principal and Fellows of Jesus College, who not only continued my exhibition after I had completed the ordinary university course, but

increased it to the full value of a scholarship, and thereby enabled me to enter upon a research course of two years' duration.

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EXPLANATION OF PLATES 35—41,

Illustrating Mr. Richard Evans' paper "On the Structure and Metamorphosis of the Larva of Spongilla lacustris.

All the figures have been drawn with the camera lucida, some of them being magnified about 350, the rest 1000 times.

SIGNIFICANCE OF THE LETTERING.

Cavities, Canals, and Membranes.

C. Cavity of the flagellated chamber. *D.* Dermal membrane. *M.* Marginal membrane. *E. C.* Exhalant canal. *I. C.* Inhalant canal. *L. C.* Larval cavity. *S. C.* Subdermal cavity. *fl. ep.* Flat epithelium.

Cells.

c. c. Collar-cell. *c. g. n.* Cell with granular nucleus. *c. v. n.* Cell with a vesicular nucleus. *fl. c.* Flagellated cell. *g. c.* Cells of the cell groups. *pl. a.* Plasmodial aggregation. *sp. c.* Spicule cell, or scleroblast.

Nuclei.

g. n. Granular nucleus. *n. fl. c.* Nucleus of flagellated cells. *n. g. c.* Nucleus of a cell of the cell groups. *n. sp. c.* Nucleus of spicule cell. *n. c. c.* Nucleus of collar-cell. *v. n.* Vesicular nucleus.

Other Structures.

c. Collar. *fl.* Flagellum. *fl. c. c.* Flagellum of a collar-cell. *sp.* Spicule. *e. p.* Apopyle (or exhalant chamber pore). *i. p.* Prosopyle (or inhalant chamber pore). *y. b.* Yolk body. *n. v.* Nutritive vacuole. *b. n. v.* Blackened nutritive vacuole.

PLATE 35.

Fig. 1.—× 1000. Portion of a section of a larva, type A, at the junction of the larval cavity and the solid posterior part of the inner mass (cf. Fig. 9). Below the flagellated cells (*fl. c.*) are seen the cells with granular nuclei (*c. g. n.*) containing a few yolk bodies, and in the interior the cells with vesicular nuclei (*c. v. n.*) full of yolk bodies. The vacuole (*n. v.*) seen in the greater number of these cells is the nutritive vacuole, which blackens with osmic or Hermann's fluid. The yolk bodies (*y. b.*) are deeply stained with bleu de Lyon, and exhibit a clear area in the centre, which in some cases has a red patch in it.

Fig. 1*a*.—× 1000. Portion of a section of the same larva as Fig. 1, showing the layers of cells at the side of the larval cavity. Note the yolk bodies (*y. b.*) in the cells with granular nuclei, and that they are few in number. Note also the absence of cells with vesicular nuclei, as well as of a flattened layer of cells lining the larval cavity (*L. C.*).

Fig. 2.—× 1000. A few cells with vesicular nuclei drawn from a larva which had been preserved in Hermann's fluid. The sections were afterwards partly bleached with chlorine, and owing to this process of treatment the nutritive vacuoles (*b. n. v.*) became decolourised, presenting the same appearance as in the previous figures.

Fig. 3.—A cell with a vesicular nucleus which has divided into two, the cell itself being as yet undivided. Note that it also represents a transitional stage from a cell with a vesicular to one with a granular nucleus. The transitional characters are—a few yolk bodies (*y. b.*), a reduced nutritive vacuole (*n. v.*), and two nuclei possessing very small central corpuscles.

Fig. 4.—× 1000. The yolk bodies as seen in a preparation stained with Bismarck brown followed by malachite green. Note the small refringent structures contained in the yolk bodies (*y. b.*), and also their variety of form.

Fig. 5.—× 1000. Portion of a section of a larva, type B, at the junction of the larval cavity (*l. c.*) with the solid posterior end of the inner mass. In addition to the flagellated cells (*fl. c.*), the cells with granular nuclei (*c. g. n.*) and with vesicular nuclei (*c. v. n.*), there is also a group of small cells with small nuclei (*g. c.*). The cells with granular nuclei are flattened to form a kind of lining to the larval cavity, and their nuclei are beginning to assume an oval form.

Fig. 5*a*.—A portion from the centre of a section from the same larva as Fig. 5. The groups of cells with small nuclei have more or less run together. The yolk bodies in many cases present no change from that represented in Fig. 1; others are greatly reduced in size and present a circular section instead of an oval one, besides showing a certain amount of structure in the interior and a tendency to stain red rather than blue, a state of things due to the older

condition of the larva on the one hand, and the method of preservation with Hermann's fluid on the other.

Fig. 5*b*.—× 1000. Section of the solid posterior end of the inner mass, containing a cell group (*g. c.*) in which the nuclei lie at the periphery, the cytoplasm being incompletely divided. There is also at the top of the cell group a curious structure, perhaps the remains of the old nucleus which gave rise to the smaller nuclei.

Fig. 5 *c*.—× 1000. Section of a cell group from the same larva as Fig. 5. In the centre is seen a structure which apparently represents the nutritive vacuole, surrounded by small nuclei.

Fig. 6.—× 1000. Portion of a section at the side of the larval cavity, type D. The cells are devoid of enclosures of any kind. The cells with granular nuclei have become quite flattened to form a lining to the larval cavity. Their nuclei are slightly compressed, but otherwise do not differ from those of the cells which lie between them and the flagellated cells.

Fig. 6 *a*.—× 1000. Part of a section of the same larva as Fig. 6. Note that the cells with granular nuclei (*c. g. n.*) are far more numerous than they were in the larva represented in Fig. 5 *a*, type B, and that the "cell groups" are far less numerous, being in reality almost a negligible quantity. The yolk bodies (*y. b.*) are also greatly reduced in size and diminished in numbers. All the cells in general as well as their nuclei are far smaller than they are in either type A or type B.

Fig. 7.—× 1000. Portion of a section of type C at the junction of the larval cavity with the solid posterior part of the inner mass. Note the cells with granular nuclei (*c. g. n.*) making their way towards the surface, and the cell groups (*g. c.*) in places arranged in chamber-like rings with developing collars. The yolk bodies are few in number, and stain red owing to the method of preservation. The nutritive vacuoles are intensely black owing to the action of Hermann's fluid during preservation.

Fig. 7 *a*.—× 1000. Two chambers drawn from the same larva as Fig. 7. One chamber (*A.*) has been cut transversely, while the other (*B.*) has been cut longitudinally. The collar-cells have well-developed collars and flagella, and are arranged to form horseshoe-like figures. The large opening towards which the collars and flagella are directed is the apopyle (or exhalant pore). It opens into the beginning of an exhalant canal (*E. C.*), which is lined by a flattened cell with granular nucleus. Note that the collars unite by their margins to form what is usually called Sollas's membrane.

Fig. 8.—× 1000. Surface view of the osculum (*osc.*) and ostia (*ost.*) of a young sponge soon after their formation. The osculum is still on a level with the general surface. The rim is formed of superposed layers of cells with granular nuclei, which form the flat epithelium. Note also the presence of an ostium (?) close to the osculum. It is a small opening measuring about

15 μ across, while the osculum measures from 60 to 70 μ. Compare the figures on Plate 39.

PLATE 36.

Fig. 9.—× 350. Entire section of a larva, type B. Note the flagellated layer (*fl. c.*) covering the whole surface; inside the flagellated layer the cells with granular nuclei (*c. g. n.*) forming a lining to the larval cavity as well as scattered about in the solid part of the inner mass; the cell groups (*g. c.*) deeply embedded, as a rule, in the inner mass; and finally the cells with vesicular nuclei (*c. v. n.*), containing numerous yolk bodies and usually only a single nutritive vacuole (*n. v.*), coloured black in one half but clear in the other half of the figure. The section from which this figure was drawn was bleached with chlorine, which rendered the nutritive vacuoles clear, as represented in the right half of the figure; in the other half they are represented as seen in the section before bleaching.

Figs. 9*a* and 9*b*.—× 1000. Figures drawn from the same larva as Fig. 9, introduced to show the groups of small cells (*g. c.*) with their small nuclei (*n. g. c.*), which are seen in the centre of Fig. 9 *a* as a patch, but in Fig. 9*b* as a ring round the periphery of the undivided cytoplasm. In these two figures the same group of small nuclei is represented in successive sections.

In Fig. 9*a* the group has been cut tangentially, and both the common membrane by which they are surrounded and the faint lines which separate them can be seen. These faint lines represent the first appearance of the cell walls in the multinucleated cytoplasmic mass.

In Fig. 9*b* the group has been cut radially; the nuclei, therefore, appear in the form of a ring round the cytoplasmic mass in which they lie. There are faint dividing lines to be seen passing between the nuclei which stretch across the central space.

Fig. 10,—× 1000. Tangential section of a larva of type D, the fourth section of the series. The nuclei of the flagellated cells (*n. fl. c.*) are cut transversely, and are therefore circular in section. The cells with granular nuclei occupying the centre of the section are branching and irregularly shaped cells, and are far from forming a complete membrane even at this stage. The nuclei of the flagellated cells in some places appear to lie within the cells with granular nuclei, but a radial section proves that this is a delusion; they merely lie close to one another.

Fig. 11.—× 350. Entire section of a larva of type C. The flagellated cells are much the same as they were in Fig. 9. The cells with granular nuclei (*c. g. n.*) are smaller, and are extremely flattened towards the larval cavity. The cell groups are in many cases completely divided and form chamber-like rings, the individual cells of which are often provided with a collar and a

flagellum. The yolk bodies (*y. b.*) are far less numerous, though always present, as will be shown in more highly magnified figures. The nutritive vacuoles are smaller, owing to the fact that the cells with vesicular nuclei which contain them are smaller. This figure was produced in the same way as Fig. 9.

Fig. 11 *a*.—× 1000. Portion of a section at the junction of the larval cavity with the posterior part of the inner mass, from the same larva as Fig. 11. The cell groups, which are very numerous, are arranged in chamber-like rings. The cells with granular nuclei (*c. g. n.*) lying towards the larval cavity are highly flattened, forming a lining to it. The nutritive vacuoles (*b. n. v.*) are blackened by the action of the preserving reagents, and the yolk bodies are few and reduced in size.

Fig. 12.—× 1000. A group of small nuclei such as is characteristic of type B, from a larva which in other respects possesses all the characters of type C. The difference between the small nuclei and the granular nuclei of the cells which surround them is most striking, and their presence in this stage of development in type C points to their production being a process which goes on continuously.

Figs. 13, 13*a*, 13*b*.—× 1000. Sections of a larva of type C to show that the larvæ contain yolk granules (*y. b.*) even in the oldest free-swimming stage. Besides those in the cells with vesicular nuclei, even the cells with granular nuclei and cell groups contain a few of them, a proof that the number of granules in type C is a variable quantity.

In Fig. 13 there is a ring-like group of cells, among which a very interesting cell occurs, showing a stage of the nucleus preparatory to division. The nucleus (*n. g. c.*) has travelled from the base of the cell to the side, and has lost its usual structure, and has an irregular blotched form.

Fig. 14.—× 1000. Section through the side of the cavity of a larva of type C when on the point of becoming fixed; showing the region where the cells with granular nuclei first make their way out, and the flagellated cells immigrate. The structure of the small nuclei (*n. fl. c.*) is in some cases already beginning to change. They become smaller in size, though the actual quantity of chromatin seems to increase. The linin threads thicken, and the chromatin shows a tendency to become aggregated into one or more irregular patches, lying close to the nuclear membrane. Owing to these changes it is already difficult to distinguish some of the small nuclei from the reduced yolk bodies, though they are easily distinguished from those that are not reduced.

Fig. 14 *a*.—× 1000. Two cells from the same larva as Fig. 14. They show how the cells with granular nuclei flatten out to enclose the rudiment of an exhalant canal in the free-swimming larva, and should be specially compared with the cell groups found in the same type of larva.

PLATE 37.

Fig. 15.—× 350. Complete section of a pupa derived from a larva type D, with consequently very few ring-like groups of small cells. Plasmodial aggregations have not yet been formed. It contains cells with vesicular nuclei, in which there is a comparatively large number of yolk bodies (*y. b.*). The flagellated cells have disappeared from the upper surface as well as from the lower, and the cells with granular nuclei have nearly formed a complete layer on both surfaces. The remains of the larval cavity (*L. C.*), not yet obliterated, are seen as small slits near the lower surface, which is a proof that this larva is fixed by the anterior pole. The flagellated cells, especially at the lower surface, present a fan-like arrangement.

Fig. 15 *a*.—× 1000. Portion of the lower surface of a section from the same larva as that drawn in Fig. 15. It shows the fan-like appearance of the immigrating flagellated cells and the cells with granular nuclei (*c.g.n.*) passing out between the groups. The nuclei of the flagellated cells which have adhered to the surface of the cell marked *c.g.n.* have already changed in character. The outline of the cells, however, can be made out. The cells with vesicular nuclei contain several yolk bodies, which, owing to their large size, are easily distinguished from the nuclei of the flagellated cells; the latter are plastered to the surface of the cell (*c.g.n.*). There are also a few cells, about half a dozen in number, which belong to the class described as cell groups (*g.c.*), which in this larva are exceedingly few in number. A portion of the larval cavity still remains (*L. C.*).

Fig. 15 *b*.—× 1000. Portion of the lower surface of a section from the same series as Fig. 15. The larger group of flagellated cells, which have travelled in, is quite exceptional, as is also the fully developed chamber situated close to it. The presence of this chamber, however, shows that the difference between pupæ derived from type D and those derived from type C is only one of degree, the flagellated chambers in the one being much more numerous than in the other. The nucleus of one of the collar-cells is preparing to divide, the chromatin having travelled to the centre of the cell after the nucleus has travelled from the base to the inner end of the cell (cf. Fig. 13, *n.g.c.*).

Fig. 16.—× 350. Complete section of pupa slightly older than that drawn in Fig. 15, derived from type D. There are no free cells in the interior, which is filled with "plasmodial aggregations" (*pl. a.*) in which no cell limit can be detected, though that of the whole group is sharply defined. Spicules (*sp.*) protrude at the upper surface.

Fig. 16 *a*.—× 1000. Portion of the same section as Fig. 16, showing both the upper and lower surfaces. The plasmodial aggregations (*pl. a.*) are seen depicted on a larger scale. They contain, as a rule, a vesicular

nucleus at the centre, together with smaller nuclei and yolk bodies nearer the surface. The yolk bodies are almost indistinguishable from the small nuclei of the flagellated cells during this stage. Note also the large granular nuclei situated on the lower surface, which owing to their large size cannot possibly be derived from the nuclei of the flagellated cells.

Fig. 17.—× 1000. Portion of a section of a pupa slightly more advanced than that represented in Fig. 16, derived from type D. The nuclei of the flagellated cells in the plasmodial aggregations are changing in character; that marked *a* has become larger in size, and the chromatin in it is evenly distributed; in that labelled *b* strands or thick threads appear; while in those marked *d* the ultimate structure of the nuclei of the collar-cells has been practically attained. All these four nuclei are still well within a perfectly definite plasmodial aggregation, proving in a most satisfactory way that the nuclei of the flagellated cells become the nuclei of the collar-cells, since the four nuclei in question are hardly distinguishable from those marked *e*, which are undoubtedly the nuclei of cells which later on become collar-cells.

Figs. 18, 18 *a*, 18 *b*.—× 1000. Section of a pupa which is slightly more advanced than the one represented in Fig. 17. The plasmodial aggregations (*pl. a.*) are losing their sharp outline and are becoming gradually united with one another. The small nuclei of the flagellated cells, which in Figs. 16 and 16 *a* are almost indistinguishable from the yolk bodies, are in this stage easily made out, owing to their loose internal structure, and the thick strands of chromatin which appear in them.

Fig. 18 represents a portion near the margin of the section, and though there are several plasmodial aggregations it does not contain a single vesicular nucleus. In Fig. 18 *a*, which is further in, an occasional vesicular nucleus is seen; and in Fig. 18 *b*, which is drawn from near the middle of the section, there are several. The cells with vesicular nuclei, therefore, are found at the centre of the young sponge, which means that those near the margin have been already transformed into cells with granular nuclei, which very likely become pore-cells.

Figs. 19, 19 *a*.—× 1000. Portions of a section of an individual which is slightly more advanced than that drawn in Fig. 18, derived from type D. The plasmodial aggregations of cells have completely lost their individuality and have become indistinguishable from one another. In Fig. 19 *a* the cytoplasm is arranging itself in the form of a ring, the cavity of which represents that of the future chamber, and the nuclei enclosed in it are those of the future collar-cells. In Fig. 19 large spaces have appeared inside the young individual. The upper one (*S. C.*) represents the subdermal cavity, which has been produced through the pushing out of the dermal membrane (*D.*) by the outgrowing spicules.

PLATE 38.

Fig. 20.—× 1000. Portion of section of a pupa slightly more advanced than that drawn in Figs. 19 and 19 *a*, derived from type D. The small flagellated cells have extricated themselves almost completely from the plasmodial aggregations, and are arranged in irregular rings (*n.fl. c.*) round the cavities of the future chambers. The cell limits are beginning to reappear. The future collar-cells, i. e. the former flagellated cells, are in some cases beginning to develop a collar which protrudes into the chamber cavity (*C.*). The subdermal cavity (*S. C.*) has increased in size considerably, and the cavities in the interior are becoming differentiated into inhalant and exhalant canals. The nucleus marked *v. n.* on the left side of the figure and the four small nuclei (*n.fl. c.*) situated close to it present a most interesting case of the nuclei of the flagellated cells acquiring the characters of collar-cell nuclei, before breaking away from the cell with vesicular nucleus.

Fig. 21.—× 1000. Portion of a section of a still more advanced pupa than that from which Fig. 20 was drawn, also derived from type D. The flagellated chambers are well defined, and the inhalant and exhalant canals are becoming lined with cells possessing granular nuclei, which are gradually flattening out. The cells with vesicular nuclei are situated close to the flagellated chambers in many cases. The collar-cells have almost completely developed collars and flagella, though the latter are difficult to trace.

Fig. 22.—× 1000. Portion of a section of a slightly more advanced pupa than that represented in Fig. 21, derived from type D. The inhalant and exhalant canals (*I. C.* and *E. C.*) communicate with the cavity of the chamber (*C.*) by means of a prosopyle (*i.p.*) and the apopyle (*e.p.*). The collars are fully developed, and unite together by their margins to form the so-called "membrane of Sollas."

PLATE 39.

Fig. 23.—× 1000. Surface view of the marginal membrane of an individual slightly more advanced than that represented in section in Fig. 15. The individual cells of the membrane are quite separate, and all contain granular nuclei. The flagellated cells, which in some cases are carried away by the cells with granular nuclei, are plastered to the surfaces of the latter, and now exhibit a definite outline.

Fig. 23 *a*.—× 1000. A portion of the same preparation slightly nearer the centre than that drawn in Fig. 23. Note how the flagellated cells become attached to both cells with vesicular nuclei and cells with granular nuclei, and that in many cases they are still free.

Figs. 24, 24 *a*, 24 *b*.—× 1000. Surface views of an individual which had

been fixed for approximately the same length of time as that represented in section in Fig. 31. Like the pupa drawn in section in Fig. 31, it had not advanced far from the condition found to be characteristic of the second larval type when first fixed.

Fig. 24 represents an irregular portion of the marginal membrane, while Fig. 24 *a* represents a portion which has already acquired an unbroken outline. In both figures several yolk bodies (*y. b.*) are seen, and it may be that there is here and there an occasional nucleus of a flagellated cell. But nearly all of them are bodies of the same nature as those marked *y. b.* in Fig. 23.

Fig. 24 *b*.—× 350. This figure represents a surface view of a portion of the larva close to the inner limit of the marginal membrane. The nuclei of the flagellated cells and the yolk bodies seen in the plasmodial aggregations are not easy to distinguish from one another (comp. Fig. 31). The small cells derived from the cell groups, which are so characteristic of the larva described as type B, are aggregated together in a loose fashion, and are proceeding to develop into collar-cells.

At the right lower corner the dermal membrane (D) alone, consisting of flattened epithelium, has been drawn, a large subdermal cavity being situated immediately below it. The developing chambers, i. e. the cell groups, are somewhat loosely held together by cells with granular nuclei (*con. cell*). The nuclei of the flat epithelium—not seen in the same focus as those of the cell groups—are drawn over or above the groups of chamber cells in the lower part of the figure, but not in the upper part. The group in the right-hand upper corner of the figure has been left clear without anything being drawn above it (*c. c.*, *g. c.*). The two exceedingly large granular nuclei are possibly nuclei of developing pore cells, which have already lost their vesicular character (*p. c. n.*).

Fig. 25.—× 1000. Portion of a section of a larva similar to that of which a part is drawn in surface view in Fig. 23. The small cells in both figures are the flagellated cells of the larva. The bodies enclosed in the plasmodial aggregations are in some cases yolk bodies (*y. b.*), and in others nuclei of flagellated cells (*n. fl. c.*); but the formation of the plasmodial stage is as yet far from complete. Note that the nuclei of the small cells (*fl. c.*) are much smaller than those of the cells *c. c.* in Figs. 24 *b* and 31. The latter have already attained the structure of the collar-cell nuclei, while the former are only in a stage of preparation for the formation of plasmodial aggregations.

Fig. 26.—× 1000. Portion of a section of an individual slightly more advanced than that drawn in Fig. 25, also derived from type D. This figure should be carefully compared with Figs. 16 and 16 *a*. The plasmodial aggregations (*pl. a.*) are fully formed, and by means of the differentiating stain the nuclei of the flagellated cells are easily distinguished from the yolk bodies, though there is no sign of cell outlines.

Fig. 27.—× 1000. Section of the lower surface of an individual which contains a few loose cells in addition to the plasmodial aggregations, and is derived from type D. The small group of loose cells (*g. c.*) should be specially compared with the similar groups in Fig. 7 *a*.

Fig. 28.—× 1000. Portion of a section of an individual slightly more advanced than those represented in Figs. 24 *b* and 31. It shows two chambers which are exceedingly different in the character of their individual cells. In the upper one all the cells and their nuclei are like one another, while in the lower one they are not so. The nuclei of the cells (*c. c.*, *g. c.*) of the upper chamber resemble those of the chamber cells of Figs. 24 *b* and 31, while those of the cells (*c. c.*, *fl. c.*) of the lower chamber are far more like the nuclei of the chamber cells seen in Figs. 19, 20, and 21. The cells *c. c.*, *g. c.*, have evidently been derived from cell groups, while the cells *c. c.*, *fl. c.*, have been produced from flagellated cells, and have not as yet attained their definitive structure, after passing through a series of changes in connection with the formation of the plasmodial aggregations.

PLATE 40.

Fig. 29.—× 350. Complete section of a pupa from a larva of type C. The flagellated layer has completely disappeared from the lower surface, while it is still complete on the upper surface. The flagellated cells which have travelled in from the lower surface are already in process of forming plasmodial aggregations. The flat epithelium of the lower surface is almost complete, but the cells with granular nuclei still remain inside the flagellated layer on the upper surface. Flagellated chambers (*C.*) are fully formed. The larval cavity is still retained, and will probably become a part of the exhalant system and gastral cavity.

Fig. 29 *a*.—× 1000. A portion of the upper surface of the same larva as Fig. 29. It shows the flagellated layer absolutely complete at the same time as fully formed flagellated chambers. The yolk bodies are rather numerous, and their remains are seen even in the collar-cells. It is quite possible that these small bodies seen in the collar-cells here are the same as the refringent bodies seen in preparations mounted in glycerine, after maceration with osmic and staining with picro-carmine (cf. Figs. 38 and 39).

The flagellated chamber marked *C.* is being constricted to form two chambers. The two chambers situated further in have been produced in the same way, the space between them becoming an exhalant canal.

Fig. 29 *b*.—× 1000. A portion from the lower surface of a section from the same larva as that drawn in Fig. 29. The flagellated epithelium has completely disappeared, and the flat epithelium is well formed. The plasmodial

aggregations (*pl. a.*) are in an advanced stage of development, but the outline of the flagellated cells adhering to their surfaces can be easily made out.

Fig. 29 *c.*—Three fully developed collar-cells from the same larva as Fig. 29. The cell *a* has a small nutritive vacuole (*n. v.*) which points to its recent origin from a cell group, as well as three other small bodies, probably reduced yolk bodies (*y. b.*). The nucleus (*n. c. c.*) is often onion-shaped, and the flagellum which protrudes out of the collar passes down to it, and has usually a small swelling at its base.

Figs. 30, 30 *a.*—× 1000. These two figures represent portions of sections of an individual slightly more advanced than the one represented in Fig. 29, derived from a larva of type C. Fig. 30 *a* is continuous with the upper corner of Fig. 30. The flagellated layer has not yet completely disappeared from the surface, and its cells are becoming widely separated owing to individual immigration, and also owing to the superficial expansion of the pupa as a whole. The cells with granular nuclei (*c. g. n.*) are in some cases at the surface, while in other cases they are seen in the act of passing to it. At the lower surface (Fig. 30) these cells form an almost complete layer. The larval cavity (*L. C.*) is still very large, and is lined by a layer of cells with granular nuclei similar to those at the surface, but smaller and more flattened. The cavity shows no sign of disappearing.

In Fig. 30 the nuclei of the flagellated cells are changing in character previous to the formation of plasmodial aggregations, the portion nearest the margin being full of them.

In Fig. 30 *a* a flagellated chamber derived from the cell groups has been cut almost tangentially (*C.*).

Fig. 31.—× 1000. Portion of a section of an individual fixed for a longer time than that drawn in Fig. 30. The flagellated layer has completely disappeared, and the flat epithelium is well formed. The flagellated cells have entered into the formation of plasmodial aggregations, and are indistinguishable from the yolk bodies. Flagellated chambers are in the process of development from the cell groups, the collars and the flagella not having been produced. This pupa here represented must have fixed during the stage of development described as type B, or else the cells of the cell groups would have developed collars before the fully formed condition of the plasmodial aggregations had been attained.

Fig. 31 *a.*—× 1000. Portion near the margin of a section of the same individual as Fig. 31. It shows how the flat epithelium of the upper and lower surfaces passes into the marginal membrane, and how all kinds of cells make their way into the cavity which exists between the two layers of epithelium close to that membrane.

PLATE 41.

FIGS. 32 *a*—*d*.—× 1000. Four successive sections of the same flagellated chamber, drawn to show the nature of the inhalant canal and pore. The canal which is drawn is an exceedingly short one, and passes straight from the subdermal cavity above into the flagellated chamber below.

a shows how the layer of flat epithelium (*fl. ep.*) forms a depression round the entrance of the canal, the cell below forming, apparently, the actual wall of the canal itself.

b shows the canal (*i. p.*) along its whole length, save a small portion near the surface, where it is covered over by the flat epithelium which lines the subdermal cavity (*S. C.* in *a*). On one side a flattened cell is seen to line the canal, but there is no nucleus anywhere.

c shows the same canal or pore (*i. p.*) opening into the flagellated chamber. It appears to be surrounded by the same cell on all sides, and the cell in question is distinct from the flat epithelium of the subdermal cavity.

d shows no sign of the canal, but a large cell with a vesicular nucleus occupies the position taken up by the canal in figs. *b* and *c*. The cell in question (*c. v. n.*) is the same cell as lines the canal in the other figures, and is perforated by it.

FIG. 33.—A flagellated chamber with a short inhalant canal and inhalant pore (*i. p.*). Apparently both the canal and pore are lined by the same cell, the nucleus of which is not seen.

FIG. 34.—× 1000. The same inhalant canal (*I. C.*) and pore (*i. p.*) in the section succeeding the one drawn in Fig. 33. In this section the nucleus of the pore cell is seen, and is evidently a vesicular nucleus in the process of transformation to the granular condition.

FIG. 35.—This figure represents a section of a flagellated chamber, and shows two things:

(1) A collar-cell which has withdrawn its collar and flagellum, and contains a nucleus in the spindle form, showing clearly that the cells of the flagellated chambers divide by karyokinesis. Note that the longitudinal axis of the spindle lies in a plane tangential to the wall of the flagellated chamber. The cell must therefore divide longitudinally.

(2) A transverse section of a spicule (*sp.*) lying in the scleroblast, a cell with a vesicular nucleus (*v. n.*) against which the spicule presses giving it a crescentic appearance.

FIGS. 36 *a*—*d*.—× 1000. Four stages of growth of the megascleres. The spicule in each case is completely enclosed by the scleroblast. In Fig. 36 *a* the nucleus is vesicular as well as in 36 *b*; but in Fig. 36 *c* it begins to lose its vesicular character, and tends to become granular. In

Fig. 36 *c* this stage has been reached, the nucleus being similar to that of any flat epithelial cell.

Figs. 37 *a*, *b*.—× 1000. Microscleres in their epithelial scleroblasts, completely enclosed by the cells which secrete them.

Fig. 37 *a* represents spicules from a larva belonging to type C, preserved immediately after fixation, i. e. while the flagellated layer was still complete at the upper surface.

Fig. 37 *b* represents two cells of the flat epithelium, one of which contains a microsclere, drawn from a somewhat advanced young sponge.

Figs. 38, 39.—× 1000. These figures represent the cellular elements of a larva which was preserved in osmic acid, stained in picro-carmine, and mounted in glycerine. Owing to the maceration that had taken place during fixation and staining it fell immediately into pieces, and the cells separated.

Figs. 38 *a*—*d* represent the flagellated cells, and show small granules which were not seen by the aid of any other method of preparation.

Fig. 39 shows a portion of the flagellated layer from within, and the granules are seen in all the cells.

Figs. 38 *a*, *b* show the difference in length which obtains between the flagellated cells, *c* the flagellum passing down to the nucleus, while in *d* it could not be traced any further than a small and irregularly shaped granule situated about halfway down to the nucleus.

The cells *e* and *f* are extremely irregular in shape, while the outline of the cell *g* is much more definite. The cells *e* and *f* contain far less granules than the cell *g*, a fact which may have some relation to their shape, the cell *g* being rendered more inert than the others by the granules.

The cells *f* and *g*, which are provided with a vesicular nucleus, contain a large nutritive vacuole, blackened by the osmic acid. The cell *g* contains several large yolk bodies (*y. b.*), while the cell *e* has only one.

All these cells contain small refringent granules, probably reduced yolk bodies, though they are not shown in the flagellated cells of the larva by any other method of preservation. Compare Fig. 29 *a*.

Figs. 40 *a*—*g'*.—× 1000. Development of the cell groups characteristic of the larva of type B. For description see Appendix B, on pp. 425—430.

Fig. 41 *a*.—× 1000. Figure showing the difference of structure between the vesicular nuclei; one, with a few but large granules, is in the same state as the cell *a* of Fig. 40; the other represents the structure of an ordinary vesicular nucleus.

Fig. 41 *b*.—× 1000. Two cells which, to judge from the number of yolk bodies contained in them, originally had vesicular nuclei. The nucleus, however, has become fragmented, and is represented by a number of small granules situated in the centre of the cells. The condition of the nucleus

in these cells probably represents a stage between the cells *b* and *c* of Fig. 40.

FIGS. 42, 1 *a*—10 *a*.—× 1000. Various stages of mitotic division found in the cells of the free-swimming larva and of the young sponge. See Appendix E, pp. 434, 435.

On the Communication between the Cœlom and the Vascular System in the Leech, Hirudo medicinalis.

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With Plates 42—44.

Contents.

The investigation, of which an account is given in the following pages, was undertaken with the object of ascertaining for certain whether, in the medicinal leech, the cavities of the so-called sinus system do, or do not, communicate with those of the contractile vascular system.

This question, which at first sight seems so simple, has for many years given rise to much controversy, some authors believing the communication to exist, others, on the contrary, holding the view that the two systems of cavities are quite distinct. Since the champions of neither theory have brought forward conclusive evidence in support of the view they uphold, the question remains to this day unanswered.

Several reasons have tended lately to arouse renewed interest in the relations of the vascular system of the leech, and to

add fresh importance to the controversy. For, since it has become gradually established that, in the Invertebrata in general, the cœlom is quite distinct from the vascular system, that even in the Molluscs and Arthropods the two systems of cavities are of quite separate origin, it is clear that a communication between the two would be a very exceptional phenomenon. Now, Bürger has shown by his embryological researches (2) that at all events the ventral sinus, the perinephrostomial sinuses, and the branches immediately deriving from them are of cœlomic nature and origin in Hirudo, just as they have been shown to be in the Rhynchobdellid leeches; and, moreover, it has recently been contended by Oka (10) and Johansson (6) that in the adult Rhynchobdellidæ the vascular system remains distinct and closed off from the cœlomic cavities. This interpretation is now supported by the evidence derived from the structure of the interesting leech Acanthobdella, in which Kowalevsky describes a closed vascular system filled with red blood, distinct from the spacious cœlomic cavity (7).

All these facts are so unfavourable to the view that in the Gnathobdellid leeches, alone amongst the Invertebrata, the cœlom is in open communication with the blood-vascular system, that many authors refuse, and I think justly refuse, to admit its truth without further and more definite proof. For instance, Mr. Sedgwick (11), in the excellent 'Text-book of Zoology' which he is now publishing, says "it is still generally held that they [the vascular and the sinus systems] are continuous through their finer branches. This view is, as we shall see, based on insufficient evidence; and having regard to the statements of Oka and Bürger, it seems safe to assert as a fact that the two systems are separate" (p. 517); and after further arguments against their communication he adds (p. 519), "A continuity between the vascular system and the undoubted cœlom of the sinuses would be a unique phenomenon in the structure of the animal kingdom." [1]

It was on reading these passages that I finally determined

[1] Apparently adopting the view that in the Vertebrates the lymphatic system is not in real continuity with the cœlom.

to try and solve the question; and although, at first, I was strongly in favour of the view held by Sedgwick, the unmistakable evidence of the facts has forced me finally to adopt the older interpretation. The result of these researches is to prove without doubt that the continuity exists.[1]

It was Leydig who first showed that in the leeches there are two systems of vessels, a contractile and a non-contractile. De Quatrefages and Leuckart compared the former with the vascular system, the latter with the body-cavity of the Chætopods. In a classical paper on the structure of leeches, Professor A. G. Bourne carefully traced out the relations of the main cavities of the two systems throughout the Hirudinea (1). He concluded that in all leeches a continuity exists between the two sets of cavities. Further, that in the Rhynchobdellidæ there are four main longitudinal blood-vessels, lying in four longitudinal sinuses of cœlomic nature;[2] the nephrostomes lie in cœlomic spaces, more or less cut off from the ventral sinus containing the nerve-cord. In the Gnathobdellidæ, Bourne believed that "(1) all trace of lateral sinus and of the dilatations connected with it has vanished; (2) all trace of the dorsal and ventral vessel has vanished; (3) the lateral vessels with their connections and the dorsal and ventral sinus system are placed in communication only through a new development, viz. botryoidal tissue."

The blood system of the leeches has been studied by a large number of investigators ever since the time of Cuvier. We need not give a detailed historical review of the work of the early authors, of which an excellent account has already been given by Moquin Tandon (9) and Gratiolet (4); it is sufficient to point out that in 1862 Gratiolet (4) gave an admirable description of the vascular system of Hirudo, to which little has

[1] The fluid contained in the channels is, therefore, a hæmolymph in the true sense of the word, and the combined contractile and non-contractile systems may justly be called the hæmolymph system.

[2] The distinction of these spaces by the terms "vessel" and "sinus" is not a very convenient one, but it is difficult to find a better. The word channel may be used as an indifferent term. There are some channels, the vascular or cœlomic nature of which cannot at present be determined.

been added since.[1] What concerns us at present is the evidence of continuity between the contractile and non-contractile systems in Hirudo.

The earlier authors all believed in the continuity, and based their conclusions mainly, if not entirely, on injection. Jaquet (5), who has recently re-investigated the blood system of Hirudo by means of injections, confirms this view, describing small branches communicating from the longitudinal vessels to the longitudinal sinuses in front, and also from the lateral vessel to the perinephrostomial sinus. But the evidence brought forward by these authors is not clear and convincing, and, in fact, they do not treat the question as one requiring definite proof.

A. G. Bourne, who also studied sections, agrees with Gratiolet in almost every particular, and believes that the two systems communicate by means of their finer branches ending in capillary networks. But here again we miss the necessary convincing evidence of the continuity.

Since considerable doubt arose as to the correctness of the generally accepted view, Mr. A. E. Shipley in 1888 re-investigated the subject with the help of sections, and wrote a short paper without figures in which he says (12), "A fragment of the brown tissue of a leech shows at once the connection of the lumen of the botryoidal tissue with that of the thin-walled vessels. And my sections through Clepsine and Hirudo show in numerous places the large openings by means of which the botryoidal tissue is put into communication with the sinuses."[2]

[1] In this work Gratiolet first showed the continuity of the botryoidal channels with the vascular system (sinus or vessel), though the botryoidal tissue was first correctly described and so named by Lankester, and its continuity with capillaries and their mode of growth in connection with it figured (8).

[2] A statement which it is difficult to reconcile with that found farther on, that "certain large corpuscles which occur in the sinuses of Clepsine and Pontobdella are not found in the blood-vessels, being . . . too large to pass through the communicating channels." As far as Clepsine is concerned, Oka's results are, of course, directly opposed to those of Bourne and Shipley.

The evidence brought forward in this paper is derived from two sources: firstly, from some injection experiments; secondly, from the study of serial sections. The former researches were carried on last summer, with the help of my friend Mr. L. J. Picton; the part dealing with injections must, therefore, be considered as the joint work of Mr. Picton and myself. For the remainder of this paper I am alone responsible.

The Evidence of Injections.

Two reasons may be assigned why the work of Gratiolet, Jaquet, and others has not carried conviction. These naturalists, who, as I have already remarked, aimed rather at tracing out the distribution of the hæmolymph channels than settling the question of the communication between sinus and vessel, do not seem to have taken any special precautions against the forcing of the injected fluid through any thin walls which might be supposed to separate the two systems, nor do they appear to have been careful as to the state of preservation of the leeches they injected. Gratiolet, indeed, frequently macerated his leeches before injecting them, finding the process easier to accomplish when the animals had begun to decay.

For these reasons we determined to avoid, as far as possible, all such sources of error.

The leeches, before injection, were not killed, but anæsthetised with a mixture of chloroform, ether, and alcohol; then spread out on a cork, and opened up so as to expose the lateral vessel, by means of which the injection is accomplished. When necessary, the leech was kept in the anæsthetised state for any length of time by placing on its head a pad of cotton wool dipped in the anæsthetic.

A filtered solution of Berlin blue was used for injecting, and the apparatus consisted of a very fine glass cannula fixed to a long india-rubber tube leading to a small funnel, into which the solution was poured. The pressure of the fluid and the flow from the nozzle of the cannula could be regulated at will by fixing the funnel at any given height. The pressure used was

so slight, and the tip of the cannula so fine, that the blue fluid only came out drop by drop from its extremity.

When the cannula is introduced into the lateral vessel, the blue fluid mixes with the hæmolymph and spreads throughout the system of channels, apparently often as much by the natural contractions of the vessels as by the pressure exerted from the cannula.

A large number of such injections were made both in Hirudo medicinalis and Aulostoma gulo, the operation lasting from thirty minutes to twenty-four hours. The leeches were then killed, hardened, and cut.

We found that the injection from the lateral vessel of one side passed easily into the lateral vessel of the opposite side. It also very soon reached the dorsal and ventral, and the peri-nephrostomial sinuses, and the capillary networks of the body-wall. On the other hand, it seemed to penetrate into the botryoidal channels only with some difficulty, and in the last place.

Transverse sections show all these spaces filled with hæmolymph tinged with the Berlin blue. The injection seems to have flowed in quite natural channels, and shows no signs of having been forced into spaces not belonging to the contractile and non-contractile systems, or through thin walls of separation.

In fact, we fully convinced ourselves that, both in Aulostoma and in Hirudo, blood-vessels, sinuses, and botryoidal tissue are in free communication. At the same time we realised that the evidence of injections alone can never be placed entirely beyond criticism, and that some other method would have to be adopted to convince the sceptical, and remove all possibility of doubt.

The Evidence of Sections.

A careful reconstruction of a series of sections seemed to me the only way of obtaining the end in view. The method I adopted was as follows. Having anæsthetised the leeches,

they were preserved in a mixture of one volume of 4 per cent. formaldehyde solution, and one volume of a saturated solution of corrosive sublimate containing 5 per cent. glacial acetic acid. This fixative gives excellent results for section cutting. The sections were stained on the slide in a mixture of methyl blue and eosin, according to a method suggested to me by Dr. A. Mann which has proved most useful. When successful, the combination stains the tissues blue or purple, and the hæmoglobinous coagulum, the hæmolymph, brilliant scarlet. This striking contrast enables one to follow out the minutest capillary with comparative ease.

The work of reconstruction had then to be undertaken. For the larger vessels and sinuses (figs. 1, 2, 3, &c.) I made use of a series of 600 transverse sections, 10 μ thick, from the middle region of the body. Camera drawings ($\times$ 25) were made of the first and every tenth section, and these were plotted out on paper ruled to scale. For this purpose arbitrary fixed lines had to be adopted; a vertical line was, therefore, drawn in each case through the nerve-cord and dorsal sinus, and another horizontal line at right angles to this through the nerve-cord. The measurements were then taken from these lines. In this way a certain element of arbitrariness is no doubt introduced into the reconstruction of the curves of the vessels; but this is only very slight, and really of no importance, since, of course, it does not in any way alter the true relations and communications of the spaces. These were always verified by examining the intermediate sections.

In reconstructing the systems of smaller vessels, sinuses, and capillaries, the difficulties were much greater. Here no fixed points were available to measure from, no practicable arbitrary lines could be drawn, owing to the extremely complicated character of the network visible only with a comparatively high power, the field of which includes but a small portion of the section.[1]

[1] Even had a fixed point been available, no purely mechanical process of reconstruction, such as the wax-plate method, could be trusted, since the capillaries are so numerous and so near to each other that it would be scarcely

Camera drawings on a large scale were, therefore, made; then combined by means of transparent tracing paper, the communications of the capillaries being verified in every case with the greatest care under the high power.

Before describing these important capillary systems, in which the communication really takes place, it is necessary to give an account of the large vessels and sinuses, in order to show that amongst these there is no continuity of the two systems.

The Lateral Vessels and their Branches.—A large longitudinal vessel, with muscular contractile walls, extends in a sinuous course along each side of the body. This is the well-known lateral vessel, and is said to communicate in front and behind with the corresponding vessel of the opposite side. In every segment the lateral vessel gives off a pair of large dorsal branches (figs. 1, 2, 6). The first of these is the short latero-lateral vessel of Gratiolet, passing almost vertically upwards to break up into smaller branches, and lead to the superficial cutaneous capillary network on the dorsal and lateral regions of the body.

The second is the more important latero-dorsal vessel of Dugès. This vessel soon divides into two large branches, the anterior of which passes over to the opposite side to join its fellow above the dorsal sinus, and the other, the posterior, runs towards the median line, but does not communicate with the vessels of the opposite side. Both these branches give off smaller vessels running outwards to the superficial capillary networks.

The anterior branch of the latero-dorsal vessel also gives off vessels passing downwards to the wall of the gut.

The entrance of the large, and contractile, latero-lateral and latero-dorsal vessels into the lateral longitudinal vessel is

possible to avoid all sorts of errors on superimposing the camera drawings. The slightest inaccuracy in such drawings—due, for instance, to the somewhat different position of the eye in making them, or perhaps to a slight shrinking or expansion of the section itself—would be sufficient to vitiate the whole result by leading any given capillary to wrongly fit on to any of its nearest neighbours.

constricted, being provided with sphincter muscles (fig. 16), and on its inner side, within the lateral vessel, is a valvular arrangement composed of a mass of long-stalked cells situated round the aperture. Although it is somewhat difficult to judge certainly as to the action of these valves, yet I think there can be little doubt that they prevent the hæmolymph from returning into the dorsal branches when the lateral vessel contracts. For the bunches of cells would block up the narrow opening if the fluid tended to return into these branches, as in the case of similar valves in other annelids. On the other hand, on the hæmolymph flowing into the lateral vessel, the valvular cells would merely hang freely in its wide lumen.

The only other branches coming from the lateral vessels are the latero-abdominal vessels of Dugès, arising about midway between the dorsal branches (figs. 2, 3, and 5). They bend downwards and bifurcate, each branch joining its fellow from the opposite side below the ventral sinus. A lozenge-shaped figure is thus formed by the right and left branches, generally more regular than in fig. 3. The lozenges are joined together by short median vessels.

The latero-abdominal vessels give off branches supplying the nephridia, and the capillary cutaneous plexus of the ventral and ventro-lateral regions.

There are no valves round the aperture of the latero-abdominal into the lateral vessel, and the entrance is not much constricted.

This description of the main trunks of the contractile vascular system agrees in all essential points with that of Gratiolet (4), excepting for the valves described above, which apparently were missed by previous observers.

Gratiolet would seem to have been mistaken in thinking that the anterior branches of the latero-dorsal vessels joined across to form a complete arch above the dorsal sinus only in the region of the intestine; this occurs also in the region of the sacculated crop,[1] and here, as elsewhere, these arches give

[1] It is possible that individual leeches vary in this respect, since Jaquet (5) also states that there is no union from side to side in the anterior region.

off on either side a branch passing downwards to spread over the wall of the alimentary canal.

The Dorsal Sinus.—Running along close to the wall of the alimentary canal (figs. 1, 2, 4, and 6) the dorsal sinus gives off a pair of branches in each segment. One of these loops underneath the anterior branch of the latero-dorsal vessel in a peculiar manner (figs. 1 and 6). Both ultimately break up into small capillaries passing into the cutaneous plexus of the dorsal and dorso-lateral regions.

Small sinuses also run ventrally from the dorsal sinus to the wall of the alimentary canal, both to the crop and intestine, although Gratiolet only found those supplying the latter (figs. 6 and 15).

According to previous observers, the dorsal sinus communicates with the ventral sinus in front and behind.

The Ventral Sinus.—This large sinus, which contains the nerve-cord, gives off two pairs of lateral branches in each segment. The most important of these are the short canals leading on either side into the perinephrostomial sinus, entering at its anterior end. From the opposite extremity of the sinus a branch is given off to the nephridium (figs. 2, 3, and 5).

In the region of the nerve ganglion a sinus branches out, following the posterior lateral nerve for some distance (figs. 3 and 4). This sinus divides into two branches, one going to the body-wall, in the ventral region, and the other passing vertically upwards to the dorsal cutaneous plexus (fig. 4). The latter is the abdomino-dorsal of Dugès.

According to Gratiolet, a similar sinus passes up from the perinephrostomial sinus; but I have not been able to find it.

A pair of vertical channels in each segment extend from the ventral cutaneous plexus to the dorsal network.

The chief point to notice about the systems of larger vessels and sinuses is that the two do not communicate with each other. It is only by means of the complex capillary systems that the continuity is established.

The Capillary Systems.—Gratiolet (4) divided the capillary systems into three sections: (1) an inner deep layer, the

vessels of the botryoidal tissue arising from branches of the lateral vessels; (2) an intermediate layer, being the capillaries winding amongst the muscles, derived from the same vessels, and communicating with the botryoidal vessels; (3) the superficial cutaneous layer, divided into a right and left plexus, communicating with capillaries arising from the intermediate layer, and also supplied by fine branches from the sinuses. This last section, the cutaneous plexus, has been shown by Professor Lankester to extend into the epidermis itself (8).

The results of my observations agree fairly closely with Gratiolet's description, the chief differences being with regard to the supply of the capillary systems.

It has already been mentioned that the latero-lateral and the latero-dorsal vessels give off small branches passing radially outwards to the skin. So far as I have seen, these radial vessels have no direct communication either with the botryoidal tissue, or with the intermediate layer of capillaries amongst the muscles, but pass right through these to near the epidermis (fig. 7). Here they branch, forming what I shall call annular vessels,[1] running round a little below the epidermis, on the one hand towards the median dorsal line, and on the other towards the ventral surface (figs. 4 and 7).

At short intervals the annular vessels give off small branches to the delicate epidermal plexus. Although the annular vessels of the right side never directly join those of the left, two such vessels from different branches of the latero-dorsal may run into each other, forming a complete loop (fig. 7).

At the sides these vessels may pass round, describing more or less complete semicircles, and reaching sometimes to the ventral surface. Exceptionally, as shown in fig. 4, they may turn inwards again so as to open into the ventral botryoidal channels.

Now the superficial epidermal plexus, into which the small branches of the annular vessels open, is

[1] These annular vessels may be the small vessels termed "branches verticales superficielles" by Gratiolet, and described as branching at both ends into the plexus.

directly continuous with capillaries coming from the intermediate intermuscular plexus, and this network amongst the muscles passes on its inner side into larger channels, which are, in fact, offshoots from the lateral branches of the dorsal sinus (figs. 7—11).

The epidermal network may, therefore, be considered as the region where the sinus system opens into the contractile vascular system. In figs. 8—11 three consecutive sections and a reconstruction of the same are drawn, showing this connection in a small portion of the region represented in fig. 7. A similar continuity can, of course, be found throughout the other parts.

There can be no doubt whatever that the dorsal sinus communicates with the lateral vessels by means of small branches given off by each system to the superficial plexus. Indeed, the distribution of the radial capillaries going to the skin being what it is (figs. 7 and 12), it is evident that such a connection must exist; the blood brought by one set of capillaries must necessarily be carried off by the other. Once the disposition of these small channels was ascertained, their continuity was a foregone conclusion. But, of course, it is satisfactory, and necessary for the sake of dispelling all doubt, to have the actual evidence of sections before us.

The communication of the contractile system with the ventral sinus must now be established.

A reconstruction of a portion of the capillary system of the ventral region is given in fig. 12. It will be seen at once that, although the actual disposition of the capillaries is quite similar to that described in the dorsal region, yet the conditions are reversed. For here the annular channel comes directly from the ventral sinus (cp. figs. 7 and 12), and the radial capillaries derive from the contractile system, being branches of the latero-abdominal vessel.

A small portion of the region represented in fig. 12 has been reconstructed, so as to be shown on a larger scale in fig. 13. Here the continuity between the two systems is again proved.

The annular channels of the dorsal region always derive from the contractile system. Those of the ventral region generally belong to the sinus system. The two sets interdigitate, an annular sinus passing upwards between two annular vessels, and they only communicate by means of fine capillaries of the epidermal plexus. There are a considerable number of annular channels to each segment.

It has already been mentioned that a dorsal annular vessel may reach round to the ventral region ; this happens in the vicinity of the nephridiopore, and is shown in fig. 14. In such cases the annular vessel comes into communication with capillaries from the latero-abdominal vessel, which then bear just the same relation to it as the capillaries from the dorsal sinus in the upper region.

These ventral annular vessels of dorsal origin bend inwards towards the ventral sinus, and pass upwards by the side of the gut to the dorsal region, where they break up into capillaries to be distributed to the botryoidal tissue and dorsal epidermal plexus. They form, in fact, the vertical channels already mentioned.

There remain to be described the relations of the botryoidal tissue. The channels are lined within with the well-known yellowish-brown cells, the outer region of which is filled with coarse pigmented granules, and the inner deeply-staining half formed of comparatively clear protoplasm.[1]

The botryoidal vessels lie chiefly in a dorsal and ventral mass on each side, between the alimentary canal and the muscles of the body-wall in the general parenchyma. On the outer side the botryoidal vessels communicate with the intermediate capillary plexus of sinus origin (figs. 7 and 8). Occasionally in the ventral region (and perhaps elsewhere) they open into capillaries of the contractile system (fig. 14). On

[1] It is, apparently, this deeply staining region of the cells which has been mistaken by Graf (3) for an inner coat in Nephelis. There can be no doubt that in Hirudo, Aulostoma, and Nephelis there is no such inner lining, and that the previous observers were quite correct in describing the brown cells as bathed by the hæmolymph.

the inner side of the botryoidal tissue its vessels open here and there into the small sinuses passing outwards to the intermediate layer.

In the ventral region the botryoidal channels also open directly into the perinephrostomial sinus (fig. 17).

I must finally describe the communications between the contractile and non-contractile systems which occur in connection with the vascular supply of the alimentary canal.[1]

Gratiolet gave a correct description of the vessels of the intestine. They are derived from short vertical branches coming off from the latero-dorsal arches, and passing into a longitudinal lateral vessel on each side, which is itself connected with a median ventral channel. From these lateral intestinal vessels a fine plexus of capillaries extends over the wall of the alimentary canal; joining again into larger trunks, the capillaries open at intervals into the dorsal sinus (such a connection is shown in the reconstruction given in fig. 15).

It may be added that a similar communication exists on the wall of the crop, but the capillary plexus is there less elaborate (fig. 6).

I have also noticed a peculiar connection between the latero-abdominal vessel and the abdomino-dorsal sinus by means of a tortuous capillary shown in fig. 4 at C. This may be exceptional.

Summary and Conclusion.

According to the foregoing account, the evidence of carefully executed injections strongly favours the view that a continuity exists between the contractile vascular system and the non-contractile sinus system in Hirudo. This continuity is proved to exist in various regions of the body by means of serial sections. The communication takes place through the capillary systems.

The hæmolymph system of Hirudo consists of four main longitudinal trunks, sending out transverse branches to the

[1] I have no doubt a similar continuity exists on the walls of the nephridium, as mentioned by Gratiolet.

body-wall. The dorsal branches of the lateral vessels pass into small annular vessels communicating with the plexus of minute capillaries in the epidermis. From these, again, arise capillaries going to small sinuses which run into the lateral transverse sinuses, and so into the dorsal sinus.

Similarly the ventral sinus sends annular sinuses along the ventral region of the body-wall opening into the epidermal plexus, whence arise capillaries joining the latero-abdominal vessels.

Continuity between the two systems has also been shown to take place by means of capillaries on the wall of the alimentary canal, and probably exists on the other internal organs of the body.

Two questions still remain to be solved: firstly, as to the circulation of the hæmolymph; secondly, as to the exact homology of the channels in which it flows.

With respect to the first of these problems, I have no direct observations to record; but it may be pointed out that the presence of the valves described above show, at least, that the hæmolymph must flow in a constant direction—that there is a real circulation, not a mere motion backwards and forwards. It seems to me extremely probable that the annular vessels collect the oxygenated blood from the epidermal plexus, and carry it into the latero-dorsal and latero-lateral vessels, whence it would be pumped into the lateral vessels. From these some of the hæmolymph must be carried by the latero-abdominal vessels to the various organs of the body, and to the ventral cutaneous plexus. The annular sinuses would collect it from this plexus and carry it into the ventral sinus. The abdomino-dorsals and the dorsal sinus would appear to supply the dorsal and lateral cutaneous plexus.

We are left in considerable uncertainty as to the true nature of some of the spaces. That the lateral vessels belong to the real vascular system, and that the ventral sinus and perine-phrostomial sinuses belong to the true cœlomic system, seems to be clearly established both by comparative anatomy and by the embryological researches of Bürger (2). This observer,

however, could not trace the dorsal sinus to a cœlomic origin, and since its branches bear the same relation to the cutaneous plexus as those of the latero-abdominal vessels, I am inclined to think that the dorsal sinus may represent the dorsal vessel of other annelids. In that case the cœlomic cavities do not persist dorsally, or have never reached the median dorsal region in the Gnathobdellidæ.

The annular channels may possibly represent the annular cœlomic lacunæ so well described and figured by Oka in Clepsine (10), and it may perhaps be through them that the chief communication between the cœlom and the vascular system has been established. The observation of the somewhat variable relations of these annular channels tends to support this view.

With the very imperfect knowledge of the development of the cœlom and blood-vessels in Hirudo at our disposal, we cannot say for certain at present where the one ends and the other begins, nor whether a given capillary really belongs to the one or the other. Nor can we safely conjecture how the continuity has actually taken place. But one thing seems fairly certain, namely, that it is not only by means of the botryoidal channels that the communication has been brought about.

It is very tempting to compare the leech with the Vertebrate, in which a third system of spaces—the lymphatic system—has been interpolated, allowing a communication to take place between the originally distinct cœlom and blood-vascular system.[1] But the botryoidal tissue is not so inter-

[1] The structural analogy between the lymphatic system of the Vertebrate and the botryoidal tissue of Hirudo is in some respects very close. The former develops as an independent set of cavities in the mesoblast, which subsequently open into the veins and the cœlom (Balfour, 'Comparative Embryology'). The latter, according to Bürger (2), develops also as a number of independent channels, hollowed out in strings of cells of mesoblastic and even peritoneal origin, which later come into connection with the hæmolymph system. The functions of the lymphatic and botryoidal systems must be quite different, since the latter is not in any way specially related to the alimentary canal.

polated in the case of Hirudo; if it were obliterated, the two systems would still be in free continuity by means of capillaries. The botryoidal channels would seem to be rather of the nature of a by-path, through which the hæmolymph does not necessarily circulate. In this connection it should be mentioned that in sections they are rarely seen to be as much distended with the fluid as the neighbouring capillaries of similar size.

Whatever may be the process whereby the continuity between the cœlom and vascular system has been established in the Gnathobdellidæ, there can be little doubt that it is a secondary condition, and that the structure of such a form as Acanthobdella, in which a closed blood-system lies in a normally developed cœlom, is really the more primitive.

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EXPLANATION OF PLATES 42—44,

Illustrating Mr. Edwin S. Goodrich's paper "On the Communication between the Cœlom and the Vascular System in the Leech, Hirudo medicinalis."

All the figures are of Hirudo medicinalis. The contractile vessels and their branches are coloured pink, the sinuses and their branches are coloured blue.

Figs. 1, 2, and 3.—Reconstructions, from a series of 600 sections, of the main trunks of the contractile and sinus systems. Figure 1 shows a dorsal view of the lateral vessels and dorsal sinus. Figure 2 shows a left-side view of the lateral vessels, dorsal sinus (without its branches), ventral sinus, and perinephrostomial sinus. Figure 3 shows a more complete view from above of the ventral sinus and its branches, with the nerve-cord visible by transparency, the lateral vessels with their latero-abdominal branches.

Fig. 4.—Reconstruction as seen in transverse section of sections 50—150, front view. A capillary, C, is seen to join the right latero-abdominal vessel with the abdomino-dorsal sinus.

Fig. 5.—Reconstruction as seen in transverse section of sections 213—360, front view. The position of the gonads is indicated by a dotted line.

Fig. 6.—Portion of section 340 partially reconstructed backwards, showing the connection, at C, between a small sinus and a branch of the latero-dorsal vessel. Dotted lines indicate the general course of the latero-dorsal vessel, which is several times cut through.

Fig. 7.—Reconstruction showing the communication between a branch of the latero-dorsal vessel, and a branch of the dorsal sinus; also capillaries opening from the intermediate plexus into the botryoidal tissue. From another series.

Figs. 8, 9, 10, and 11.—Camera drawings of three consecutive sections, and a reconstruction of the same, showing the continuity between the two

systems in a small portion of the epidermal network included in Fig. 7 between two ×.

Fig. 12.—Reconstruction showing the communication between a branch of the ventral sinus and the latero-abdominal vessel.

Fig. 13.—Reconstruction on a larger scale of a small portion of the superficial plexus included in Fig. 12 between two ×. The dotted line represents the basal limit of the epidermis.

Fig. 14.—Reconstruction of a portion of the ventral capillary plexus in the neighbourhood of the nephridiopore, showing the connection (not followed out in detail) between the annular vessel of dorsal origin and capillaries derived from the latero-abdominal vessel; also the communication of the botryoidal channels with the intermediate plexus.

Fig. 15.—Reconstruction showing the communication between the two systems on the wall of the intestine, the position of which is represented by a dotted line.

Fig. 16.—Section showing the opening of the latero-dorsal into the right lateral vessel (445th section of the first series).

Fig. 17.—Section showing a botryoidal channel opening into the perinephrostomial chamber.

Fig. 18.—Section showing the opening of a small sinus into the botryoidal channel (one of those represented in Fig. 7).

Balanoglossus otagoensis, n. sp.

By

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With Plate 45.

Hitherto no example of the Hemichorda has been recorded from New Zealand; and, indeed, it is only quite recently that any member of the group has been demonstrated to occur on the shores of Australasia. In 1893, Mr. T. P. Hill,[1] of Sydney University, recorded the capture of a specimen of Ptychodera Australiensis, of which he gave a detailed account in 1895. But the genus Balanoglossus has hitherto not been discovered in the Southern Hemisphere (fide Spengel, 1893), hence the species about to be described gains in interest.

On February 16th of the present year, Mr. Hamilton, the Registrar of the University, accompanied me on a short collecting expedition to the Otago Harbour at Port Chalmers; we were examining, in a boat, seaweeds, &c., from a depth of about one fathom, close inshore, which here descends abruptly into the sea to about this depth; while overhauling a piece of great kelp, Macrocystis, we observed a bright red worm creeping along the stem. At first I thought it a Nemertine, till I had placed it in a bottle of water, when I was delighted to find it to be an Enteropneust. A few minutes afterwards we obtained a second specimen, smaller, and of an orange tint, which

[1] 'Proc. Linn. Soc., N.S.W.,' 1895.

turned out to be a quite young individual of the same species. A few days afterwards I returned to the spot, but was unsuccessful in repeating the capture.

The following account is founded on an examination of the larger individual, which, after examining it alive and making a sketch of it in natural colours, I killed and stained. I then examined it in oil of cloves, and finally cut it into a series of transverse sections.

The animal measured during life, when moderately extended, 30 mm. from the tip of the proboscis to the anus,[1] its greatest diameter at the collar 2·4 mm.; the proboscis was 9·5 mm., about one third the whole length (fig. 1). The colour is rich carmine-red, the proboscis and collar being deepest in tint, the colour fading gradually towards the hinder end; in the last half of the body the thin body-wall allows the brown intestine to show through. The hinder part of the body is flecked with white (? glands), visible through a hand lens. The extreme tip of the proboscis is brown, which passes gradually and imperceptibly into the red.

The proboscis is the most remarkable feature in this species, for it is traversed throughout its length by a deep groove along its dorsal surface (figs. 1, 2, A, and figs. 5, 6, 7, G). It was only after I had killed the worm that I became aware of the interest attaching to this groove, and I regret that I did not examine it in greater detail during life. I am not quite certain whether it is a permanent structure, or whether it can be flattened out during the movement of the animal. My notes read as follows:—"The animal was freely creeping on the weed, . . . creeping by the use of its very contractile proboscis, which becomes deeply grooved along one surface." However, this fact can easily be ascertained on the next occasion on which the worm is captured.

The worm, at any rate, appeared to use its proboscis as a temporary organ of fixation during progression, for it attached

[1] The measurements of the specimen when killed are as follows:—Proboscis 6·25 mm., branchial region and collar 2·4 mm., genital region 3 mm., total length of worm 20 mm.

the tip of it to the surface of the weed or bottle, and drew its body after it, with a good deal of wriggling; probably this act of fixation was effected by the abundant sticky secretion which is discharged by the epidermis.

The collar is moderately long and is triangulate, being marked by two transverse furrows, of which the second is the deeper.

The branchial region is relatively short: I counted twelve double gill-slits on each side of the pharynx, i. e. there are twelve pairs of branchial apertures opening into the branchial grooves (fig. 1). I was unable to see these apertures in life, but I counted the gills when the animal had been stained and cleared (fig. 2); the gill-bars are without synapticula (fig. 3). I further confirmed this observation by counting the gill-pores in this series of sections.

The post-branchial region is not "winged;" the dorsal surface is slightly depressed along the branchial and post-branchial regions, and the gonads lie, as usual, in the lateral ridges. Of these there are about sixteen on each side, in this case ovaries.

There are no hepatic diverticula; the post-genital region of the body is cylindrical, and exhibits a narrow, but deep, ventral furrow for some distance (fig. 11).

In the genital region the intestine is narrow and its wall is a good deal folded, but posteriorly it widens out and comes to fill the body-cavity (fig. 12).

The internal anatomy, as derived from study of the sections, agrees in all points with those on which Spengel[1] lays stress in characterising the genus Balanoglossus, viz.—

The absence of circular muscles in the trunk; the absence of synapticula in the gill-bars; the length of the divergent limbs of the subnotochordal skeleton, which in the present species extend backwards to the hinder end of the collar, being cut through in the sections that include the collar-pore (fig. 4, F). The longitudinal muscles of the collar region have a fan-shaped arrangement around the end of this limb (fig. 4, G).

[1] Naples monograph, 1893.

So much for its generic characters. There is no doubt that it belongs to the genus Balanoglossus. With regard to specific characters, it belongs to the same section of the genus as B. Kowalevskii and B. Mereschkovskii, as is shown by the following features:—The great relative length of the proboscis; indeed, this length is greater in B. otagoensis than in either of these; there is only a single proboscis pore; there are no median gonads. Further, it possesses paired intestino-tegumentary canals (Darmpforte of Spengel), as do these two species; but whilst in them there is at least six pairs of these peculiar structures, there is but a single pair in B. otagoensis (as in the genus Schizocardium). It may not be quite safe, perhaps, to place much reliance on this point from an examination of so few specimens, since Spengel states that the number is not constant in B. Kowalevskii, and suggests that it increases with age, as do the gills and gonads. Nevertheless it seems to me probable that a single pair is constant in B. otagoensis from the following fact: not only is there only a single pair observable in the sections of the adult animal, but in the smaller younger specimens, in which no gonads are as yet developed, the canals are already present, although the gill-slits have not reached more than half their total number; the canals, then, appear early in life.

Another distinction of specific importance is noticeable in the sections, in regard to the longitudinal muscles of the proboscis. In both the species referred to above, Spengel describes the muscles as being arranged in several concentric layers, encroaching considerably on the connective tissue which more or less fills the proboscis. In B. otagoensis, however, the longitudinal muscles of this organ are confined to a very narrow band (fig. 5, D), close to the circular muscles, which, as in Balanoglossus generally, are very feebly developed.

From these facts there is no doubt but that the New Zealand species is quite distinct from the American and European species. But there is a Japanese species, briefly described by Spengel, which agrees with B. otagoensis in the most

noticeable external character, namely, in having a grooved proboscis. Spengel had portions of three specimens of this species, B. sulcatus, Spengel, which he was unable to further investigate owing to an accident that happened to them. He gives a drawing, however, on page 347, from which it will be seen that it bears a considerable resemblance to the New Zealand species. All that is known of B. sulcatus is as follows:—It has a groove along the proboscis; it has no synapticula; it appears, from the drawing, to possess ten or eleven pairs of gill-slits. This drawing is about eight times natural (preserved ?) size, from which we may conclude that the proboscis is about 8 mm. long and the width of the collar 1·5 mm., both measurements agreeing fairly well with the proportions of these organs in B. otagoensis.

It is, from consideration of geographical distribution, possible that they are identical [1]—there is nothing to oppose identity in this respect. Nevertheless, as we know practically nothing of the anatomy of B. sulcatus, I give a new name to the New Zealand species, which may be characterised as follows:

Balanoglossus otagoensis, n. sp.

Hab., coast of Otago, New Zealand.

The proboscis is deeply grooved along the whole of its dorsal surface. The proboscis cavity extends right to the tip of the organ. The longitudinal muscles of the proboscis form a very narrow band close to the wall. There is but a single proboscis pore. There are no median gonads. There is a single pair of intestino-tegumentary canals. The arms of the subnotochordal skeleton reach backwards to the level of the collar-pore.

I have considered it unnecessary to enter into details of anatomy or histology, for these are to be found in Spengel's

[1] I hear from Prof. Dendy, of Christchurch, N.Z., that he has captured a species of Echiurus on the coast, which agrees very closely with the peculiar Japanese E. unicinctus.

great monograph; but there is one small point to which I would draw attention, as I do not find anything quite like it recorded by Spengel. It has reference to the relation of the cardiac vesicle (Herzblase, Spengel: "sac of proboscis gland" of Bateson) and central sinus (Bateson's "heart").

In the anterior region of the "basal complex" of organs, the condition of affairs in B. otagoensis is quite in agreement with that described for other species; the central blood sinus projects upwards into the cardiac vesicles, so that the cavity of the latter is more or less crescentic in section. The greater part of the sinus appears, in section, as a subcircular or semicircular space filled with blood; but it is prolonged right and left into an arm, which passes upwards outside the cardiac vesicle, and downwards around the notochord. This arm gives off a number of blind diverticula, arranged one above the other (fig. 9). The whole series of outgrowths is covered by a layer of cells, the nuclei of which take the stain deeply. It constitutes Spengel's "glomerulus" (the "proboscis gland" of Bateson). Such is the normal arrangement, and such it is in the anterior part of B. otagoensis; but further back the relative sizes of the parts undergo a peculiar change—the central sinus becomes greatly dilated, bulging upwards more and more into the cardiac vesicle, which it almost entirely fills, so that its cavity is reduced to a very narrow cleft (fig. 10). Further back still, the usual condition is again assumed till the central sinus disappears as such.

Looking through the 'monograph,' I have been unable to see any account of this condition for Balanoglossus; but in Ptychodera minuta it occurs, and is figured on pl. iii, fig. 18.

I do not know that any great importance is to be attached to this greatly dilated condition of the central sinus; it may be that the worm happened to be killed during a local contraction which drove the contents of the sinus into this temporarily dilated region, but I did not observe any corresponding restriction of its dimensions, and since the condition of these parts has been dealt with by Spengel at length, and some stress is

laid on the variations presented by the different genera, it seems worth while to note the peculiarity in the present species.

EXPLANATION OF PLATE 45,

Illustrating Mr. Blaxland Benham's paper on "Balanoglossus otagoensis, n. sp."

Fig. 1.—Dorsal view of Balanoglossus otagoensis (× 8) from a sketch of the living animal; the gill-pores have, however, been inserted from later study. (A) The characteristic groove on the proboscis.

Fig. 2.—View of the left side of the specimen after staining and clearing. The depth of the groove (A) is indicated. (B) The "cardiac vesicle." (C) Branchial region. (D) Genital region.

Fig. 3.—Sketch of gill-bars.

Fig. 4.—Half a transverse section through the region of the collar-pore, to show the end of the subnotochordal skeleton. × 125. (A) Dorsal nerve-cord. (B) Dorsal blood-vessel. (C) First gill-sac. (D) Collar funnel and pore opening into it. (D) Parts of gill-bar. (E) Pharynx. (F) Limb of subnotochordal skeleton. (G) Longitudinal muscles. (H) Collar cavity (camera).

Fig. 5.—A transverse section of the proboscis near the anterior end (stained with picro-carmine). × 125 (camera outline). (A) Epidermis. (B) Nerve tissue. (C) Basement tissue. (D) Longitudinal muscles, confined to a very narrow area. (E) Connective tissue. (F) Cavity of proboscis. (G) Dorsal groove.

Fig. 6.—Outline of transverse section of proboscis (camera) near the middle. × 125.

Fig. 7.—Transverse section of proboscis near the base (camera). × 125. (A), (C), (D), (E), (F), as before. (G) Central sinus. (H) Cardiac vesicle. (I) Glomerulus. (J) Notochord.

Fig. 8.—A portion of the section of the wall of the proboscis in the region of the square on Fig. 5. × 450 (drawn with camera). (A) Epidermis. (B) nerve layer. (C) Basement tissue and circular muscle. (D) Longitudinal muscle-fibres. (E) Connective tissue with nuclei.

Figs. 9, 10.—Two transverse sections of the "basal complex" to illustrate modifications in form of cardiac vesicle. (G), (H), (I), (J), as in Fig. 8 (both drawn with camera). × 150.

Fig. 11.—A diagrammatic transverse section through the genital region in the region of the single pair of "intestino-tegumentary canals" (B) (compiled for a series of outline drawings). (A) Intestine. (B) "Canal." (C) Its pore. (D) Gonad. (E) Ventral blood-vessel. (F) Ventral nerve. (G) Ventral groove. (H) Dorsal nerve. (I) Dorsal blood-vessel.

Fig. 12.—Sketch of anterior part of the body of the small specimen, stained and mounted entire, to show the position of the single pair of intestino-tegumentary canals (F). (A) Collar. (B) Branchial region. (C), (D) Intestine in genital region. (E) Post-genital intestine.

The Movements of Copepoda.

By

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Since the time of Brady it has been generally supposed that amongst the Copepoda the most important locomotor organs were the antennules. This belief is categorically stated in Huxley's text-book on 'The Anatomy of Invertebrated Animals,' p. 235.

During a stay of several weeks in the Plymouth Biological Station I had almost daily opportunities of examining the numerous Copepoda captured in the tow-net, and my observations are by no means consonant with the popularly accepted idea. The movements of the species I examined were of two kinds; there was a slow gliding movement, and a sudden dart of lightning swiftness. During the prosecution of movements of the first description the antennules, or first antennæ, are held rigidly extended at right angles to the long axis of the body, and their appearance suggests the idea that one of their functions may be to act as hydrostatic sense-organs. Movement is effected principally by means of the second antenna, the gnathites likewise assisting, notably the second maxilla. It seems probable that feeding is carried on during these slow movements.

The quick movements are effected, on the other hand, entirely by the simultaneous action of the thoracic feet. A sudden blow executed by all the four powerful pairs of paddles is sufficient to propel the animal for a very considerable distance. The animal moves so quickly during the longer darts that it is

impossible to see exactly what happens to the antennules, but by carefully examining the shorter darts, which are carried out at a more moderate speed, it is seen that the antennules are held as rigidly as during the slow movement, and there is therefore no ground for attributing any share in the production of the movements of these animals to the first antennæ. Naturally when the animal is suddenly propelled forward the tips of these appendages will be mechanically dragged back by the resistance of the water; and a careless observation of this phenomenon, joined to the undoubted fact that in the fresh-water Cyclops the first antennæ do assist in the slow movements of the animal, may have given rise to the belief that it was the rule among Copepoda to propel themselves by means of the first antenna.

In a paper published some years ago (Sedgwick's theory of the embryonic phase of ontogeny as an aid to phylogenetic theory, 'Quart. Journ. Micr. Sci.,' 1895) I put forward the view that the progressive development of the Crustacea was correlated with a passing of the function of locomotion backwards along the series of appendages. Thus, in the ancestor represented by the Nauplius, the first, and more especially the second antennæ were the main locomotor organs; in the stage represented by the Zoœa the maxillipeds had acquired the function; whilst in the ancestral condition corresponding to the Mysis larva, motion was effected chiefly by the hinder thoracic legs, as is still the case with Schizopoda; finally, the lower Macrura swim by means of the abdominal appendages alone. The result of this process has been that the appendages of the anterior segments have been one by one relieved of the function of locomotion, and have become specialised for masticatory and sensory purposes. Now in the Nauplius the main brunt of the work of locomotion is borne by the second antenna; the first is already mainly a sensory organ, and if the Copepoda really did propel themselves chiefly by the first antennæ they would have retrograded from the condition represented by the Nauplius. It is interesting to note that not only is this not the case, but that the second antenna is still an important locomotor organ. Func-

tionally, indeed, Copepoda seem to stand on pretty much the same plane of development as the Protozoœa larva.

Phylogenetic hypotheses have too often been based on mere resemblances in form, apart from a consideration of function. This seems to me to be a wrong method of attacking the problem. Function is the all-important thing—that which determines structure; and I hold that if ever we are able to sift the primary from the secondary elements in ontogeny, it will be by the recognition of the fact that the persistence of ancestral structure is caused by the retention of ancestral habits, and that the habits at all periods of the life-history demand the closest study.

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