A Description of Ephydatia blembingia, with an Account of the Formation and Structure of the Gemmule.

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With Plates 1—4.

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Part I.—The Morphology, etc., of Ephydatia blembingia.

I. Introduction.

Ephydatia blembingia is a fresh-water sponge which Mr. Annandale came across in a small pool of water while in search of snails. It was collected and preserved by me on the 23rd of July last year.

The specific name blembingia has been applied to it on account of its locality. Blembing is a small Malay village which was visited by the members of an expedition sent out by Cambridge University to the Siamese Malay States, and which is situated on a small river of the same name. The river Blembing is a tributary of the Pergau, which in its turn empties itself into the Kelantan River.

The pool of water in which the sponge, now described for the first time, was found, was situated in a comparatively dense jungle at a distance of a few yards from the bank of the river. The trees growing around it were so big, and their foliage so thick, as to admit of only a small amount of light ever passing through them. Consequently the pool of water in which Ephydatia blembingia was found was always in a deep shade.

The material which I collected was preserved in the following reagents:

(1) Flemming's solution (weak fluid).

(2) Saturated solution of corrosive sublimate (92 volumes) and glacial acetic acid (8 volumes).

(3) Absolute alcohol.

(4) Rectified 70 per cent. spirits.

II. Description of Ephydatia blembingia.

(1) Colour, Habits of Growth, and External Form.—Ephydatia blembingia is almost colourless, or to use a term which was used many years ago by Professor Lankester (**11**) to describe the colour of Spongilla from the Thames, it is "pale flesh-coloured." Knowing as we do that Spongilla

lacustris and Ephydatia fluviatilis, denizens of our own rivers, are green in colour only when they grow in bright sunlight, this is what we would have expected in the case of a sponge which grew in a pool of water scarcely ever brightened by direct sunlight.

The habits of growth of Ephydatia blembingia are peculiar. In reality it is an encrusting sponge, though some specimens have a massive appearance. But this is due to the habit of growing on such supports as blades of grass and branching weeds of various kinds which inhabit the same pool of water as the sponge. It never seems to produce independent branches, which, when present, give a sponge a kind of bush-like appearance, as Spongilla lacustris does. If, at first, a specimen appears to branch, on closer examination the apparent branching reveals itself as the result of creeping over a branched support. Consequently, in spite of its massive appearance, Ephydatia blembingia is an encrusting sponge. The biggest specimens measure no more than about an inch across (Pl. 1, fig. 1).

The surface texture of the preserved sponge is somewhat woolly, an appearance caused by the spicule fibres which support the otherwise smooth dermal membrane. The fibres often penetrate the membrane, owing undoubtedly to its being rubbed off their extreme points.

To sum up, Ephydatia blembingia may be described as a pale flesh-coloured sponge, with encrusting habits, creeping over branched vegetable supports, and consequently irregular in shape and woolly in texture.

The oscula, not to speak of the dermal ostia, are so small as to be invisible without the aid of the microscope. The openings represented in fig. 1 are those of the inhalant canals seen through the dermal membrane.

(2) Skeleton.—The skeleton consists almost entirely of spicules, which I shall now proceed to describe.

A. Spicules.—In order to facilitate the description of this most important element of the skeleton, I shall arrange the spicules under three heads.

(*a*) The first group of spicules consist of diactinal monaxons or amphioxea, which are usually curved, though straight specimens are occasionally seen (Pl. 1, fig. 3, *a*—*e*).

(*b*) The second group also consists of curved amphioxea, but for reasons which will be stated further on they are separated from the first group (Pl. 1, fig. 3, *f*).

(*c*) The third group consists of amphidiscs, which may be present in a fully developed or in an immature form (Pl. 1, figs. 3, *g*—*m*, 4, *a*—*c*).

(*a*) The amphioxea belonging to the first group taper gradually to a sharp point. They are never provided with a swelling at the middle point of the shaft, and scarcely ever are they malformed or modified in any way. In both respects, therefore, they differ most strikingly from the spicules of Spongilla moorei, a description of which was published in this journal a year and a half ago. They appear to be invariably covered with small spines.

(*b*) The amphioxea belonging to the second group are invariably curved and covered with small spines. In fact, they present the same characters as the spicules of the first group, but differ from them in being only half as long and less than half as thick. They are not found in the general tissues of the sponge or in its membranes, but are grouped together round small bodies[1] which are embedded in

[1] The bodies above mentioned seem to possess a definite outline, and to lie in cavities of their own, much in the same way as the gemmules (Pl. 4, fig. 17). I have no conception what these bodies are, but several solutions have suggested themselves. Unfortunately I have been unable to find them in thin sections, and consequently cannot speak of their internal structure.

The first suggestion, with regard to their nature, to present itself was that they were a second kind of gemmule. The arrangement of the spicules round them reminds us of that of the spicules round the gemmules of Spongilla lacustris, and is, so far, in favour of the supposition that these bodies are some kind of gemmules. But apart from the fact that Ephydatia blembingia possesses another kind of gemmule, these structures are much more transparent than ordinary gemmules are at any stage in their development or when they are mature. If they were gemmules their basket-like shape could be easily explained as the result of contraction under the action of preserving reagents, owing to the cuticular coat being extremely thin. Apart from the

the deeper tissues, and which present a kind of basket-shaped form.

(*c*) The last class of spicules to be considered consists of the amphidiscs (Pl. 1, fig. 4, *b*). The diameter of the hat-shaped disc is about three times that of the shaft. The two discs are exactly similar in size and shape. The surface situated away from the shaft is smooth and convex, while the other surface is concave. Their margins are very finely serrated. The shaft is covered with spines which are conical in shape and placed at right angles to the axis of the amphidiscs.

In addition to the fully mature forms, all the stages of development are represented, from the simple rod slightly

fact that, if the supposition here made were true, it would cut at the very root of the system of division into sub-families, now adopted, of the so-called Spongillidæ, the reasons given above seem to be sufficiently weighty to compel us to lay aside this possible view of the nature of these enigmatical bodies.

The second supposition that suggests itself as a solution of the problem is that these structures are a kind of symbiotic or parasitic sponge. This supposition is not so unreasonable as it would at first appear, for we already know that Spongilla bohmii is parasitic on Spongilla nitens (**17**). Besides, it must be remembered that all the spicules in connection with these bodies are quite different from those which form the sponge skeleton, being, as has been stated already, only half as long and less than half as thick. It is no argument to say that *they are incompletely developed*, for they are all of equal size, which would not be the case if they were merely young spicules. However, if these bodies are of the nature of a parasitic sponge, there are, at present, no data by which its position among the Spongillidæ can be determined.

There is still left another possible solution of the problem, namely, that the bodies here discussed are the result of parasitism on the part of some animal other than a parasitic sponge. If this supposition were true, these bodies would have to be considered as a kind of gall, by means of which the sponge endeavoured to protect itself from the action of an unwelcome intruder. But there are two facts which go against this view. In the first place, though I have examined several of these bodies, I have so far failed to find any animal inside them. In the second place, though there are many parasites in the sponge, not one of them has as yet been found to possess such a coat as these bodies would provide.

Though it must be left an open question what the nature of these bodies are, for the reasons given above I am inclined to adopt the view that they are parasitic sponges.

swollen at both ends to the fully-formed amphidiscs. Their development, however, will be considered along with that of the gemmule.

B. The Arrangement of the Spicules to form Fibres, etc. (Pl. 1, fig. 2).—The spicule fibres are poorly developed, and consequently stand in a most marked contrast with those of some other fresh-water sponges. I have never seen more than three spicules situated side by side in a spicule fibre, and scarcely ever saw more than two. As often as not, the spicules seem to be arranged end on in a single file. In the deeper parts of the sponge, fibres are almost non-existent, the spicules lying about freely and presenting no particular arrangement. Nearer the surface, however, the fibres are better developed, and traverse the strands of tissue which separate the various compartments of the sub-dermal cavity from one another. On the outer ends of the fibres is situated the dermal membrane, which is often pierced owing merely to the wear and tear of the life which the sponge lives. Owing to the absence of flesh spicules or microscleres, the skeletal fibres formed of megascleres present an evident tendency to run in the vicinity of the membranes which line the canals and cavities of the sponge.

As has been stated above, spicules of the class *b* take no part in the formation of the skeleton, but this is not true of those belonging to class *c*, i. e. the amphidiscs. The latter are found in all stages of development scattered about in the general tissues of the sponge, while the former are limited to the walls of the enigmatic bodies described above. Special stress must be put on the fact that the developing stages of the amphidiscs have been seen in the sponge tissues, and not in the gemmule wall.

C. Spongin.—It is scarcely necessary to mention spongin in connection with Ephydatia blembingia, for it is almost completely absent. In this respect the sponge here described strongly contrasts with some fresh-water sponges. In Spongilla moorei the spicule fibres and the dermal membrane are covered with this substance (7), but in Ephydatia blembingia there is no spongin on the surface, and the spicule fibres are, at most, provided with a very small amount at the junction of the spicules.

This difference is explained by the dissimilarity in the conditions of life. On the one hand, Ephydatia blembingia lives in a small pool of water which probably dries up for the greater part of the year; while Spongilla moorei, on the other hand, lives at the bottom of Lake Tanganyika. Therefore, the former may be described as an annual, while the latter—so to speak—is a perennial spongilla. If this difference in the conditions of life under which these two sponges live were to have any effect at all, we would naturally expect the spongin part of the skeleton to suffer most.

(3) The Canal System.—Owing to the presence of gemmules in all stages of development, the canal system could hardly be in such a condition as to be capable of minute description, for the formation of gemmules is accompanied by the breaking down of the sponge tissue. Besides, we know of no preserving fluid that does not admit of a considerable amount of disassociation of the tissue cells of the Monaxonida. Though they be preserved with the greatest care, and with the best reagents known, free cells are found in great abundance in the interior of the sponge tissues. The presence of so many amœboid cells is conducive to this state of things. Consequently our remarks on the canal system must be meagre at best.

As has been stated above, the dermal ostia are microscopically small but comparatively numerous. They open as usual into the subdermal cavities, which are large and extensive (fig. 2), and which are lined by cells which possess granular nuclei. These in their turn open into the inhalant canals, which are also well developed, but decrease in size towards the surface of fixation of the sponge. The flagellated chambers are small and numerous, lying about in the extremely loose tissues of the sponge. The exhalant canals, though at first of fine calibre, assume comparatively huge proportions. The oscula, however, by which they open to the exterior are small. The membranes which line the canals are not provided with special spicules, but are supported by the spicule fibres, which are situated close to the lining membranes.

4. The Structure of the Gemmule.—The gemmules are scattered about singly throughout the whole tissue of the sponge. They are found, on the one hand, near the surface, and on the other hand, quite close to the vegetable supports of the sponge. They are never found in groups. Each gemmule occupies its own cavity (Pl. 1, fig. 2, *gem.*).

I shall here describe only the structure of the mature gemmule, the development of which will be described in Part II. Nevertheless, it must be remembered that the sponge contained gemmules in all stages of development at the time it was collected.

The gemmule is oval in shape, being, as a rule, slightly flattened on the side on which the opening is situated. The external opening or pore is placed at the bottom of a small depression surrounded by a rosette-like structure, which is raised up, and into the composition of which all the layers of the gemmule coat enter (Pl. 1, fig. 7).

The contents of the gemmule consist of a number of globular cells which are full of oval-shaped food granules. The cells are all alike, and the whole mass possesses no membrane of any kind save the gemmule coat, which I shall now proceed to describe.

The gemmule coat consists of three layers which differ from one another, to a considerable degree, both in structure and extent of development.

The inner layer of the gemmule coat completely surrounds the cells which are situated in the interior. It presents the general shape of the gemmule and is prolonged round the aperture to form a kind of a tube, the passage through which is interrupted by a chitinous membrane situated about the middle. The cellular contents of the gemmule extend into the inner half, and the second layer of the gemmule coat to the outer half of this tube. In structure this layer is chitinous, and resists the action of all ordinary reagents, save the mineral acids. It often happens, that, in sections, it splits in two, a result brought about by the weakening produced through the inner ends of the amphidiscs being embedded in it, and not lying upon it as is usually described in the gemmules of Ephydatia. The line along which the splitting takes place is that in which the discs are situated (Pl. 4, fig. 17, *a*).

The middle layer of the gemmule coat is by far the thickest and approximately extends over the whole length of the shafts of the amphidiscs. It is clear in structure, and presents in section the appearance of ordinary parenchyma with very small granules at the nodes. In the fully developed gemmule there are no lines of division indicative of the different cells out of which it was originally formed (Pl. 4, fig. 17, *a*).

The outer layer of the gemmule coat is thinner than either of the other two, and in it are embedded the outer ends of the amphidiscs. It consists of the same substance as the inner layer but is much more granular. In the mature gemmule it is often rubbed off, and consequently the outer discs of the spicules protrude from the gemmule coat (Pl. 4, fig. 17, *a*).

The amphidiscs lie partly in the three layers. The inner disc lies in the corresponding layer, the shaft in the middle layer, and the outer disc in the thin outer layer. They are so closely packed that the discs overlap one another and consequently are not on the same level. Their shafts never seem to cross one another, but lie approximately parallel.

III. The Affinities of Ephydatia blembingia.

The presence of gemmules in the material at my disposal made the task of determining the systematic position of the fresh-water sponge here described a comparatively easy one. The possession of gemmules excludes it from the sub-family Lubomirskinæ, which is a sub-family created for the purpose of grouping together a number of fresh-water sponges in which the gemmule, if it does exist, has not yet been discovered. Further, the existence of the thick coat which surrounds the gemmule cells and which contains, embedded in it, a thickly-set layer of amphidiscs separates it, on the one hand, from the sub-family Spongillinæ, and on the other hand places it among the Meyeninæ. Again, its generic position is not difficult to determine. The equality of size of the amphidisc rotules separates it from both Tubella and Parmula, the serrated edge of the rotules from Trochospongilla, the equality in length of all the amphidiscs from Heteromeyenia, and the absence of any kind of filament or appendage, attached to the chitinous tube, from Carterius. Consequently the sponge, which is described in this paper, belongs to the genus Ephydatia. Of the species contained in this genus, the sponge to which the name Ephydatia blembingia has been given seems to approach Ephydatia plumosa (Carter, **2**) more closely than it does any other well-marked species. Several species of the genus Ephydatia are provided with amphioxea, which are covered with small spines, and are the constituent elements of the skeletal fibres. In Ephydatia fluviatilis (**17**) both smooth and spined spicules occur together. It follows, therefore, that the presence or absence of small spines on the skeletal spicules is not distinctive as a specific character. Potts (**17**) seems to consider this difference so unimportant that he describes an American sponge, to which he has given the name palmeri, as a mere variety of the Indian sponge plumosa; though the skeletal spicules in the former are covered with small spines, while in the latter they are smooth. The skeletal spicules of Ephydatia blembingia agree with those of palmeri, and not with those of plumosa.

The amphidiscs seem to be closely similar in plumosa, palmeri, and blembingia, though the rotules appear to be more deeply notched in the two sponges mentioned first than they are in blembingia. If these were all the differences that could be enumerated the sponge now discussed would have to be considered a slight variety of the species plumosa, if, indeed, not actually identical with the variety palmeri. However, there still remains to be mentioned another most important difference, namely, the absence from blembingia of the flesh spicules so characteristic of both plumosa and palmeri. Though this is a negative character, combined with the other differences it seems to be a sufficient reason for the formation of a new species, to which I have given the name blembingia.

IV. Summary.

Ephydatia blembingia is an encrusting sponge which grows on vegetable supports. It is pale flesh in colour, and loose in texture. The skeletal spicules are covered with small spines. Flesh spicules are absent unless the small amphioxea (*b*) be considered to belong to such a category. The spicule fibres are poorly developed, and in the deeper parts of the sponge the spicules, as a rule, lie about irregularly arranged in the tissues. Spongin is present only in very small quantities. The gemmules are numerous, but not aggregated in groups. They are situated—each one occupying a cavity of its own—near the surface as well as deeper down in the tissues of the sponge. They are oval in shape, and possess an opening resembling that of a bottle, which is obstructed by a chitinous septum. They are provided with a thick and well-developed coat, in which amphidiscs of equal lengths are arranged in a single layer. The shaft of the amphidiscs is furnished with conical spines, large in size and situated at right angles to the longitudinal axis. The outer surface of the discs is convex, and the margin is slightly serrated. Amphidiscs, in all stages of development, are scattered about in the sponge tissue where they are formed.

Part II.—The Formation of the Gemmule of Ephydatia blembingia.

I. Introduction.

When I took the description of Ephydatia blembingia in hand I had no intention of describing the development of the gemmule; but when I saw that the material at my disposal contained gemmules in all stages of development I thought it would be a mistake not to describe it. Further, I was encouraged to do so by Professor Weldon, to whom I am greatly indebted both for the free use of his laboratory and all its resources, and for much invaluable assistance, especially in connection with the literature on the subject. I shall first give a summary of what is already known of the gemmule. I shall then proceed to describe my own observations, the method followed being that of tracing the origin and subsequent changes of the various cells which take part in the process, this method being considered simpler and more intelligible than that of giving a complete description of the different stages of development. The reader can easily make out for himself, by examining the figures 8, 9, ... 17, the true relation of the changes in the different parts of the developing gemmule much better than by reading the best possible description. Finally, I shall review previous accounts and compare my own conclusions with them.

II. Historical Review.

Carter (**2**), who was the first to attempt an explanation of the origin of the gemmule, which he terms the seed-like body, writes as follows:—"At the earliest period of development in which I have recognised the seed-like body it has been composed of a number of cells, united together in a globular or ovoid mass (according to the species) by an intercellular substance. In this stage, apparently without any capsule, and about half the size of the full-developed seed-like body, it seems to lie in a cavity formed by a condensation of the common structure of the sponge immediately surrounding it. It passes from the state just mentioned into a more circumscribed form, then becomes surrounded by a soft, white, compressible capsule; and finally thickens, turns yellow, and develops upon its exterior a firm crust of siliceous spicules." He says with regard to the origin of the gemmule, "I do not wish it to be inferred that I am of opinion that the seed-like body is but an aggregate of separate sponge-cells;" and further, after describing certain cells of the sponge, he says: "It may, perhaps, be one of these cell-bearing cells which becomes the seed-like body."

Lieberkühn (**12**), in the year 1856, published an account of the origin and structure of the gemmule. He found in the deeper parts of the sponge shiny white gemmules, which on the whole appeared like ordinary brown gemmules, and which possessed exceedingly plain amphidiscs. He also found other gemmules, distinguished by their very delicate transparent shells, also possessing very obvious amphidiscs. These, he said, had a superficial layer of a substance feebly refractile, and a central mass brilliantly refractile. The feebly refractile cells separated easily, while the others only did so with difficulty. In these bodies he was not able to find the delicate transparent encrusting layer, which he had seen round the white gemmule; but found a layer of cell-like spherules which resembled the ordinary sponge-cells in the arrangement of their granules and of their nucleolus; while others contained the amphidiscs. Some of the enclosed amphidiscs had exactly the shape of those found surrounding the ordinary gemmule. Others, he said, did not possess the two discs, but in the interior of each cell-like structure there was a delicate rod with a slight knob-like swelling at each end. In others a series of very fine spicules radiate from the terminal swelling. He derived the amphidiscs by imagining these spicules to become broader, and the axial rod to become thicker. The contours of the cells containing the spicules were described as being as sharp as those of ordinary sponge-cells. He could find no nuclei in these cells. He finally concluded that these bodies were incompletely developed gemmules. He also found certain bodies which he described as white aggregations of sponge-cells, possessed of the same size and shape as ordinary gemmules. In the same year he published a second paper, in which he summed up as follows (**13**):—"That the gemmules are derived from a heap of ordinary sponge-cells we can very plainly see in that branched sponge which has gemmules with smooth shells. In a longitudinal section of a suitable piece we find—(1) Gemmules which are completely developed, and possess a smooth shell containing a large number of the rounded masses accurately described by Meyen. Each of these masses is spherical, and contains in its interior an albuminous fluid and many strongly refractive spherules. It is about as large as a sponge-cell, and quickly disintegrates in water. (2) Gemmules with an obvious shell, which contains Meyen's spherical masses and also contains bodies which have Meyen's masses, but are distinguished from these by sending pseudopodia like the ordinary sponge-cells. (3) Gemmules in which the shell and the pore are obvious, containing only cellular bodies which send out pseudopodia. Some of these contain a nucleus and a nucleolus like sponge-cells, and are distinguished from these only by the fact that they contain in their interior the refracting spherules already alluded to. (4) Spherical heaps corresponding in size to the gemmules which consist of the above-mentioned bodies, sending out pseudopodia, and of undoubted sponge-cells. The sponge-cells have an obvious nucleus and nucleolus, and they contain besides a mass of very fine granules, which may be scattered through the whole cell-body or may be collected in small spherical masses. These spherical masses are of the same size as the refracting spherules already described, and one or two such spherules are often found in the sponge-cells. Round some of these spherical heaps .of cells a very fine structureless membrane can be recognised. The spherical masses of Meyen which are commonly found in gemmules are nothing else than altered sponge-cells; by compressing the contents of the gemmule under the cover-slip we can find a nucleus and a neucleolus in every such mass; but nucleus and nucleolus are so hidden by the strongly refractile contents of the Meyen's masses that they can only be demonstrated by a process of pressure. These nuclei and nucleoli do not especially differ from those of ordinary sponge-cells."

Lieberkühn again, in a third paper (**14**), speaks as follows of the gemmule :—"The gemmules are not eggs, but a sort of cyst or capsule, out of which the same individual which built them ultimately creeps through the pore."

In a later publication (**3**) Carter describes the seed-like bodies as being globular in shape, and consisting of a coriaceous membrane enclosing a number of delicate, transparent, spherical cells, more or less filled with ovules and granular matter, while an incrustation of gelatinous matter charged with small spicules peculiar to the species surrounds the exterior of the coriaceous membrane. "It has also been shown," he adds, "that at an early period of development the spherical masses, which we shall henceforth call ovi-bearing cells, are polymorphic—identical, but for the ovules, with the ordinary spongo-cells—and surrounded by a layer of peculiar cells equally polymorphic, which I have conjectured to be the chief agents engaged in constructing the capsule."

Again, in a later publication (**4**), he speaks of the "ova"—preferring the term "ovum" to "seed-like body"—of Spongilla as follows:—"At an early period of the ovum the spherical cells, though already filled with the refractive granules, are few in number and sub-polymorphic; hence it may be reasonably inferred that their multiplication as the ovum increases in size is produced by fission; the younger the ovum the more polymorphic and resistent are these cells, while the older it becomes the more they are attenuated, and the more rapidly they burst by endosmose after liberation."

In the year 1874 he further writes of the gemmules as follows (**5**) :—"It may be a question whether the entire body may not be the ovarium of a Spongozoon in the first place; while, as in hundreds of instances of the same kind in the animal kingdom, all the other parts have perished, their function having ended when sufficient nutriment had been gathered and assimilated to support the reproductive elements until they could do this for themselves." Further on he adds, "It is an assemblage of ova which are at once developed together into a young Spongilla."

In his final communication (**6**) on the gemmule, he views it "as a simple ovum with modified form to meet the requirements of the case."

It seems Carter was always uncertain as to the origin of the gemmule, and at one time or another he appears to have had four views. First, that the gemmule was a mere aggregation of sponge-cells; secondly, that it was an aggregation of cells produced from one cell, the "ovi-bearing" cell; thirdly, that it was a single ovum, which was his final view; and fourthly, that it was a single "ovarium" of a dead "spongozoon."

In the year 1884 Marshall published an account of the development of the gemmule of Spongilla lacustris (**15**). He says that the first sign of the gemmule consists of a number of amœboid cells, which are found in the neighbourhood of the inhalant canals and the ciliated chambers, and which he terms the "trophophores." They fill themselves with reserve material, and wander together in groups. They become round and give up water, so that they look like masses of reserved food material. Very early round the pseudomorula formed in this way there appears a delicate structureless membrane, a cuticle, the matrix of which should be probably looked for on the surface of the pseudomorula itself. The "mesoderm" outside this cuticle builds at first an endothelium which deposits on the cuticle further layers of horny substance and delicate siliceous structures, in this case spiny tangential needles.

In the year 1886 appeared Goette's account of the development of the gemmule of Spongilla fluviatilis (**10**). He says that the first rudiment of the gemmule is formed by an aggregation of ordinary parenchyma cells in a nearly spherical area of 36—44 μ in diameter; really, the flagellated chambers and canals of this region become enclosed in the aggregation, which is produced through hypertrophy of the cells. In this aggregation of cells the formation of two layers quickly takes place; a central mass of cells, containing a great number of yolk-granules, and an outer sheet of cells, which become club-shaped and form a kind of columnar epithelium round the central mass. This sheet secretes a cuticle round the central mass, and its cells form the amphidiscs. Subsequently the club-shaped cells migrate outwards, and secrete a second cuticle outside the amphidiscs.

In the same year as Goette, and independently of him, Wierzejski described the development of the gemmule (**19**). He describes the first rudiment of the gemmule as a group of naked amœboid cells. He says that the cells of the mother-sponge can migrate to the body of the gemmule and thus increase its volume. The heap of cells brought together through migration from the sponge tissue become differentiated into a central mass and a peripheral layer. Shining spherules and granules are deposited in the cells of the central mass, those of the peripheral layer becoming columnar. The amphidiscs are not developed in the peripheral cells, but in the surrounding tissues, and only subsequently migrate to the columnar layer.

In the year 1892 Zykoff published an account of the development of the gemmule (**21**). This account adds little, if anything, to what was known before of the formation of the gemmule. He found, among the ordinary amœboid cells of the parenchyma, cells which contained a number of refractive granules of a very definite form, which he describes as boat-shaped. He considers the appearance of refractive yolk-substance in a few amœboid cells of the mesenchyme as the first step in the development of the gemmule. These amœboid cells have the protoplasmic structure of Fiedler's amœboid "Fresszellen," but the nuclear structure of his "Nahrzellen." He disagrees with Goette and supports Wierzejski on the question of the origin of the first rudiment of the gemmule. He denies Goette's statement that the flagellated chambers and the epithelial lining of the canals participate in the formation of the gemmule. The rudiment of the gemmule soon becomes differentiated to a central mass of yolk-cells, among which amœboid cells of the mesenchyme occur, and a peripheral stage which consists of one or two concentric layers of mesenchyme cells of the sponge. The peripheral cells become club-shaped and not columnar. This change takes place gradually, not all at once. The club-shaped cells secrete the inner cuticle, and the amphidiscs migrate from the sponge and take up their position among the club-shaped cells, which subsequently migrate outwards, secrete the outer cuticle, and, finally losing their club-shaped form, gradually become resorbed.

In the year 1893 Weltner published a short paper (**18**), in which he brings together the different views expressed as to several important points in connection with the structure and development of the gemmule, and from his own observations draws his own conclusions. Having discussed the use of the protective coat; the presence of a thin membrane, which he does not believe to exist, round the reproductive portion of the gemmule; the number of nuclei in each cell, of which he has seen more than one in several cases, he finally deals with the question of the origin of the cells of the gemmule, in the first rudiment of which he finds three kinds of cells, namely cells which have yolk-bodies alone, cells which display fine granules of equal size and a distinct nucleolus, and cells which have large granules of unequal size. The third class of cells are different from the cells with granules of unequal size found in the parenchyme.

He comes to the conclusion that the development of the gemmule is not yet sufficiently known, and that a fresh inquiry should be instituted as to two main points: first, the origin and nature of the cells which form the first rudiment of the gemmule; secondly, to ascertain the fate of these cells.

He suggests that their origin and nature should be examined with a view to the following possibilities:

Is the first rudiment of the gemmule formed from a single cell which has the value of an egg? Then the gemmule should be a group of segmenting cells.

Or, does the inner mass of the gemmule arise from one class of cells derived from the previous mesoderm?

Or does it arise from more than one class of mesoderm cells?

Or, finally, is it built from different germ layers (two or three)? The gemmule should then be considered a bud.

In Section III of the second part of this paper I shall give an account of the development of the gemmule in Ephydatia blembingia, reserving criticism of whatever kind to Section IV. In Section III I shall include nothing but a simple description, followed by a few conclusions. This course will be pursued in order to make the account more available and more intelligible to the reader than it would be if it were mixed up with critical remarks and conclusions scattered about throughout the paper.

III. Descriptive Account of the Development of the Gemmule of Ephydatia blembingia.

(1) Origin and Further Development of the Reproductive Part of the Gemmule.—The first sign of preparation for the formation of the gemmule consists in the presence of single cells or small groups of cells scattered about chiefly in the dermal membrane; the strands of tissues which support the dermal membrane; and in the tissues situated immediately below the subdermal cavity.

The protoplasm of the cells in question is uniformly clear, and the nucleus is granular and not vesicular (Pl. 2, fig. 8). I have been unable to detect a karyokinetic figure in any of these cells. Consequently I am of opinion that the constituent cells of these groups seldom divide during the early stages of formation of the gemmule, which is contrary to what must have been the case if the cells of the reproductive part of the gemmule were derived from one mother-cell.

The cells in virtue of their power of wandering travel through the dermal membrane, and strands of tissue which support the membrane, and become aggregated in groups situated either deep in the tissues of the sponge or even in the strands of tissue above mentioned (Pl. 2, fig. 8).

The protoplasm soon loses its uniformly clear appearance and becomes unevenly granular (Pl. 2, fig. 9), a feature which rapidly becomes more accentuated (Pl. 2, fig. 10). The contained granules or irregular blotches at this stage lie in round, clear spaces in the protoplasm, but they soon increase in size to such an extent as to fill the spaces above mentioned. At the same time they acquire an oval or spherical form and exhibit a certain amount of internal structure, in the form of unevenly distributed granules of very small size (Pl. 2, fig. 11*a*). The subsequent change in the interior of the spherical granules or yolk bodies, as they may be termed henceforth, consists in the differentiation of a peripheral layer or coat which sometimes, though not always, contains fine granules, from a centre which invariably seems to possess a finely granular structure (Pl. 3, fig. 13*d*). The yolk bodies have at this stage attained their ultimate structure, and fill the cell in which they have been formed.

While these changes are going on a curious change takes place in the character of the nucleus. At first granular, it now becomes vesicular, or perhaps more correctly it presents an appearance intermediate between the typical vesicular nucleus with a solid nucleolus and a granular nucleus (Pl. 3, fig. 13*d*, *nu.*). The cells seem never to possess more than one nucleus.

The yolk cells, as they may be termed henceforth, have increased slightly in size during the changes above described. However, they retain their individuality, though owing to the pressure which they exert on one another they are often polygonal in shape. In the fully developed gemmule they are so pressed against one another that their individual outline can be seen only with difficulty, which is in no way a remarkable thing seeing that at no stage do they possess a definite cell wall though having a well-defined cell limit.

The yolk cells collectively, or the reproductive part of the gemmule, as they may be termed, at no stage possess a membrane, though in the fully mature gemmule they are so pressed against the inner chitinous layer of the protective coat as to present a perfectly smooth and membrane-like appearance.

(2) The Origin and Subsequent Changes of the Cells which produce the Ground Substance of the Protective Coat of the Gemmule.—These cells, after having wandered from the general sponge tissues, appear in the neighbourhood of the gemmule as a loosely arranged layer situated outside the future yolk cells. Fig. 11*a* (Pl. 2) shows how they travel towards the developing gemmule and how they become concentrated to form a layer.

Their general protoplasm is clear, but they contain a number of granules or yolk bodies which resemble those of the yolk cells. In addition, they often contain a much bigger spherical body which seems to be of the same nature as what I have described in my account of the structure of the larva of Spongilla lacustris as nutritive vacuoles. The cells which develop to yolk cells seem never to contain either of the above bodies at their first appearance. At first they are spherical in shape, but soon become columnar, though never club-shaped. However, their outer end may be round and not flat during certain stages (Pl. 3, fig. 13, and Pl. 4, fig. 15*a*). They assume the columnar form, at first, only on one side of the reproductive mass of cells, the columnar layer so formed gradually increasing in extent until it completely surrounds the yolk cells. The point at which the columnar layer is finally completed marks the position of the future pore of the gemmule.

Subsequent to the assumption of the columnar form, these cells begin to secrete the inner chitinous layer, which in its formation follows the same course as the columnar layer did, which is a proof that the layer in question is secreted by the columnar cells and not by yolk cells (Pl. 3, fig. 13; Pl. 4, figs. 14 and 15).

Soon after the amphidiscs have taken up their position among the columnar cells—a phenomenon which takes place soon after the formation of the columnar layer—the latter grow out and before long appear outside the outer ends of the amphidiscs (Pl. 4, fig. 16). While this is going on their inner ends situated between the amphidiscs become transformed to the parenchyma-like substance situated in the mature gemmule between the inner and outer chitinous coats. During the elongation of the columnar cells outwardly the nucleus is carried along. After their inner moiety has been modified and the nucleus has passed to the outer portion they secrete the outer chitinous layer and ultimately break off, and so becoming liberated they pass back again to the sponge tissue (Pl. 4, fig. 16*a*). The nucleus at the close of these changes, as at the beginning, is vesicular.

The outer chitinous coat is much thinner and less homogeneous than the inner. In the fully mature gemmule the greater part of it is lost, so that the outer ends of the amphidiscs are uncovered.

(3) The Origin, Migration, and Final Modification of the Scleroblasts, inside which the Amphidiscs are developed, and their Migration from the Sponge Tissue into the Columnar Layer.—At the outset special emphasis must be laid on the point that incompletely developed amphidiscs were never seen in the protective coat of the gemmule, whether during the early or later stages. The amphidiscs situated in the gemmule coat are always fully developed, while in the sponge tissues incompletely developed stages as well as fully developed ones are plentiful.

The first stage observed in the formation of the amphidiscs consists of a rod-like structure swollen at both ends (Pl. 1, fig. 3, *m*, and fig. 6, *a*), in which respect they differ essentially from the young stages of the amphioxea, which are always pointed (Pl. 1, fig. 5). Both kinds make their first appearance in cells with vesicular nuclei, which soon become transformed and become granular, especially in the mother-cells of the amphidiscs. The next change consists in the development of a more or less conical form by the ends of the above-mentioned rods, the cone-shaped end at the same time becoming surrounded by a rim (Pl. 1, fig. 6, *b*). The cone-shaped end, together with its slightly developed rim, ultimately grows to the hat-shaped disc. Throughout the process of formation of the amphidisc both ends are of the same shape. If one end is incompletely developed, the other is equally so. The spicules retain their position inside the scleroblast until they have reached their definitive form, and there seems to be no reason for supposing that, were the scleroblast in any way injured, the spicule could ever attain full development. The amphidiscs thus described assume their ultimate form while yet in the general tissues of the sponge. It is important to remember that they are developed in cells which are essentially amœboid. When gemmules are being developed, the scleroblasts in virtue of their inherent power of locomotion move towards them. They travel along the strands of tissue which have been described above as passing from the general sponge tissue to the somewhat loose membrane which surrounds the gemmule. Ultimately they make their way among the columnar cells which surround the gemmule (Pl. 4, figs. 14 and 15*b*). Fig. 15*b* (Pl. 4) is particularly interesting in that it shows the last spicule that has entered the columnar layer as well as one situated in a strand of tissue close by. The latter is on its way to take up its position alongside the former spicule among the columnar cells. When the amphidiscs, still situated inside the scleroblasts, have reached their final position, at first they are longer than the columnar cells, which lie completely inside their outer ends. At this stage the scleroblasts, though already considerably modified, can be distinctly seen. In the fully grown gemmule, however, they are indistinguishable from the parenchyma-like substance produced from the modified inner ends of the columnar cells.

The scleroblasts with their contained amphidiscs first push their way in among the columnar cells at that point where the columnar layer and the inner chitinous coat made their first appearance. They become more numerous and gradually increase in number until finally they envelop the whole gemmule (Pl. 4, figs. 15 and 16). There are, therefore, three distinct structures at least which first appear on the same side of the central cells, i. e. on the side opposite the point which later on will be occupied by the pore, and all three increase in extent in a similar way. They ultimately form complete layers, though one of them, viz. the columnar layer, is no longer found in the mature gemmule.

The migration of scleroblasts, or cells that would become scleroblasts, is not a new idea to zoological literature. Mr. Bourne described such migration of the calicoblasts in *Heliopora cœrulea* (**1**), and Professor Minchin has given a full account of the migration of the epithelial cells in the Ascons to the interior, and the subsequent formation of spicules inside them (**16**). It is true that in both these cases the migration to the interior is previous to the formation of spicules, while in *Ephydatia blembingia* the amphidiscs are fully formed before the change of position takes place. This difference does not in any way tend to minimise the importance of the facts described above. The amphidiscs are so small as compared with ordinary spicules, and their ends are rounded, consequently there is no inherent improbability in the view that they are carried from one place to another by the scleroblasts.

(4) *The Origin, Structure, and History of the Trophocytes.*—The trophocytes are large round cells with vesicular nuclei, the chromatin of which is for the most part aggregated in small granules either round the spherical central corpuscle or against the nuclear membrane, the intervening space being, as a rule, occupied by only a few small granules. In the immediate neighbourhood of the nucleus there are innumerable small and irregularly shaped granules which give the cell a dirty-looking appearance, the peripheral portion being exceptionally clear and devoid of granules of any kind. A negative feature of these cells is seen in the absence of both yolk bodies and nutritive vacuoles.

The trophocytes originate from the sponge as a separate class of cells, like the three other classes which have been already considered. They migrate from the sponge tissue at the same time as, and along with, the cells which become columnar. While the columnar cells always remain outside the yolk-cells, the trophocytes pass in among them. They are incapable of passing through the columnar layer after it has been completely formed, but seem to be able to push their way through when the cells in question are arranging themselves and becoming elongated. Not all of them pass among the yolk-cells, some, as it appears, only entering among the developing columnar cells and turning back. The majority of them, however, seem to pass among the yolk-cells. As a rule, they pass through the developing columnar layer singly, but occasionally groups of several cells are witnessed making their way in. After the trophocytes have entered among the yolk-cells they distribute nutritive material to them, probably in solution. They take no part in the formation of the reproductive portion of the gemmule further than to supply it with nutritive material which the yolk-cells store up in the yolk-bodies. When the inner chitinous layer is about half formed (Pl. 3, fig. 13[1]), the few remaining trophophores are seen travelling towards that part of the gemmule where the pore will appear. They pass out and become scattered about round the gemmule (Pl. 3, fig. 13*c*). It is not difficult to understand why the trophocytes travel all in the same direction, i. e. away from the portion that is already formed of the inner chitinous layer, for it is undoubtedly the direction of least resistance.

5. Summary of Conclusions.—(1) Four classes of cells, each of which is derived independently from the sponge, take part in the formation of the gemmule; first, the mother-cells of the yolk-cells which, alone, constitute the reproductive portion of the gemmule; secondly, the mother-cells of the columnar cells which pass back to the sponge; thirdly, the mother-cells of the amphidiscs, "scleroblasts," which

[1] The sections represented in figs. 13—13*d* (Pl. 3) were cut from material preserved in Flemming's weak solution, while those represented in all the other figures were from material preserved either in absolute alcohol or in a mixture of 92 parts of saturated solution of corrosive sublimate and 8 parts of glacial acetic. This explains the absence of the dirty-looking granules from all the trophocytes except those represented in figs. 13—13*d* (Pl. 3).

become modified and form a part of the intermediate layer of the protective coat of the gemmule; and fourthly, the trophocytes, whose function is to supply both the columnar and the yolk cells with food material, and which, like the columnar cells, pass back to the sponge.

(2) The yolk-cells and the columnar cells draw their food material in solution from the trophocytes; the yolk-cells storing it up as a reserve in the yolk bodies; the columnar cells using it in such a way as to enable them to secrete the inner chitinous layer, to grow and pass out between the outer ends of the amphidiscs, their inner ends being modified to form the greater part of the ground substance of the protective coat of the gemmule, and finally to secrete the outer chitinous layer; processes which mean that there is an enormous amount of metabolism going on.

(3) The amphidiscs are developed in cells, the scleroblasts, which carry them through strands of the sponge tissue to their ultimate position in the protective coat of the gemmule.

IV. Critical Review of Previous Accounts.

On perusal of the historical section of this paper it will be seen that the views which have been expressed as to the first appearance of the gemmule are numerous and conflicting. The only thing certain is that a group or aggregation of cells is formed. How it is formed and whence it is derived no one seems to know, though every one has a theory to put forward. Again, it is equally uncertain whether the gemmule is formed from the group which first appears, or whether this group in order to build up the gemmule structure acquires recruits from among the sponge cells and tissues.

Probably the first question that should be discussed is whether the group of cells above mentioned is the product of cell migration to one spot, or of cell division either of a single cell or of a group of cells.

In his first attempt to explain the origin and structure of the gemmule in the year 1849 (**2**), Carter expressed himself in favour of the view that the gemmule is derived from what he calls an "ovi-bearing cell." In the year 1886 Goette supports the view that this group of cells is the product of cell proliferation (hypertrophy) (**10**).

In reply to both of these views it will suffice to point out that at no stage during the early development of the gemmule are there any signs of cell division. Though during the very earliest stages the cells are absolutely clear (Pl. 2, fig. 8) I am totally unable to find the least sign of nuclear division, not to speak of fragmentation. In all cases the nuclei seem to be well formed, and in no way modified. Carter had not the facts required to support his view, while Goette seems to have merely figured a piece of ordinary sponge, indifferently preserved, as the first rudiment of the gemmule. For fig. 31 (**10**) can hardly be explained in any other way. It must be admitted as certain that he saw flagellated chambers and canals in the specimen represented in the above-mentioned figure, but it seems almost equally certain that what he saw was not the rudiment of a gemmule, for the gemmule at its first appearance offers no points of comparison with Goette's representation. From the consideration of the absence of cell division, the view that the gemmule rudiment is formed by that means may be set aside—to say the least—as a most highly improbable one.

The second view of the origin of the gemmule rudiment to be considered is the one according to which it contains collar cells and flat epithelium cells, or, as Weltner expresses it, that it consists of cells from two or three germ layers. This view has its most influential advocate in Goette. It was held by Carter also at one time, and probably by Lieberkühn, who says of the spherical heaps of cells he found in the sponge tissue, that, besides containing Meyen's masses, they also contain undoubted sponge cells.

If the explanation given above of Goette's fig. 31 (**10**) is correct—and it seems that it must be—it easily explains how he arrived at the conclusion that all the sponge layers participate in the formation of the gemmule. Besides, it is quite possible that those who hold this view of the origin of the gemmule are mentally dominated by the principles of the "Germ Layer Theory." If so, this would be a splendid example of an otherwise good theory leading to false conclusions. Further, it is more than probable that Carter, Lieberkühn, and Goette never saw the first signs of the formation of the gemmule. This is undoubtedly the most charitable view to take of the conclusions they arrived at.

Now that the above-mentioned views have been disposed of, there remain for consideration two more views, one of which can be set aside after only a few remarks. The view in question is the one according to which the gemmule is derived from a group of cells, all of which are alike. This view has not found favour with those who have investigated the structure and formation of the gemmule. Carter at one time held it (**5**), thinking that the gemmule was an ovarium of a "Spongozoon," a name which he gave to his imaginary sponge-animal. However, in a later publication he gave his support to another view. In fact, a single glance at a good section of the gemmule during some of the early stages is enough to cause one to recoil from the idea that only one class of cells take part in its formation. Consequently there remains only one view, namely, that the gemmule originates from a number of cells belonging to various classes. This view, in one form or another, is supported by Marshall (**15**), Wierzejski (**19**), Zykoff (**21**), and Weltner (**18**).

Marshall's account does not concern us as much as those of the other authors above mentioned do, for the reason that he worked on the gemmule of a species belonging to a different genus. There is, however, one point which must be mentioned. The point in question is that he derives the gemmules from two classes of cells at least; namely, the cells which he terms "trophophores," and which give rise to the contents of the gemmule, that is the reproductive part, as well as to the delicate structureless membrane surrounding it; and the "mesoderm" cells, which give rise to the outer shell as well as to the spicules. The importance of this discovery lies in the fact that the reproductive part of the gemmule and its protective coat are respectively formed from classes of cells which are absolutely different, a conclusion which is endorsed in the present paper, though the existence of Marshall's "delicate membrane" round the central mass is here denied.

Wierzejski, evidently, has observed some phenomena which he did not understand. According to this author a first heap of naked amœboid cells becomes differentiated to a central mass of yolk containing cells, and a peripheral layer of columnar cells. But he also states that the cells of the mother sponge can even migrate to the body of the gemmule, and thus increase its size. In the light of the facts which have been described in the foregoing section of this paper, it seems certain that Wierzejski discovered the migration of cells, on the one hand, to form the columnar layer, and on the other hand to feed the mother-cells of the reproductive cells of the gemmule. Wierzejski in describing the first group of cells uses the term "pseudomorula," and, probably knowing that a true morula always becomes differentiated to two classes of cells, he comes to the conclusion, as it appears, that his "pseudomorula" must do the same. Consequently he commits the mistake of describing the columnar layer of cells as originating by differentiation from his pseudomorula instead of by further migration from the sponge tissue. Not only this, he was also unfortunate in not being able to discover the true nature of the cells which migrated to the interior of the gemmule, as he says, to increase its size. His failure was probably due to the method of preservation he used.

Before proceeding any further, it is necessary to refer to Fiedler's account of the cells which he found during his investigations of Ephydatia fluviatilis (**9**). Fiedler describes and figures two kinds of cells (**9**, pl. xi, figs. 3 and 4, and pl. xii, figs. 36 and 37). One kind, which he terms "amœboid Fresszellen," has granules of equal size in its protoplasm, and a nucleus the chromatin of which is arranged in a network. The other kind, which he terms "amœboid

Nahrzellen," has granules of unequal size in its protoplasm, and a nucleus with a distinct nucleolus.

Zykoff, who writes in the light of Fiedler's discoveries, considers the appearance of refractive yolk substance in a few amœboid cells of the mesenchyme as the first development of the gemmule. He finds these cells belong to neither of Fiedler's classes of cells, for they have the protoplasm of the "amœboid Fresszellen" and the nucleus of the "amœboid Nahrzellen." These cells, together with others like them, but without yolk substance, are described as creeping together to form a spherical heap of cells, which differentiates to a central mass which consists of yolk-cells, amongst which here and there are scattered amœboid cells of the mesenchyme, and to a peripheral sheet of mesenchyme cells without yolk, which pass to the general mesenchyme of the sponge. Zykoff has described Wierzejski's figures as being diagrammatic and far from the truth. His figures, however, might with a certain amount of propriety be described in the same terms, and Weltner's criticism that they are not natural is quite true. Zykoff, however, is in error when he says the cells of the peripheral sheet above mentioned do not contain yolk. It is true that there are cells among them without spherical bodies in them, the trophocytes of the present paper; but it is equally true that the greater number of them contain bodies which are in all respects similar to the "refractive yolk substance" which Zykoff professes to have seen "in a few amœboid cells of the mesenchyme," which he describes as the appearance of the first development of the gemmule. Zykoff has here failed to distinguish between two classes of cells, and consequently the description he has given of them is not true of either. The cells which he found to contain yolk-bodies in the sponge tissue, and which, he assumes, become the yolk-cells, develop, as it appears, to the columnar cells, and do not fall under the category of "amœboid Fresszellen," or that of "amœboid Nahrzellen." They form a separate class, while the other cells found among them as well as among the yolk-cells must be placed in a class by themselves. In the present account they have been termed "trophocytes," and seem to be identical with Fiedler's "Nahrzellen." Zykoff found the cells in question among the yolk-cells, but does not properly account for their absence from that position in later stages. It seems certain that Zykoff never saw the first stages in the development of the gemmule. His first figure has not the remotest resemblance to the first rudiment of the gemmule. If he never saw the first stages, this explains how he missed the cells with nuclei the chromatin of which is arranged in a network at first, but later on presents the appearance of a modified vesicular nucleus. However, there seems to be little doubt but that these cells form a different class from the above-mentioned classes, and correspond when they are coming together in all respects to Fiedler's "Fresszellen." Consequently, at the time the peripheral layer of cells appears the whole group consists of three classes of cells: first, the mother-cells of the yolk-cells; secondly, the mother-cells of the columnar cells; and thirdly, the "trophocytes." The first class consists of Fiedler's "amœboid Fresszellen," the third class of his "amœboid Nahrzellen," while the second class consists of those cells which, according to Weltner, belong to neither of Fiedler's classes, and, according to Zykoff, occupy a position between the two.

Now that the somewhat difficult questions of the origin and fate of the cells above discussed seems to have been solved, there remain but few points to be considered in connection with the formation of the protective coat of the gemmule.

At no stage in the formation of the gemmule was a delicate coat or membrane, situated internally to the inner chitinous layer, found to exist. It often happens, however, that the outer limit of the reproductive portion of the gemmule is sharp, smooth, and well defined. But there is no membrane, the sharpness of contour being merely the result of the pressure exerted by the mass of cells on the inner chitinous layer.

The cells of the outer layer are columnar in form, and not club-shaped. This, however, is a small point hardly worthy of all the importance attached to it by Zykoff. The columnar cells during their transference from the inner to the outer side of the external ends of the amphidiscs grow out rather than migrate out. The result is that the spaces between the amphidiscs are partly occupied by the inner moiety of the cells, which moiety, being more or less cut off by the outer ends of the amphidiscs, becomes transformed to the parenchyma-like substance which occupies that position in the mature gemmule. That this is true can be easily seen on examination of fig. 15*b* (Pl. 4), where the inner ends of the columnar cells are already undergoing the above-mentioned transformation, though the amphidiscs are not yet in position. Consequently the origin of this layer need no longer be considered unknown, as has been done by Goette and Zykoff.

The next question to be considered is the origin of the amphidiscs. Lieberkühn describes the amphidiscs as being developed in some of the cells of the peripheral layer (see p. 83). Goette figures a developing amphidisc in one of these cells, and describes these spicules as being formed from within outwards. As has already been pointed out, incompletely developed amphidiscs are never seen in the gemmule coat (p. 92), but are abundant in the sponge tissue. They seem to be invariably symmetrical in form, one end being the exact counterpart of the other. Goette seems to have been in error on both these points.

Zykoff merely confirms Wierzejski's view that the amphidiscs are formed outside the gemmule, but neither of them was able to find the scleroblast, which is most surprising, seeing that Lieberkühn says that the outlines of the cellular structures containing the amphidiscs are as sharp as those of ordinary sponge cells. Zykoff discusses at considerable length the mode of migration of the amphidiscs from the sponge tissue to the gemmule coat, and arrives at the somewhat amusing conclusion that they are pushed from one position to the other by the sponge cells, much in the same way, I should imagine, as a colony of ants carries away bits of food which are too heavy a bundle for one. The presence of amphidiscs in the scleroblasts, both in the sponge tissue and in the gemmule coat, disposes of the necessity of such a supposition. It seems that it may be considered as finally established that the amphidiscs are carried to their ultimate position by the scleroblasts which secrete them.

In conclusion I wish to offer my sincerest thanks to Professor Weldon for the free use of his laboratory and all its resources, as well as for much invaluable assistance in relation to the literature of the subject; to Professor Minchin for reading the proof sheets; to the Government Grant Committee of the Royal Society for their kind and timely assistance; and to the Principal and Fellows of Jesus College, Oxford, for further help.

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21. Zykoff, W.—"Die Entwicklung der Gemmulä bei Ephydatia fluviatilis, Auct." 'Bull. Soc. Imp. Natur., Moscow' (1892), pp. 1—16, Taf, I, II; Abstract in 'Zool. Record,' vol. xxix (1892); also in 'Journ. Roy. Micr. Soc.' (1892), p. 378.

EXPLANATION OF PLATES 1—4,

Illustrating Mr. Richard Evans' paper on "Ephydatia blembingia, and the Development of the Gemmule in the same Species."

All the figures from 2—17 *a*, both inclusive, have been drawn with the camera lucida.

SIGNIFICANCE OF THE LETTERING.

am. wand. cell. = *fut. yo. cells.* Amœboid wandering cells which later on in the development become the yolk-cells. *Am. wand. cells* = *fut. col. cells.* Amœboid wandering cells which later on in the development become the columnar cells. *Amphid.* Amphidiscs. *chit. sept.* Chitinous septum. *col. cells.* Columnar cells. *col. lay.* Columnar layer. *d. m.* Dermal membrane. *in. chit. lay.* Inner chitinous layer. *nu.* Nucleus. *ou. chit. lay.* Outer chitinous layer. *mod. sclerob.* Modified scleroblast or spicule cell. *sclerob.* Scleroblast. *s. d. c.* Sub-dermal cavity. *spic.* Spicule. *sp. fib.* Spicule fibre. *tropho.* Trophophore. *v. s.* Vegetable support of the sponge. *yo. body* Yolk-body. *yo. cell.* Yolk-cell.

PLATE 1.

FIG. 1 (× $1\frac{1}{5}$).—Ephydatia blembingia growing on vegetable supports, *v. s.*

FIG. 2 (× 28).—A section showing diagrammatically the structure of the sponge, especially the dermal membrane (*d. m.*) and its supports, which are traversed by the spicule fibres; the large sub-dermal cavities (*s. d. c.*); the poorly developed spicule fibres (*sp. fib.*), the great number of spicules, both amphioxea and amphidiscs, scattered about more or less loosely in the tissues, and the gemmules (*gem.*) which are never found aggregated together in groups.

FIG. 3 (× 225).—A representation of the various kinds of spicules. *a—e* represent the amphioxea which on the one hand form the spicule-fibres, and on the other hand lie about loosely in the sponge-tissues. *f* is one of the spicules which are seen grouped together in fig. 7. *g—i* are the amphidiscs. *g* and *h* are fully formed; *i* and *k* are intermediate in size, while *l* and *m* represent the early stages.

Fig. 3 was drawn from specimens cleaned with nitric acid.

FIG. 4 (× 575).—A more highly magnified representation of some of the spicules shown in Fig. 3. *a* represents *m* of Fig. 3; *b* is an enlarged drawing of *g* of Fig. 3; and *c* shows the end of the amphidisc when looked down upon. Note the serrated edge.

FIG. 5 (× 665).—A young amphioxea shown inside the scleroblast. It should be specially compared with the Fig. 6, *a*, which represents the early stage of the amphidisc. Both are drawn on the same scale, and on comparison it will be clearly seen that the spicule represented in Fig. 6, *a*, cannot be a young amphioxea.

FIG. 6 (× 665).—A representation of an early stage, *a*; intermediate stage, *b*; and a fully-grown stage, *c*, of the amphidisc. Each spicule is situated inside the scleroblast which produced it. The cells themselves are amœboid in character.

FIG. 7 (× 225).—This figure represents a side view of the somewhat problematic body discussed in the footnote on p. 74, and described as having a basket-like form. It is covered with spicules (*sp.*) of the amphioxea type belonging to group *b* (compare Fig. 3, *f*).

PLATE 2.

FIG. 8 (× 565).—A representation of the cells which later on in the development become the yolk-cells of the gemmule. On the left side of the figure the cells are seen coming together in virtue of their power of wandering. On the right side a portion of a much bigger group of cells is seen. The group in question is situated in the interior of one of the columns of tissue which support the dermal membrane. Note that the protoplasm of the cells is absolutely clear, and that the nucleus is not vesicular.

FIG. 9 (× 950).—A representation, more highly magnified, of a slightly later stage than that shown in Fig. 8. Note that the protoplasm is becoming slightly granular.

FIG. 10 (× 950).—A representation of a slightly later stage than that shown in Fig. 9. Note that the protoplasm has become still more granular.

FIG. 11 (× 130).—A representation of the gemmule at a stage slightly later than that shown in Fig. 10. Note that the granules in the cells have increased to a considerable extent in size, but that their internal structure is not so dense as at later stages. Also note that a great number of cells possessing different characters are aggregated round the central cells (*yo. cells*). These cells seem to have been derived from the sponge at a later stage than the yolk-cells, and constitute a different class both as regards their origin and fate.

FIG. 11 *a* (× 950).—A representation, more highly magnified, of a portion of the section shown in Fig. 11. Note the yolk-cells (*yo. cells*) with their yolk-bodies (*yo. body*), also the columnar cells which are as yet only an aggregation of amœboid wandering cells with food vacuoles and yolk-bodies, also the "trophophores" which possess clear protoplasm, but no yolk-bodies or food vacuoles.

Fig. 12 (× 130).—A representation of a stage slightly later than that shown in Fig. 11. Note that the amœboid wandering cells outside are becoming columnar, especially on one side, also that the outer cells are becoming separated, from those which are becoming columnar, to form a kind of loose membrane (cf. Fig. 14).

Fig. 12*a* (× 950).—A representation, more highly magnified, of the amœboid wandering cells, which are becoming columnar on one side of the section shown in Fig. 12, and also, on the left side of the figure, of the cells which begin to form the loose membrane.

PLATE 3.

Fig. 13 (× 130).—A representation of a stage slightly later than that shown in Fig. 12. Note that the columnar layer is complete except over a small portion at which, later on, the pore will appear, and at which alone the "trophocytes" (*troph.*) are found; also that the inner chitinous layer is being formed from the same position, i. e. from the bottom, as the columnar layer of cells was formed.

Fig. 13*a* (× 1150).—A representation of a portion of a section similar to the one shown in Fig. 13. Note the group of six trophocytes (*tropho.*) which are making their way to the interior of the gemmule, also the "trophocyte" which has already reached that position. This group is an unusually large one, and was found opposite one of the strands of tissues which pass from the sponge tissue to the loose membrane which surrounds the gemmule. The "trophocytes" appear to travel chiefly along these strands of tissue.

Fig. 13*b* (× 1150).—A representation of a similar portion to that shown in Fig. 13*a*. Note that the "trophocytes" (*troph.*) are scattered about among the amœboid wandering cells, which later on become the columnar cells. On the left of the figure is seen the end of one of the strands of tissue along which the trophocytes travel. Also note the trophocyte on the right of the figure. This cell is just passing among the yolk-cells (*yo. cell*).

Fig. 13*c* (× 960).—A representation of a portion from the top of a section similar to the one shown in Fig. 13. Note that the "trophocytes" (*troph.*) are arranged chiefly outside the columnar cells, but that there are some situated still among the yolk-cells. Those outside have already travelled out while those inside are in the process of doing so. In the lower part of the section there were no "trophophores."

Fig. 13*d* (× 1150).—A representation of a yolk-cell from the same section as Fig. 13*c*. Note the large, central, vesicular nucleus (*nu.*).

PLATE 4.

Fig. 14 (× 130).—A representation of a stage slightly later than that shown in Fig. 13. Note that the columnar layer is complete, and that the loose membrane which surrounds the gemmule is almost complete and is connected by strands of tissue, in which amphidiscs are found, with the general sponge structure. Further, note that there are neither amphidiscs nor trophophores among the columnar cells.

Fig. 14*a* (× 960).—A representation, more highly magnified, of a portion of the columnar layer and loose membrane shown in Fig. 14.

Fig. 15 (× 130).—A representation of a stage slightly later than that shown in Fig. 14. Note that there are amphidiscs (*amphi.*) over half the extent of the columnar layer of cells, while the other half is as yet free of them. Also note that the inner chitinous layer is still incomplete at the point where the pore will be formed; but the yolk granules in the interior are fully formed. The outer end of the amphidiscs extends beyond the columnar cells.

Fig. 15*a* (× 665).—A representation, more highly magnified, of the amphidiscs (*amphid.*) lying inside the modified scleroblast (*mod. sclerob.*) which has lost its nucleus, and of the columnar cells lying between the amphidiscs. Note that the inner ends of the columnar cells are becoming clear, as is shown in Fig. 15.

Fig. 15*b* (× 665).—A representation of the inner chitinous layer (*in. chit. lay.*), the columnar layer (*col. cell.*), the loose membrane, and one of the strands which pass to the membrane, the whole being taken from the region intermediate between the one occupied by amphidiscs and the one devoid of them in a gemmule similar to that shown in Fig. 15. Both amphidiscs are shown inside their scleroblasts, and the one in the strand of tissue outside is being carried to its position alongside the other amphidisc among the columnar cells.

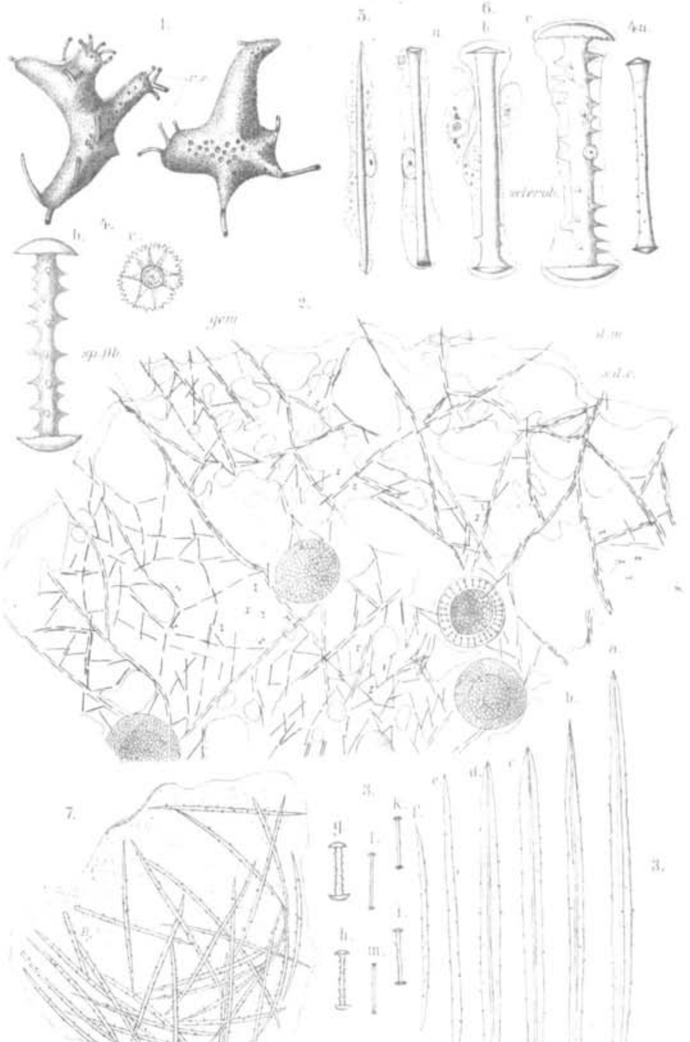
Fig. 16 (× 130).—A representation of a stage slightly later than that shown in Fig. 15. Note that the spicular layer and the inner chitinous layer are complete. Further, note that the columnar cells have passed out and are situated externally to the outer end of the amphidiscs, their inner ends having been modified to form the parenchyma-like substance situated between the amphidiscs.

Fig. 16*a* (× 665).—A more highly magnified representation of the gemmule coat shown in Fig. 16. Note that the columnar cells are forming the outer chitinous layer and are becoming separated preparatory to their passing back to the sponge.

Fig. 17 (× 130).—A representation of the fully-developed gemmule, showing the contents passing up the pore as far as the chitinous septum (*chit.*

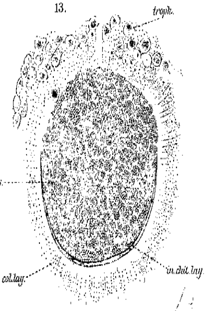
sept.); the inner chitinous layer (*in. chit. lay.*); the amphidiscs (*amphid.*); and the not strongly developed outer chitinous layer (*ou. chit. lay.*). Note that the columnar cells are no longer present.

FIG. 17*a* (× 665).—A more highly magnified representation of the gemmule coat, showing the several parts indicated in the description of Fig. 17. Note the parenchyma-like structure of the substance situated between the amphidiscs (*inter. lay.*), that is, the intermediate layer of the protective coat of the gemmule.

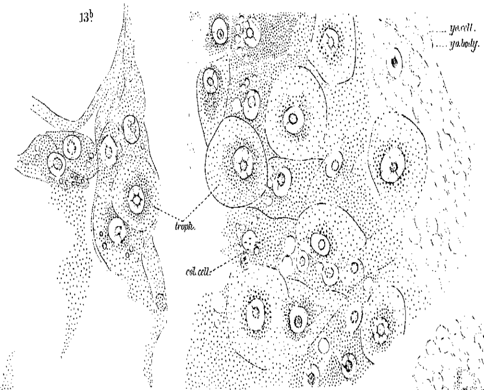



8.
d.m.
am.wand.cells = fut. yo.cells
11a.
am.wand.cells = fut.yo.cells
9.
10.
am.wand.cells = fut.yo.cells
12a.
troph.
col. cells
col. cell.
11.
12.
yo. cells
troph.
col.cells.
am.wand. cells.fut. col.cells
yo.cells
yo.cells
yo.body

13.

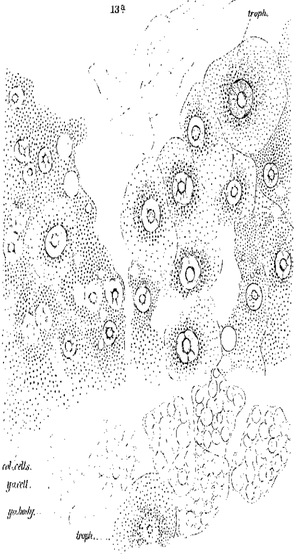


13b

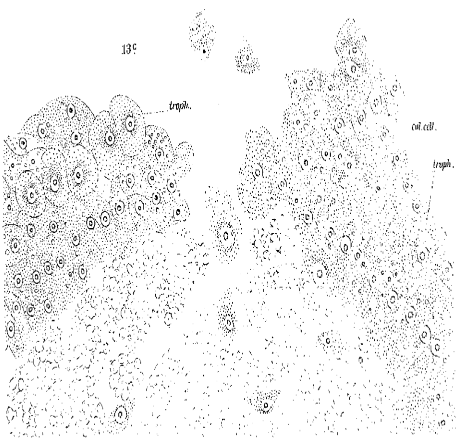


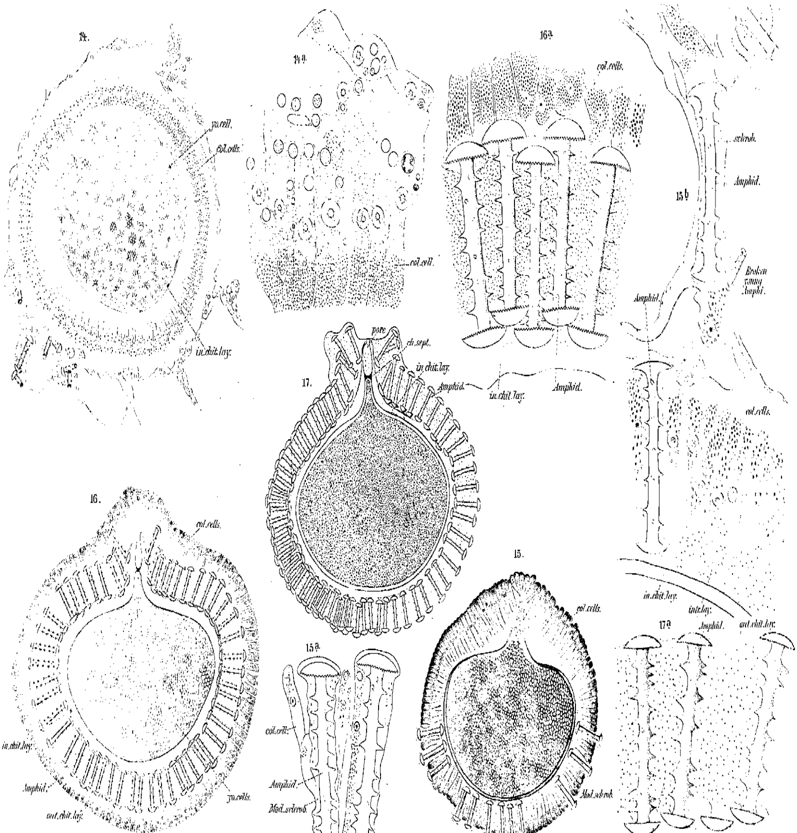
13d

13a



13c




14.
yo.cell.
col.cells.
in.chit.lay.
14 a.
col.cell.
16 a.
col.cells.
Amphid.
in.chit.lay.
Amphid.
15 b
sclerob.
Amphid.
Broken young Amphi.
Amphid.
col.cells.
in.chit.lay.
int.lay.
17.
pore
ch.sept.
in.chit.lay.
Amphid.
16.
col.cells.
in.chit.lay.
Amphid.
out.chit.lay.
yo.cells.
15 a.
col.cell.
Amphid.
Mod.sclerob.
15.
col.cells.
Mod.sclerob.
Amphid.
17 a
Amphid.
out.chit.lay.
in.chit.lay.
int.lay.