

**On the Early Stages in the Development of
Flustrella hispida (Fabricius), and on the
Existence of a "Yolk Nucleus" in the Egg
of this Form.**

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With Plates 22—25.

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INTRODUCTION.

IN the following paper an attempt has been made to trace out the earlier stages in the development of *Flustrella hispida*. The larva of this familiar Bryozoan has previously

been studied by Hincks (13), Redfern (22), Joliet (14), Barrois (2), and Prouho (20), but the first four authors studied only the living larva, of which Barrois has given an excellent account with numerous figures, while Prouho, who has described the later stages of the larval history in detail, has paid but little attention to its earlier stages.

The research was undertaken at the suggestion of Dr. S. F. Harmer, with the view to determine whether that structure overlying the internal sac in the mature larva of *Flustrella* is to be regarded as a stomach comparable to that which he had described (11) as present in *Alcyonidium*. As the work proceeded it has seemed expedient somewhat to extend its scope, and to follow out the history of the egg from its first appearance; and the presence of a "yolk-nucleus" being detected, this structure has also been studied in some detail.

The work, which has been conducted partly at Cambridge, partly at Plymouth, and partly at Brighton, has been greatly assisted by a grant from the Government Grant Committee of the Royal Society, to whom my best thanks are due. I would also take this opportunity to express my thanks to Dr. Harmer for his kindly interest and criticism, and for the loan of some of his own preparations of later larval stages, and for permission to reproduce one of his drawings (Pl. 25, fig. 65 *a*), to Dr. E. J. Allen for granting me the use of a table at the Plymouth Laboratory of the Marine Biological Association, to the authorities of Newnham College, Cambridge, for permission to work at the Balfour Laboratory, and to Prof. J. Graham Kerr, Dr. E. G. Gardiner, and Mr. W. Wallace, for advice on technical points.

MATERIAL AND METHODS.

Collecting Material.—The material on which this paper is based was collected at the following places and dates: Swanage, March, 1902; Totland Bay, Isle of Wight, April, 1903; Plymouth, February to April, 1903, February to July, 1904; Brighton, May to July, 1903.

At each of the above places *Flustrella hispida* grows abundantly between tide marks on *Fucus*, and occasionally also on other Algæ. The colonies form characteristic, dark, mossy-looking patches encrusting the algal fronds: the *Fucus*, when growing near low water mark, is often almost entirely covered with this Bryozoan, but nearer high water mark the *Flustrella* is not nearly so abundant, nor is it so well developed. Young colonies occur mainly on *Fucus* of the same season's growth.

For the study of the larval development, colonies of one or two seasons' growth taken from close to low water mark have proved the most suitable. Such colonies contain abundance of spermatozoa or of ova and larvæ, according to the season. Older colonies contain larger masses of dead zoœcia, larvæ being relatively less abundant, while these latter are wanting in such colonies as are presumably of the current year's growth. Again, in colonies taken near high water mark, larvæ and eggs are comparatively scarce, thus suggesting that the conditions of life are not so favourable as at a lower tide level, possibly because the colonies are uncovered by the water for a considerable part of the day. *Flustrella* colonies containing ripe reproductive elements or larvæ may be recognised by the presence of numerous dark-brown blotches.

The reproductive period commences early in February and continues until the beginning of August. Generally speaking, it appears that spermatozoa are abundant in February and March, and that they are not in evidence after the latter month. Young ova are scarce early in February and increase in number in March; during this latter month segmenting eggs and young larvæ are also abundant and some mature larvæ are present. Sections of the younger portion of a colony taken in March have shown the presence of segmenting eggs and larvæ in the older zoœcia, and of spermatozoa, together with a few young ova, in the younger zoœcia near the apex of the colony (Pl. 22, fig. 1). The maximum development of young embryos

is from April to June, while in July and August larvæ in advanced stages are still abundant, but at this time young stages and ova are rare. On the other hand, even in March and April, colonies may be found which contain only mature larvæ. It should be stated that in any one colony the majority of the larvæ are at approximately the same stage of development.

Flustrella hispida will readily live in standing water for from one to five days according to the time of year, while in running water it has been kept in good condition for over a week,¹ but it is best whenever practicable to work with quite fresh material, as the larvæ appear less healthy in colonies which have been kept even a few days.

Methods.—The work has been done partly by the study of entire larvæ and partly by means of sections. The larvæ were examined both in the living state and after fixation. For the latter purpose large numbers of eggs and larvæ were removed from the colonies and preserved and in addition portions of colonies were fixed entire in order to study the larvæ in situ.

Fixing Reagents.—The preservatives used were:—

- (1) Cold saturated solution of corrosive sublimate, with the addition of 5 per cent. glacial acetic acid.
- (2) 100 parts 5 per cent. chromic acid, with five drops of glacial acetic acid.
- (3) Flemming's solution.
- (4) Hermann's solution.
- (5) Dr. Allen's chromo-nitro-osmic acid mixture.
- (6) Acetic alcohol containing sublimate to saturation.
- (7) Kleinenberg's solution.

Preserving Larvæ in situ.—For preserving larvæ in situ it has been found best to cut the colonies with the seaweed on which they are growing into small portions, and to immerse these in the fixing solution for some time to allow

¹ At Millport, owing to the exceptional purity of the water, there appears to be no difficulty in keeping *Flustrella* alive in the tanks for a quite indefinite period.—R. M. F.

complete penetration. As soon as possible after fixation the colonies were removed from the *Fucus* and after washing transferred to 70 per cent. alcohol. Chromo-acetic acid and corrosive acetic have given the best results when dealing with material fixed in bulk.

Isolated Larvæ.—The removal of larvæ from the colonies is best effected by slicing off the front wall of the colony with a sharp razor; the larvæ lie immediately below this wall, enclosed in the tentacle-sheath of the polypides, and they can then be readily removed by means of a scalpel. Before attempting to preserve the larvæ a considerable amount of washing is necessary in order to free them from a mucus-like substance in which they lie imbedded.

The best fixing reagents for the isolated larvæ appear to be corrosive acetic, and acetic alcohol saturated with sublimate; chromo-acetic acid sometimes gives good results; and the fixing reagents containing osmic acid have proved useful, especially in the study of entire eggs and larvæ before clearing. Material preserved in chromo-acetic requires very prolonged washing and frequently proves difficult to stain.

Entire Eggs and Larvæ.—The external characters of isolated eggs and larvæ of all stages have been studied during life. After fixation the larvæ were again examined unstained in 70 per cent. alcohol, and were then stained either in borax carmine followed by acid alcohol, or in safranin, and re-examined. After clearing with cedar-wood oil or clove oil—both of which reagents gave good results—the larvæ were either mounted entire in Canada balsam or imbedded in paraffin for sectioning. Staining the larva with borax carmine after acetic alcohol and corrosive sublimate or corrosive acetic brings out the nuclear spindles and also the yolk nucleus very clearly, and so greatly assists in the interpretation of the external appearance of a segmenting egg. In a few cases the embryo was removed from Canada balsam after having been examined and drawn, and was imbedded in paraffin for sectioning.

Preparation of Sections.—Sections were made both of the isolated larvæ and of portions of colonies containing larvæ. The watch-glass method of imbedding was found the most convenient, especially when dealing with isolated larvæ. Groups of from twenty to thirty isolated larvæ were imbedded en masse and sectioned, sections thus being obtained in a variety of planes. The larva at nearly all stages has a definite axis, which renders it possible to orientate it and so to obtain sections in any desired plane. To serve as a guide in determining in which direction unorientated larvæ had been sectioned, a set of standard sections was prepared by carefully orientating single larvæ which had been first studied entire.

Finally, portions of colonies were imbedded and cut with the larvæ in situ. To insure thorough impregnation, it was found best to soak the material in xylol for about a week, then to leave it in a mixture of xylol and paraffin for about six hours in a warm place, such as the tray of the water-bath, and finally to transfer to pure paraffin for about an hour. In cutting such material great difficulty has been experienced owing to the fact that the larvæ lie close under the front wall of the colony: this wall, being beset with chitinous spines, renders it difficult to imbed and to cut in such a manner that the razor encounters none of the spines when passing through the larvæ, since the chitin is sufficiently hard to notch the razor, thereby of course causing the section to tear. This difficulty is less marked in the case of transverse than of longitudinal sections.

Staining.—The most useful stain for sections appears to be Heidenhain's iron hæmatoxylin, followed by eosin dissolved in 90 per cent. alcohol. By this treatment the structure of the yolk nucleus and of all nuclear bodies is brought out very clearly. Borax carmine and safranin have given good results, and double staining with methyl blue and eosin has also been found useful. Mayer's alcoholic cochineal, picronigrosin, hæmatoxylin with a few drops of Kleinenberg's solution, hæmatoxylin and methyl orange have also been utilised; Mayer's mucicarmine was used for the detection of mucus.

Spermatogenesis and Oogenesis.

In *Flustrella hispida* the zoecium is hermaphrodite, but the spermatozoa are chiefly developed earlier in the year than the ova. In February and early in March, however, ova and spermatozoa are found to occur simultaneously in the zoecium; the spermatozoa are in such cases fully developed, while the ova are immature. Pl. 22, fig. 1, shows a section through a very young colony taken early in March; ova are seen to be present in one zoecium, and in the anterior portion of the same zoecium spermatozoa also occur.

Early in February the colonies assume a very puffed and spotted appearance, large dark-brown patches becoming visible. On cutting a section of such a colony these brown patches are found to be due to the presence of an immense number of spermatozoa, which can be removed in the same way as the ova by slicing off the front wall of the colony. As has already been described by Calvet (8), the spermatozoa are developed from the mesenchyme lining the lateral walls of the zoecium, and the mother cells lie in masses close to the front wall in the region of the tentacle sheath (Pl. 22, fig. 1, *T*). When ripe the spermatozoa have the typical flagellate form. Frequently masses of spermatozoa are seen to be lying with their heads imbedded in a central mass of protoplasm, and with their tails vibrating at the periphery. No attempt has been made at present to work out the details of spermatogenesis. The spermatozoa decrease in number towards the middle of March, and they are not in evidence after the end of that month.

The ovary lies at a point to the rear of and at a lower level than that at which the spermatozoa are developed. It is situated on a funicle passing from the mesenchymatous lining of the lateral zoecial wall to the intestine (Pl. 22, fig. 1, *Ov*). At first the young ovary shows no indication of cell-walls, but consists merely of a protoplasmic mass containing numerous large nuclei (Pl. 22, figs. 1 and 4, *N*). Cell-walls subsequently arise in this protoplasmic mass, four or five of the ovarian

cells being differentiated in this manner (Pl. 22, fig. 5) and developing into ova, while the remainder give rise to the follicle cells. As maturation proceeds, the follicle cells increase in number and appear to grow in among the primitive ova, so that when these latter are ripe each ovum is surrounded by a follicular membrane (Pl. 22, figs. 2 and 3, *Fo.*).

Generally speaking, all the ova contained in the ovary are of about the same age (Pl. 22, figs. 2-5).

THE MATURATION OF THE EGG: THE YOLK NUCLEUS.

The chief point of interest in the process of the maturation of the egg of *Flustrella* is the appearance of a "yolk nucleus," apparently homologous with that type described by van Bambeke (1) as occurring in the egg of *Pholcus phalangioides*. The existence of a yolk nucleus does not appear to have been hitherto recorded in any of the Ectoproctous Bryozoa, although a similar structure has been figured by Braem (4 and 6) and by Kraepelin (17) as being present in the egg of *Plumatella* among the Entoprocta.

The Yolk Nucleus.—The history of this body in the egg of *Flustrella hispida* is briefly as follows:

In very young ovaries in which the ovarian cells are only just recognisable, there are, in addition to the germinal vesicle, certain darkly-staining granules surrounding the nucleus and lying in close contact with the latter (Pl. 22, figs. 4-5; Pl. 23, fig. 29, *Y.N.*). These granules at a later period coalesce to form the structure, which, following van Bambeke, may best be termed the "yolk nucleus." They originate, as has been said, quite close to the germinal vesicle, and their appearance is so very similar to that of certain intra-nuclear elements as to suggest that they have originated from the nucleus. In fact, in one case (Pl. 22, fig. 5; Pl. 23, fig. 29) these granules seemed to be in process of actually passing out from the germinal vesicle. The granules at this period are homogeneous in appearance, and their behaviour with any of

the staining reagents employed exactly resembles that of the chromatin granules of the nucleus.

Shortly after this stage these extra-nuclear granules coalesce, and eventually come to form a crescentic body—the yolk nucleus—which (Pl. 22, figs. 8, 11, 13) becomes surrounded by what appears to be a clear space; and at an earlier stage a similar clear zone frequently also occurs around individual granules, or groups of granules, prior to their complete coalescence (Pl. 22, figs. 6-7, *x*). This clear area may abut directly on to the germinal vesicle, or it may be separated from it by a thin layer of protoplasm (Pl. 22, figs. 11, 13, 14). At about this time also, vacuoles begin to appear in the body of the yolk nucleus (Pl. 22, figs. 11 and 13).

The appearance of the yolk nucleus as seen in sections depends, of course, upon the point through which the section is taken (Pl. 22, figs. 9-12). In figs. 9 and 10 the position of the yolk nucleus is marked only by the clear area which usually surrounds it; in fig. 11 the yolk nucleus shows a crescentic cross section, and it is seen to contain numerous vacuoles; while in Pl. 22, fig. 12, it has the appearance of a cap overlying the nucleus.

The yolk nucleus gradually passes from a crescentic to a hemispherical form (Pl. 22, fig. 14); and its growth being proportionately more rapid than that of the egg as a whole, this hemispherical form becomes still more marked in later stages, so that in certain sections the yolk nucleus may even appear as a complete ring encircling the nucleus (Pl. 22, fig. 15). The vacuoles increase in number, and frequently contain crystalloid bodies at this stage (Pl. 22, figs. 13, 14, *cr.*).

The yolk nucleus next loses its originally homogeneous appearance and shows signs of degeneration. This is evidenced by the appearance of a peculiar reticulate structure, the substance between the meshes staining less deeply than the network (Pl. 22, figs. 15, 16). The yolk nucleus then loses its regular outline (Pl. 22, figs. 16, 17), and it finally breaks up into more or less finely-divided, darkly-staining fragments, surrounded each by a clear zone. The process of fragmenta-

tion of the yolk nucleus appears to vary somewhat in its details in different eggs (Pl. 22, figs. 18-21). The fragments of the yolk nucleus retreat towards the periphery of the egg, and there for a time form a disconnected ring of darkly-staining substance, each segment of which is still surrounded by a clear zone (Pl. 22, figs. 22, 23). Soon after this the fragments of the yolk nucleus lose their identity, and at about the same time the first indications of the true yolk make their appearance as small granules, which are scattered about in the cytoplasm (Pl. 23, fig. 24, Y). At the same time vacuolisation of the cytoplasm occurs; the yolk spherules come to lie within these vacuoles and rapidly increase in size until they appear to occupy the entire egg (Pl. 23, fig. 25).

The whole process of the growth and disintegration of the yolk nucleus and the formation of the yolk takes place some time before the egg is released from the ovary.

That the yolk nucleus is a true cell organ, and not merely an appearance due to the coagulation of proteid by the fixing reagent used, is proved by the fact that it is visible in the living egg as a dark mass surrounding or overlying the nucleus.

The nature and origin of the clear zone or space which has been described as surrounding the yolk nucleus are somewhat doubtful. It may possibly contain a fluid, but all attempts to prove by means of staining reagents that this is so have failed; and it would seem perhaps more probable that this apparent space is only an artificial one caused by the contraction of the substance of the yolk nucleus during fixation. This latter view is supported by the fact that the clear zone has not been detected in the living egg, nor in those eggs (Pl. 23, figs. 26, 27) which have been fixed by chromo-nitrosomic acid, and also by the fact that the shape of the supposed space corresponds so exactly with that of the yolk nucleus which it surrounds.

With the view to determine whether the formation of oil globules, which has been described and figured by von Bamberke (1) as preceding yolk formation in the case of *Pholcus*

phalangioides, occurs also in the egg of *Flustrella*, a number of eggs were preserved with Dr. Allen's chromo-nitro-osmic mixture; these were sectioned without previous bleaching, so that any fatty matter might remain intact, but the results obtained have so far proved somewhat difficult of interpretation. Even in very young eggs in which the yolk nucleus is still in quite the initial stages of development, large drops of a fatty substance are found to be present in the region of the developing yolk nucleus; and as the egg enlarges, the number of the fatty drops also increases, but the latter always remain in the immediate neighbourhood of the yolk nucleus, while similar globules are also visible within that body itself (Pl. 23, figs. 26, 27). The amount of fatty material increases as degeneration proceeds. It collects especially towards the periphery of the egg, and at the same time the development of the yolk commences. The two substances increase in quantity side by side, so that the mature egg has the appearance of a mass of yolk spherules interspersed with fat globules (Pl. 23, fig. 28). This condition, the presence of fat and yolk side by side, continues so long as any yolk is discernible in the larva—that is until after the degeneration which precedes metamorphosis has commenced. At no period are the fat globules arranged in any definite relation to the nucleus. It is hoped that it may be possible to elucidate this question of the relation of the fat globules and the food yolk to one another and to the yolk nucleus, by investigating in greater detail the history of the yolk nucleus in the eggs of such other Bryozoa in which it may prove to be present.

Prolonged treatment with xylol will cause the fat globules to disappear from the yolk.

The Germinal Vesicle.—To determine whether there is any connection between the yolk nucleus and the germinal vesicle, the latter body has also been carefully studied. Pl. 23, fig. 29, shows a young germinal vesicle to which particles of the yolk nucleus are in close approximation, and it will be seen that one of these latter appears to be actually in process

of passing out through the nuclear membrane. The chromatin network is somewhat dense, and it has at its nodes deeply staining granules, which are similar in their appearance to the original elements of the yolk nucleus, while a faintly staining substance occupies its interstices.

The germinal vesicle at first grows relatively more rapidly than the egg as a whole, and the chromatin network becomes more attenuated (Pl. 23, figs. 30-32); but this latter fact is probably due rather to the increased size of the nucleus than to any emission of chromatin from it. No further changes take place in the germinal vesicle until after the formation of yolk has been completed, although the irregular contour of the nuclear membrane observed in certain sections (Pl. 23, figs. 24, 25) during the period of yolk formation may possibly denote amœboid movements in connection with the latter process: such amœboid movements have been described by Bambeke (1) in the case of the egg of *Pholcus phalangioides*.

After the completion of yolk formation the chromatin network begins to thicken (Pl. 23, fig. 33), and the substance between its meshes now stains more deeply (Pl. 23, fig. 34). The nucleolus also becomes relatively very large, and at the same time the nuclear membrane loses its regular outline (Pl. 23, figs. 34, 35). These processes continue until all trace of the chromatin network has disappeared, and the nucleus stains uniformly throughout (Pl. 23, fig. 36). At this stage, which is immediately prior to that of the formation of the polar bodies, the nucleus begins to decrease in size relatively to the rest of the egg and becomes amœboid: the nucleolus is still present.

The Nature and Function of the Yolk Nucleus.—The term "yolk nucleus" has been applied by various authors to bodies which appear to be totally different in their origin, development, and appearance, and which would seem to have only this much in common, that all have been regarded as being in some way connected with the phenomenon of yolk formation. In the present instance,

the body described approximates very closely to that type of yolk nucleus described by Bambeke (1), Crampton (9), Wallace (26), and Calkins (7) for the eggs of *Pholcus phalangioides*, *Molgula manhattensis*, *Zoarces viviparus*, and *Lumbricus* respectively.

It is not proposed in the present paper to enter into a detailed consideration of the former work bearing on this subject. A very complete bibliography of the yolk nucleus is furnished by the papers of Jordan (15), Mertens (18), Henneguy (12), Calkins (7), Wilson (27), Bambeke (1), and Crampton (9).

As has already been stated, the yolk nucleus of the egg of *Flustrella hispida* is closely comparable with that type described by Bambeke, Calkins, Crampton, and Wallace, but on comparison with these apparently closely related bodies certain points of difference may be noted.

Bambeke (1) describes in the egg of *Pholcus phalangioides* a type of yolk nucleus which corresponds closely in its appearance and in its mode of growth and degeneration with that occurring in *Flustrella hispida*. He recognises four stages in the history of the yolk nucleus and nutritive yolk of the egg of *Pholcus*: (a) the appearance of small, darkly staining granules, which he believes to be of nuclear origin, and which coalesce to form a crescentic structure containing vacuoles, with included crystalloid bodies, and surrounded by a clear zone; (b) the degeneration of the yolk nucleus; (c) the appearance of oil drops; (d) the formation of the true yolk. The most important difference between Bambeke's account of the yolk nucleus and the present one is that in the case of the egg of *Pholcus* the nucleus appears to take an active part in the process of yolk formation, and that the yolk nucleus itself gives rise to the vitellus by first undergoing a metamorphosis leading to the formation of oil globules, these latter becoming resorbed by the protoplasm from which the true yolk is then elaborated. Now, as has already been pointed out, the presence of oil globules in the case of the egg of *Flustrella hispida* bears but little

obvious relation to the process of yolk formation. In this form oil globules are present in very young eggs, both in the yolk nucleus itself and also scattered throughout the surrounding protoplasm; and, although they are certainly present in increasing numbers as the yolk develops, they do not disappear with the completion of yolk formation, but persist throughout larval life. So far, also, in *Flustrella* it has not been possible to confirm Bambeke's views as to the importance of the nucleus as a factor in yolk formation. The irregular shape of the nuclear membrane, which is sometimes observed in the early stages of yolk formation in the egg of *Flustrella* (Pl. 23, fig. 24), may possibly indicate amœboid movements; but these have no apparent reference to the distribution of the oil drops, which collect chiefly towards the periphery of the egg rather than in the immediate neighbourhood of the nucleus; and neither the oil globules nor the yolk spherules show the radial arrangement which is described by Bambeke.

Crampton (9), in his paper on the early history of the Ascidian egg, described a yolk nucleus of similar type in the egg of *Molgula manhattensis*; and he attempted to determine its chemical nature by differential staining. The general account given by Crampton of the history of the yolk nucleus in *Molgula* agrees with the above description of this structure in *Flustrella*. Crampton, among other stains, made use of Heidenhain's iron-hæmatoxylin, and states that this stain is taken up by the chromatin of the germinal vesicle, but that it has no effect on the yolk nucleus; he also mentions the difficulty experienced in washing out this stain from the yolk after yolk formation, without at the same time decolourising nuclear structures. In the case of the egg of *Flustrella*, iron-hæmatoxylin stains both the yolk nucleus and the chromatin elements of the nucleus with equal intensity, and a similar difficulty with regard to the washing of eggs containing yolk has been experienced. Pl. 23, fig. 25, shows a section of an egg in which, even after prolonged washing with iron-alum, the centres of many yolk spherules remained darkly stained. Crampton's researches led him to

the conclusion that the yolk nucleus, although of nuclear origin, does not consist of chromatin, as has been maintained by several writers, owing to its apparent origin from and similarity of appearance to the chromatin granules of the germinal vesicle; but that it is either purely albuminous or consists of nucleo-albumin containing a large percentage of nucleic acid constituents. According to this author, accounts of the origin of the yolk from the cytoplasm at a point distant from the nucleus, or from several centres, or of its formation all over the egg, refer only to its later history, and do not take into account an earlier stage, which is marked by the appearance of the yolk nucleus. If Crampton's view be the correct one, a study of younger eggs should in such cases lead to the discovery of this supposed early stage.

Wallace (26) describes in the egg of *Zoarces* a yolk nucleus of the type occurring in *Flustrella*; and by Mr. Wallace's courtesy, I have been enabled to examine many of his preparations and drawings, which show that the yolk nucleus agrees in almost all respects with that of *Flustrella hispida*. Wallace, however, found that fixing reagents containing nitric acid dissolved out the yolk nucleus, while, as has already been stated, this is not the case with the egg of *Flustrella*. Further, Wallace agrees with Bambeke that the formation of oil-drops precedes true yolk formation.

The Yolk Nucleus and Yolk Formation in Bryozoan Eggs.—As has been stated earlier in this paper, no yolk nucleus has hitherto been noted in the eggs of any of the Ectoprocta.¹ Among the Entoprocta, however, a structure, which appears to be similar to the yolk nucleus occurring in the egg of *Flustrella hispida*, has been figured by Kraepelin and by Braem as present in the ovum of *Plumatella*.

Kraepelin (17) points out that shortly before the egg

¹ It has lately been possible by the courtesy of Miss A. Heath to examine some preparations of a species of *Alcyonidium* which contained young ova. The material, which was collected at Millport in September, 1905, contained abundance of young ova, and these were found to contain "yolk nuclei" apparently similar to that which has been described in *Flustrella*.

ripens, the germinal vesicle becomes more or less surrounded by a differentiated mass of protoplasm, which persists until after fertilisation, but subsequently vanishes.

Braem (4 and 6) mentions the presence of a light zone of protoplasm surrounding the nucleus, which makes its appearance in the ovum at an early stage. This zone is at first not clearly defined from the outer layer of the protoplasm, but later on it becomes sharply demarcated. The outer zone takes no part in segmentation. Small, darkly-staining bodies next arise in the outer zone of the protoplasm. These bodies are of varying sizes, and are each surrounded by a clear area. Braem states that they are similar in appearance to the nucleolus, and suggests that they resemble the latter in chemical composition. He believes that they originate from the outer zone of the cytoplasm, but similar bodies may also occur in the inner zone. Braem suggests that these bodies in the ovum of *Plumatella* may be homologous with the yolk nucleus of certain other animal ova.

In the case of the eggs of *Flustrella hispida*, the facts so far obtained all point to the conclusion that the yolk nucleus is a true cell organ originating from the nucleus at an early stage in the history of the ovum; that after undergoing a series of changes it finally disintegrates; and that the process of disintegration is in some manner intimately connected with yolk formation. So far as can be seen, the germinal vesicle plays no direct part in the formation of the yolk. It has not yet been practicable sufficiently to investigate the presence or otherwise of a yolk nucleus in the eggs of other Bryozoa, and the results of this investigation must be reserved for a future paper.

The Centrosphere.—No trace of a centrosphere has yet been detected in the ovum of *Flustrella hispida*.

The Polar Bodies.—The formation of the polar bodies had only been observed in one case. The egg (Pl. 24, fig. 51) had already passed into the tentacle sheath, and the first polar body (*P.B.*) was lying on the surface of the ovum, while the second was in process of formation.

FERTILISATION, AND THE PASSAGE OF THE EGG INTO THE
TENTACLE SHEATH.

Fertilisation.—The act of fertilisation has not yet been observed, only such ova as were either preparing for, or had already undergone, fertilisation having been obtained. It has already been stated that the spermatozoa and ova ripen for the most part at different times, and no ripe spermatozoa have been observed in the zoöcial cavity after March.

Passage of the Eggs into the Tentacle Sheath.—The development of the ova takes place within the tentacle sheath, and the eggs pass from the zoöcial cavity into the tentacle sheath in the interval between the degeneration of a polypide and the formation of a new bud. This bud attains complete maturity, and not, as has been described by Joliet (14) in the case of *Valkeria cuscuta*, only partial development. As has been mentioned, from four to five eggs generally ripen at the same time, and these enter the tentacle sheath together and develop side by side. As development proceeds, the larvæ, while still enclosed in the tentacle sheath, increase in size, and gradually come to fill the entire cavity at first occupied by the polypide, and the latter now ceases to exist. Those zoöcia occupied by advanced larvæ contain large quantities of a slimy, mucus-like substance, which surrounds the developing embryos.

SEGMENTATION, AND THE FORMATION OF THE GERMINAL LAYERS.

The earlier stages of segmentation have been studied in detail in the hope of elucidating the problem of the origin and subsequent history of the mesoderm and of the endoderm in this form. So far, however, the formation of mesoderm has not been definitely traced, but it is hoped that it may be possible to determine this point more satisfactorily at a later date.

The Primitive Cleavages.—After fertilisation the egg becomes separated from the vitelline membrane by a wide space (Pl. 23, fig. 37). The first cleavage (Pl. 23, figs. 37-42)

divides the egg into two symmetrical halves, each containing equal quantities of yolk: it is completed in about twenty minutes. This division is followed by another, in a plane at right angles to the first, dividing the egg into four spheres, which are to all appearance equal in all respects (Pl. 23, fig. 43; Pl. 24, fig. 52).

To simplify the following account of the cell-lineage of *Flustrella*, these four first-formed cells have been distinguished in the figures by the letters A, B, C, and D. Cells arising from these are denoted by the letter of the particular cell from which they have been derived, with the addition of a negative index to indicate the generation to which the cell belongs, and of a positive index to denote the number of cells in that generation at the moment of the formation of any given cell, and the order of their formation: thus A_4^4 denotes the fourth cell derived from A in the fifth generation.¹

The 8-cell Stage.—The four cells A, B, C, and D, again dividing in a plane at right angles to each of the former divisions, give rise to eight cells: of these, the four lower cells are larger, and contain more yolk than the four upper ones (Pl. 23, fig. 44).

The polar bodies are seen in the living egg to lie on the surface of the smaller cells, and these smaller cells and their derivatives always lie on the upper surface of the egg, which later on becomes the dorsal or aboral surface of the larva.

The 12-cell Stage.—The four small upper cells next divide (Pl. 23, figs. 45 *a-b*) each into two unequal cells by a cleavage lying at an angle of 45° to the primitive cleavage plane. The ring of small cells which is thus formed becomes shortly afterwards rearranged into two rows of four cells each, so that the embryo now consists of two parallel series of small cells overlying the four large yolk-laden ones.

Owing to this new arrangement of the embryonic cells, it is

¹ In view of the use which has been made of a somewhat similar system of notation by other authors, it should be stated that the symbols here used have been adopted only for the sake of clearness, and that they have no reference to any of the theories of cell genesis which have been put forward.

now possible to distinguish between a longitudinal (long) and a transverse (short) axis of the larva. At a later period, however, cell division is found to occur more rapidly along the transverse than along the longitudinal axis, and the larva assumes a spherical shape; so that it is not therefore practicable to establish any direct correlation between the long and short axes of these early stages and the long and short axes of the mature larva.

The 16-cell Stage.—Each of the four large lower cells next divides into two cells of unequal size. The plane of cleavage is a vertical one lying more or less at right angles to the long axis of the embryo, and the four central cells formed are the larger; so that now the oral surface of the larva also consists of two rows, each of four cells, which immediately underlie those of the upper series. The larva (Pl. 23, figs. 47*a-c*) is now, therefore, built up of sixteen cells arranged in four parallel rows, which are disposed in two tiers; that is, it consists of an upper, aboral tier of two rows, each of four cells, and of a lower, oral tier of larger cells, also of two rows, each of four cells. All of these sixteen cells belong to the fifth generation (the unsegmented ovum being regarded as the first generation). The four central cells of the oral series are much larger than the lateral ones of the same series, and these again are larger than those of the upper tier.

At this stage, the segmentation cavity (*S.C.*) becomes visible, and it is noteworthy that it contains a substance which stains feebly with eosin, safranin, borax-carmin, etc.

It may be mentioned that the arrangement of the cells in two tiers of two parallel rows each of four cells is characteristic of many Polyzoan larvæ at the 16-cell stage.

The 20-cell Stage.—The next division (Pl. 23, fig. 48) also takes place in the four large central oral cells, which each again divide into two unequal cells— $A \frac{1}{6}$ and $A \frac{2}{6}$, $B \frac{1}{6}$ and $B \frac{2}{6}$, $C \frac{1}{6}$ and $C \frac{2}{6}$, and $D \frac{1}{6}$ and $D \frac{2}{6}$ —cleavage taking place in a vertical plane at right angles to the previous division. The embryo (Pl. 23, figs. 49*a-c*) now consists of

twenty cells; that is to say, there is an aboral series of eight small cells which are disposed in two parallel rows and belong to the fifth generation; below these a ring of eight larger cells, four of which— $A \frac{3}{6}$, $B \frac{3}{6}$, $C \frac{3}{6}$, and $D \frac{3}{6}$ —are of the fifth generation, and four others— $A \frac{1}{6}$, $B \frac{1}{6}$, $C \frac{1}{6}$, and $D \frac{1}{6}$ —of the sixth generation; while the oral surface is formed by four large central cells— $A \frac{2}{6}$, $B \frac{2}{6}$, $C \frac{2}{6}$, and $D \frac{2}{6}$ —which are also of the sixth generation, and which project upwards into the segmentation cavity (Pl. 24, fig. 53). The aboral series of cells contains, generally speaking, less yolk than the lower series of cells, of which the before-mentioned four large oral cells are particularly rich in yolk (Pl. 24, figs. 53, etc.).

The 32-cell Stage.—The beginning of the next stage is shown in Pl. 24, figs. 54 *a-b*, and 55 *a-b*. The small cells composing the two rows of the upper tier first divide horizontally, giving rise to four rows each of four cells, and arranged in two tiers (Pl. 23, fig. 50 *b*, and Pl. 24, figs. 54 *a-b*, 55 *a-b*). This cleavage is followed almost immediately by the division of the four cells— $A \frac{3}{6}$, $B \frac{3}{6}$, $C \frac{3}{6}$, and $D \frac{3}{6}$ —which were the first cells originally budded off by the large oral cells. These four cells divide (Pl. 24, figs. 54 *b*, and 55 *b*) by a vertical cleavage lying at an angle of 45° to the primitive cleavage of the segmenting ovum. The embryo (Pl. 23, figs. 50 *a-c*, and Pl. 24, fig. 56) now, therefore, consists of thirty-two cells, which are all of the sixth generation, and which are arranged in the following manner. The aboral surface of the larva is composed of sixteen small cells, disposed in two tiers, each tier consisting of two parallel rows of four cells each. Below these sixteen aboral cells is a ring of twelve larger cells, overlying and partially surrounding the four large oral cells, which still occupy the lower surface of the larva. The upper halves of these four oral cells, being surrounded by the ring of twelve intermediate cells, are thus enclosed by them within the segmentation cavity, and the latter is almost entirely obliterated at this stage (Pl. 24, fig. 55 *a*).

The Formation of Endoderm.—Pl. 24, fig. 57, illustrates a somewhat later stage than that just described. The small aboral cells and the ring of larger cells underlying these have divided, and the latter are now seen to enclose about two thirds of the four large central oral cells within the segmentation cavity. These four large oral cells have also again divided, but this time the plane of cleavage has been a horizontal one; and the four larger upper segments resulting from this division lie within the segmentation cavity, and represent the primitive endoderm. The four lower segments retain their original oral position.

The Ectoderm.—From this time onwards, cell division becomes less regular, and for a time at least, it takes place more rapidly in the transverse than in the longitudinal direction, so that the larva tends to become spherical in form. The small aboral cells divide repeatedly, forming the aboral ectoderm; while the ring of larger intermediate cells, which were shown to have been initially derived from the large oral cells, $A \frac{2}{4}$, $B \frac{2}{4}$, $C \frac{2}{4}$, $D \frac{2}{4}$, in like manner give rise to the oral ectoderm. A study of the living embryo and of sections (Pl. 24, figs. 58–60) shows that the cells of the aboral ectoderm tend to remain, throughout embryonic development, smaller than those of the oral ectoderm, the difference becoming more marked as development proceeds, but it is not possible at this, or at any later stage, to distinguish any definite ring of cells which can be correlated with the ciliated ring of the mature larva. The true origin of this structure will be dealt with later, but it may not be out of place to say here a few words in order to explain how it has come about that the existence of such a ring of cells has been supposed by Barrois and other authors to occur at this stage. It is true that observations made on the entire egg at this period, especially when it is viewed from the aboral surface, give somewhat the appearance of there being an equatorial ring of cells, but this appearance is a deceptive one. As stated above, an equatorial ring of twelve cells actually did exist at the thirty-two-cell stage, but the cells of this ring, as has already been described, have

divided to form the oral ectoderm. The cells of the oral ectoderm are all equal in size, though they are larger than those of the aboral ectoderm. A study of sections shows that the ring-like appearance seen at this stage, when viewing the egg from the aboral surface, is due simply to this difference in size between the cells of the oral and aboral surfaces causing the former to project out beyond the latter (Pl. 24, figs. 58-60). In later stages this appearance is enhanced by the development of the aboral groove or mantle cavity just above the line of junction of the two sets of ectoderm cells (Pl. 25, fig. 61). Barrois (2) was misled by this deceptive appearance, and, not having checked his observations on the living egg by the examination of sections, published figures (2, pl. xii, fig. 6), purporting to represent the larva at this stage, showing a prominent equatorial ring of large cells, while the remaining cells are represented as being of the same size both above and below this supposed ring.

The Mesendoderm.—Owing to the rapid growth of the oral ectoderm, the four central oral cells eventually become surrounded and enclosed in the segmentation cavity, thus forming, together with the four cells originally segmented off, eight mesendoderm cells in all. These eight cells divide repeatedly, and give rise to a solid mass of tissue, which in time comes to fill the segmentation cavity. Pl. 24, figs. 57-60, represent stages in this process. The asymmetrical position of the mesendoderm cells in Pl. 24, fig. 58, seems somewhat peculiar, and might well be considered to be abnormal, but the arrangement shown in this figure has been met with in all the sections examined, and must, therefore, be regarded as quite normal.

The Blastopore and Gastrulation.—The oral ectoderm remains for some time open at the point of enclosure of the mesendoderm cells, and this opening may be regarded as the blastopore (Pl. 24, figs. 58, 59, *Bl.*). All trace of the blastopore vanishes in later stages (Pl. 24, fig. 60). It is impossible to say whether the enclosure of the second set of mesendoderm cells be due to the pressure of the surrounding

cells—that is to say, whether it represents a process of invagination, or whether the oral ectoderm simply grows over and encloses the mesendoderm. In either case the process may be regarded as a form of gastrulation, but no trace of an archenteron has been observed.

Later Segmentation Stages.—At the stage represented in Pl. 24, fig. 60, except that the oral surface is slightly flattened, the embryo has become almost spherical in form. Aborally, it is covered by a layer of small, flat, ectoderm cells, while the oral surface is composed of large, high cells; and from these latter a mass of endoderm or mesendoderm cells projects into the segmentation cavity, which is still visible at this stage. A shallow groove, bounded below by a slight protuberance, is noticeable on the exterior of the larva rather above the equatorial line. A study of sections shows that this groove marks the junction of the oral and aboral ectoderm, and that it is due to the difference in the size of the cells in this region. It is to be again noted that there are no specially enlarged cells, such as have been described by Barrois, in this region.

Up to this stage in the development, the oral surface has been relatively larger and more convex than the aboral, but now cell division becomes more active on the latter surface, so that it in turn becomes relatively larger and more convex than the oral surface. Owing to the increased growth of the aboral surface, the slight groove and the protuberance already noticed now lie below the equatorial line, and in sections this groove is now seen to be the result, partly of the difference in size of the ectoderm cells at the line of junction of the oral and aboral series, and partly of an actual bulging out of the oral ectoderm (Pl. 25, fig. 61, *M.C.*). This encircling groove represents the initial stage of the aboral groove or mantle cavity.

The stage thus briefly described marks the close of the segmentation period. The larva now passes gradually from a spherical to an elongated form, which is typical of the mature larva. When viewed in section (Pl. 25, fig. 61), the

difference in size and character between the cells of the oral and aboral ectoderm is now seen to be very marked, and the mesendoderm forms a solid mass entirely obliterating the segmentation cavity.

Summary.—To sum up the results obtained from the foregoing study of the cell division:—

Four equal cells—A, B, C, D—result from the primitive cleavages. These four cells divide and give rise to eight cells, arranged in two series, four small upper cells, and four large lower cells, each of which series is destined to play a distinct part in the subsequent history of the cell layers. The four small upper cells, A_4^1 , B_4^1 , C_4^1 , D_4^1 , give rise only to the aboral ectoderm. The four large lower cells, A_4^2 , B_4^2 , C_4^2 , D_4^2 , give rise in part to the oral ectoderm, in part to a tissue, which may for the present be best termed “mesendoderm.” The cells from which the oral ectoderm is derived are cut off from the four large oral cells by two successive vertical divisions at right angles to each other. The four large oral cells then divide a third time horizontally, and the four upper products of this cell division pass into the segmentation cavity, and there give rise to the primitive endoderm, or rather, mesendoderm. Owing to the rapid growth of the surrounding ectoderm, the four remaining oral cells also eventually become enclosed within the segmentation cavity, giving in all eight mesendodermic cells, which, by subsequent division, eventually form a solid mesendodermic mass.

A true blastopore, which does not close until after the formation of a considerable mass of mesendoderm, is present during the stages referred to.

The formation of mesoderm has not been actually observed; it seems probable, however, that the mesoderm is derived from the primitive mesendodermic mass at a later stage than those hitherto dealt with, and this point will be further discussed (p. 467) when dealing with the degenerating larva.

In view of the fact that the most essential of the larval organs are developed from the oral ectoderm, it seems of

especial interest to note that this tissue is from the first distinct from the aboral ectoderm; the difference in the relative sizes of the oral and aboral ectoderm cells, which is so marked in early stages, is noticeable throughout larval life.

Comparison with other Bryozoa.—As yet, comparatively little has been written on the early development of the Bryozoa; the most important papers on the subject being those by Barrois, Joliet, Repiachoff, Vigelius, Harmer, Braem, Prouho, and Calvet.

Barrois has published descriptions of the early stages of several Bryozoan larvæ, and among others he dealt with (2) that of *Flustrella hispida*. His observations, however, were made solely upon entire eggs and larvæ. His descriptions and figures are in entire agreement up to the thirty-two-cell stage with the general results described in the present paper, but he does not make any special mention of the lineage of these cells, and he was also unable to study the formation of the endoderm. As has already been pointed out, Barrois erroneously describes a later stage, in which both dorsal and ventral ectoderm are said to be composed of small equisized cells, the two series being separated by an equatorial ring of large cells (2, pl. xii, fig. 6), and he figures this ring as being present in all subsequent stages. Had Barrois sectioned any of his material, instead of relying solely on external appearances, he would have seen that no equatorial ring of single cells, such as he described, is present at any of the stages figured.

In another paper (3) Barrois describes the enclosure, in *Schizoporella unicornis*, of four primitive endoderm cells by epiboly. He also describes the formation of two bands of mesoderm, which at a later period fuse with the endoderm to form a single mesendodermic mass.

Repiachoff (23), in a paper on *Tendra zostericola*, also describes the endoderm as originating by the enclosure and division of four large oral cells. He, however, states that the process is followed in *Tendra* by the formation of an

archigastrula with an opening to the exterior: no such stage occurs in *Flustrella hispida*.

Vigelius (25) studied the early stages in the development of *Bugula*, and noted the presence of four large dorsal cells within the segmentation cavity and the subsequent division of these to form the endodermic mass.

Harmer (11), in his paper on *Alcyonidium*, mentions the presence of a blastopore as occurring in that form.

Prouho, in a paper (20) on *Flustrella hispida*, makes no mention of the early stages of this form. In a later paper (21), however, he describes the formation of endoderm in the *Cyphonautes* larva. He states that at the thirty-two-cell stage the embryo is flattened along an axis perpendicular to the plane of the first segmentation, the four oral cells being larger than the other cells and especially rich in yolk; these four large cells subsequently become enclosed by the rapid growth of the ectoderm. In the case of *Alcyonidium albidum*, Prouho records that the endodermic cells each divide into two before becoming enclosed by the ectoderm, and he defines the blastopore in this case as the point at which the ectodermic cells close over the four large cells.

Braem, in his account (5) of the embryology of *Paludicella Ehrenbergi*, points out that segmentation in the larva of this species is total and almost equal. At the eight-cell stage the segmentation cavity is visible, and the four upper cells are somewhat larger than the lower cells. The sixteen-cell stage is similar to that which occurs in *Flustrella hispida*. At the thirty-two-cell stage the embryo is spherical, and forms a typical blastula; the blastula has a large segmentation cavity, and the cells of the vegetative pole are larger than those of the animal pole and do not increase in number as rapidly. The four central cells of the vegetative pole then become surrounded by the ectoderm cells, and are pressed into the segmentation cavity. After their enclosure, these four cells segment to form other endoderm cells, which multiply and give rise to a many-celled archenteron opening to the exterior. A cell layer containing muscle fibres lies

between the ectoderm and endoderm, and Braem considers that this tissue may represent the mesoderm, and that it is possibly derived from the initial cells separated off from the original four large endoderm cells.

Calvet (8), in his general account of the embryology of Cheilostomes and Ctenostomes, makes the general statement that segmentation is equal and regular up to the thirty-two-cell stage; but, from the above description of the process in *Flustrella hispida*, this is obviously not invariably the case. Calvet describes the formation of endoderm as taking place in a manner similar to that in which it originates in *Flustrella*, and he arrives at the conclusion that the endoderm arises partly by endocytulation, partly by planulation.

The endoderm, therefore, appears to originate in a similar manner in *Flustrella* and in the few other Bryozoa in which its formation has so far been investigated.

THE DEVELOPMENT OF THE LARVAL ORGANS.

The formation of mesendoderm being completed, the first traces of larval organs soon appear in the shape of a two-fold invagination of the oral ectoderm, and this is followed soon after by a third invagination at a point anterior to the previous ones. Both ectoderm and mesendoderm cells have by this time begun to lose their definite cell outlines.

Ectodermic Organs.—The internal sac is the first organ to be formed. It arises as a median invagination of the oral ectoderm; and in Pl. 25, fig. 62, it is seen as an elliptical space (*I.S.*) communicating with the exterior by means of a narrow opening. The cells lining this sac are short and flat, and their nuclei lie close to the periphery. These cells are seen to contain large globules of a substance closely resembling the yolk spherules in their general appearance and in their reactions to staining reagents; and drops of this substance are also found to be exuding from many cells (Pl. 25, fig. 62 *g.*). This substance subsequently disappears entirely

from the cells of the internal sac (Pl. 25, fig. 65). The significance of its appearance will be discussed in a later section.

The second oral ectodermic invagination is destined to form an organ representing the pharynx; it occurs in front of that from which the internal sac arises, and appears at a slightly later period. The cells bounding its opening are large and high, their nuclei lie on the side nearest the opening, and the cells themselves are much vacuolated. The vacuoles (Pl. 25, figs. 62-64) in the walls of the pharynx are filled with a substance similar in appearance to that which has been described as occurring in the cells of the internal sac (Pl. 25, fig. 62), and, as in the latter case, this substance also entirely disappears at a later stage (Pl. 25, fig. 65). From its mode of origin, the pharynx is to be regarded as a true stomodæum.

The pyriform organ arises, at a somewhat later period than the internal sac and the pharynx, as an oral ectodermic invagination anterior to the latter organs. In Pl. 25, figs. 62, 63 *a*, the internal sac and the pharynx are shown well developed, while at this stage the pyriform organ is represented only by a slight invagination (*Py.*).

The aboral organ, the "calotte" of French authors, is at the stage figured in Pl. 25, fig. 62, already visible as a thickened mass (*Ca.*) of aboral ectoderm overlying the pharynx and provided with numerous nuclei. From this organ a delicate network of fibres and nuclei passes to the developing pyriform organ (Pl. 25, figs. 62, 63 *a*).

Organs of Mesendodermal Origin.—The three-fold ectodermic invagination leading to the formation of the internal sac, pharynx, and pyriform organ has the effect of compressing the mesendoderm into a solid mass, which lies in the posterior part of the larva with its anterior end overlying the inner end of the pharynx (Pl. 25, fig. 62, *Ed.*). This mesendodermal tract consists of a mass of yolk spherules with scattered nuclei and it rapidly loses all trace of definite cell structure. At a slightly later period, the mesendodermic mass becomes hollowed out and forms what,

from its origin, appearance, and position, there can be no doubt is a vestigial stomach (Pl. 25, figs. 63 *a-b*, 64, 65 *a-c*, *St.*). This supposed stomach is identical in its structure with that described by Harmer (11, p. 446, Pl. XXVII, figs. 1, 2) as occurring in the larva of *Alcyonidium* only, unlike the stomach in that case, communication with the exterior by the pharynx is never established. As is the case also in *Alcyonidium*, the stomach is lined simply by a protoplasmic mass in which nuclei and numerous yolk spherules are embedded. The lining epithelium shows no trace of any glandular character, and the organ itself remains entirely vestigial and disappears before free life commences.

In describing the development of the stomach in the *Cyphonautes* larva, Prouho (21) says: "La depression orale devient de plus en plus profonde, pendant que la région aborale devient de plus en plus conique . . . Les cellules de la masse endodermique, qui se sont un peu multipliées pendant que l'embryon subissait les modifications ci dessus, se desposent autour d'un axe et forment une masse, pleine, allongée, oblique, dont une extrémité vient s'appuyer contre le fond de l'invagination orale; cette masse endodermique, définitivement rejetée à l'arrière de la larve occupe d'ores et déjà la position de futur estomac."

The organ which has been regarded as the stomach in the larva of *Flustrella hispida* has been shown by its origin and position to correspond to the endodermic mass which ultimately gives rise to the stomach in the *Cyphonautes* larva, and it is also closely comparable to the rudimentary stomach of the larva of *Alcyonidium*, except for the fact that it never communicates with the pharynx. The exceedingly slight development of the stomach in *Flustrella* is easily explained when the short duration of free larval life, and the correlated abundance of the supply of food yolk are taken into consideration.

According to Prouho (20) the organ which has been here termed the pharynx is to be regarded as a rudimentary intestine much less differentiated than that of *Alcyonidium*. But, as

we have seen, this structure is a true stomodæum, and from the above account it will be evident that the alimentary apparatus is much better developed in *Flustrella* than Prouho had supposed to be the case, in that it really comprises both stomach and pharynx, though both it is true are of a rudimentary character.

It will be remarked, when viewed in relation to the vestigial character of the stomach, that the considerable development of the pharynx is somewhat surprising; but may it not be that the pharynx has, in accordance with the exigencies of larval life, assumed another function? Mention has already been made of the globules which are present in the vacuoles of the cells of the pharynx. At about the time that these disappear, the larva becomes freed from the vitelline membrane, and at the same time the slimy, mucus-like substance already noted becomes very abundant around the embryos. Possibly, therefore, the pharynx has assumed a glandular function, and it may well be that this mucus-like substance has been derived from the globules previously contained in the pharyngeal cells. Attempts to prove, by treatment with Mayer's mucicarmine, that the globules in the pharynx and internal sac really consist of mucus, have so far given negative results; and this point must, therefore, for the present remain unsolved. The suggestion here put forward may also afford an explanation of the existence of the drops previously noticed as exuding from the cells of the internal sac.

In Pl. 25, figs. 63 *b*, 64, are shown two bands of tissue marked * which appear to be budding off from the sides of the mesendodermic mass. Their significance will be discussed later.

THE MATURE LARVA.

In Pl. 25, figs. 65 *a-c*, the larva is represented at a stage shortly anterior to that at which degeneration commences. It is now enclosed in a chitinous bivalve shell, and has escaped from the vitelline membrane. From this point

onwards the development of the larval organs, with the exception of the stomach, has been already so fully described by Prouho (20) as to make detailed description unnecessary.

The internal sac has become much elongated, so that it now occupies the greater part of the interior of the larva. Its lining epithelium has become much thickened, and has already lost all trace of cell structure.

The pharynx has altered somewhat in appearance, owing to the loss of the large globules already described.

The pyriform organ is now fully developed. On the exterior two depressions are noted—an anterior depression (Prouho's "fossette supérieure") and a posterior one (Prouho's "fente ciliée"), and between these lies a tuft of cilia, the "papille du plumet vibratile." Internally, corresponding to the "fossette supérieure," is the "système glandulaire supérieure," which consists of a single mass of cells lying in the longitudinal axis of the larva, while, similarly corresponding to the "fente ciliée," are two masses of cells, which are placed in the transverse axis of the larva, one on either side of the posterior part of the "système glandulaire supérieure," and which represent the "système glandulaire inférieure."

The aboral organ (Pl. 25, fig. 65 *a*) at this stage is fully developed. It consists of a tuft of long cilia arising from the thickened patch of aboral ectoderm which has already been mentioned. This organ is best seen in the living larva, in which it is visible protruding between the two valves of the shell; and connecting the aboral organ and the pyriform organ is seen the neuromuscular cord. In the living larva the jerking movements of the neuromuscular cord are distinctly visible, but it has not been possible to draw any conclusions from these movements as to the functions of either the pyriform or the aboral organs. The structure of the aboral organ and neuromuscular cord have already been fully described by Prouho (20). As stated by this author the nerve-muscle tract, on reaching the pyriform organ, breaks into three strands, one of which passes to that organ

between the cells of the two glandular systems, the other two passing to the cells of the ciliated crown.

The ciliated crown (the "couronne" of French authors), first visible at this stage, does not consist of a single ring of cells as it has been described in other Bryozoan larvæ, and as figured by Barrois (2) for *Flustrella hispida*, but of two or three rows of cells, as is shown in Pl. 25, figs. 65 *a-c* (*C.*). All trace of definite cell walls in the ectoderm has vanished by this time, but certainly the ciliated crown contains at least three series of nuclei, corresponding presumably to originally three rows of cells.

The cells from which the cilia originate lie rather below the aboral groove, and can at will be retracted within the valves of the shell (Pl. 25, fig. 66). Later on the ciliated crown certainly does have the appearance described by Prouho (20), of a single series of flat discoidal cells with long vibratile cilia, each imbedded in a cuticle, and prolonged below this into a triangular mass of protoplasm (Pl. 25, fig. 66); each of these cells contains a single large nucleus: this appearance does not, however, arise until quite late in larval life. No traces of the ciliated crown, or of any specially enlarged cells, are visible before the larva escapes from the vitelline membrane, despite Barrois' assertion to the contrary. It is evident, therefore, that in *Flustrella* the ciliated crown is formed by a series of cells, and that it is only late in larval life that these unite to form a single row of large cells.

The chitinous bivalve shell is developed as a secretion of the aboral ectoderm, and is closely adherent to the latter.

The aboral groove, which is now strongly developed, occupies its original position above the ciliated crown (Pl. 25, figs. 65 *a-c*, *M.C.*). It is best seen in transverse section.

The stomach, as a result of the growth of the larva, has become much more elongated, and, owing partly to this, partly to the absorption of food material, the yolk spherules surrounding the stomach have become much reduced in number.

THE DEGENERATING LARVA.

Shortly after the stage which has been briefly described above, the degeneration of the larval organs commences. The initiation of this process is shown in Pl. 25, fig. 66.

The internal sac becomes enormously thickened, and its lining ectoderm highly modified, especially near the opening of the sac to the exterior, where it now assumes a granular character. The pharynx gradually loses its cellular structure; the pyriform organ is at this stage still fully developed, as are also the aboral organ and neuromuscular tract. The ciliated crown, as has already been stated, consists at this stage of a single ring of large cells. The stomach has practically vanished, its position being marked only by a number of scattered yolk spherules and of nuclei lying between the internal sac and the aboral ectoderm.

Origin of the Mesoderm.—Among the above-mentioned scattered elements of mesendodermic origin, and apparently developed from them, occur fibres (Pl. 25, fig. 66, *M*), which are presumably muscular in nature. Others of these supposed muscular fibres occupy the former position of, and are probably developed from, the lateral bands of tissue previously noted as budding off from the stomach. It is this mesendodermic mass of yolk spherules, nuclei, and fibres which Prouho (20) regards as representing the mesoderm in *Flustrella hispida*. He maintains that the mesoderm occurs as a distinct layer of cells lying beneath the aboral ectoderm, generally thickened at the aboral pole; that a similar membrane overlies the internal sac, and also that all the muscular elements of the larva are of mesodermic origin. But, as has already been shown, it is quite impossible at any early larval stage to differentiate the mesoderm from the general endodermic mass, and Prouho's so-called "mesoderm" is, therefore, undoubtedly not simply mesoderm, but endoderm, or perhaps rather mesendoderm, since it is, of course, possible that in this endodermic mass lie enclosed the elements of the future mesoderm, from which the muscles are

now formed. The two bands of tissue apparently budding off from the main mesendodermic mass have already been noticed; possibly these may represent the true mesoderm, which in that case would appear to develop only late in larval life. Prouho, owing to the fact that he only studied the later stages of larval life in which the stomach had already begun to disappear, was led to regard the whole mesendodermic mass as mesoderm, or as mesoderm containing some endodermic cells, but he himself suggests the necessity of a detailed study of the larva in its earlier stages in order to decide the correctness of his view.

THE ALIMENTARY CANAL IN LARVAL ECTOPROCTA.

The presence of an alimentary canal in Ectoproct larvæ has been described by Barrois, Vigelius, Repiachoff, Prouho, and Harmer.

Barrois (2 and 3) at one period regarded larval Ectoprocts as having an alimentary canal, owing to his mistaken supposition that the internal sac represented the stomach. Later on he saw reason to modify his views, but he pointed out and depicted (2, pl. vii, fig. 13) an invagination between the pyriform organ and the internal sac, which he regarded as the rudiment of a pharynx. He therefore believed that many Ectoprocts were originally provided with a digestive tube, and that in cases where no such system is formed the endoderm arises as in other Bryozoa where the digestive system is better developed, but that later on it degenerates to a mass of yolk spherules filling the interior of the embryo.

Vigelius (25) stated that in the early larval stages of *Bugula*, a slight split occurs in the mesendodermic mass filling the interior of the larva; this split he regards as representing a primitive stomach, which however, plays a purely passive rôle, and does not open to the exterior. Later on the supposed stomach vanishes, and the endoderm forms a simple cell mass.

Repiachoff (23) describes for *Tendra zostericola* the

formation of an archigastrea with a definite opening to the exterior: this communication becomes obliterated at a later stage. The fully-developed larva has a stomach which communicates with the exterior, similar to that which has been described by Harmer (11) as present in the developing larva of *Alcyonidium*.

Prouho (21) describes the presence of a functional alimentary canal in the "Cyphonautes" larva, the stomach being developed from the internal endodermic mass in the manner described above. In an earlier paper on *Flustrella hispida* (20), he regards the pharynx as a rudimentary digestive tube less differentiated than that of *Alcyonidium*, and representing either an attempt to form a digestive organ or a vestige of one which has vanished.

Harmer (11) found, in a species of *Alcyonidium*, a definite alimentary canal closely resembling that occurring in *Flustrella hispida*, but communicating by a narrow opening with the exterior. In the same paper he points out the probability that a similar structure occurs in *Flustrella hispida*.

From its endodermic origin, its position, appearance, and mode of development, and from the close agreement in structure with the stomach described in *Alcyonidium*, there can be no doubt that the larva of *Flustrella hispida* possesses a vestigial stomach, and that this and the associated pharynx must be regarded as vestiges of a digestive system in which degeneration has proceeded a stage further than it has in *Alcyonidium*, since no communication with the exterior is ever established at any stage.

GENERAL SUMMARY.

The main points in the foregoing paper may be summarised as follows:—

(1) A "yolk nucleus" of the type described by Bambeck, as occurring in the egg of *Pholcus*, is present in the developing egg of *Flustrella hispida*.

(2) Segmentation and cell-lineage have been followed out in detail up to the 32-cell stage.

(3) The formation of the endoderm has been traced.

(4) The oral and aboral ectoderm are differentiated as early as the 16-cell stage, and remain quite distinct from that time onwards.

(5) The ciliated ring of the larva is formed by the coalescence of several originally distinct rows of cells, and not by the hypertrophy of a single row.

(6) A stomach, comparable to that of *Alcyonidium*, is present also in *Flustrella*.

REFERENCES.

1. BAMBEKE, C. v.—“Contributions à l'histoire de la constitution de l'œuf,” iii, “Recherches sur l'oocyte de *Pholcus phalangioides* (Fuessl.),” ‘Arch. Biol.’ vol. xv, pp. 511-598, pls. xxiii-xxviii, 1898.
2. BARROIS, J.—‘Recherches sur l'Embryologie des Bryozoaires,’ Lille, 1877.
3. BARROIS, J.—“Mémoires sur les métamorphoses des Bryozoaires,” i, “Métamorphose des Escharines,” ‘Ann. Sci. Nat.’ ser. 6, Zool., vol. ix, No. 7, 67 pp., pls. xiii-xvi, 1880.
4. BRAEM, FRITZ.—“Untersuchungen über die Bryozoen des süßen Wassers,” ‘Bibliotheca Zoologica,’ No. vi, Cassel, 1890.
5. BRAEM, FRITZ.—“Die geschlechtliche Entwicklung von *Paludicella Ehrenbergii*,” ‘Zool. Abz.’ vol. xix, pp. 54-57, February, 1896.
6. BRAEM, FRITZ.—“Die geschlechtliche Entwicklung von *Plumatella fungosa*,” ‘Zoologica,’ Heft 23, Stuttgart, 1897.
7. CALKINS, G. N.—“Observations on the Yolk-nucleus in the Eggs of *Lumbricus*,” ‘Trans. New York Acad.’ vol. xiv, pp. 222-230, 1895.
8. CALVET, L.—“Contributions à l'Histoire Naturelle des Bryozoaires Ectoproctes Marins,” ‘Trav. Inst. Zool. Montpellier,’ ser. 2, No. 8, 1900.
9. CRAMPTON, H. E.—“Studies upon the Early History of the Ascidian Egg.” Part I.—“The Ovarian History of the Egg of *Molgula manhattensis*,” ‘Journ. Morphol.’ vol. xv, suppl., pp. 29-56, pl. iii, December, 1899.
10. FOOT, K.—“Yolk-nucleus and Polar Rings,” ‘Journ. Morphol.’ vol. xii, pp. 1-16, pl. i, May, 1896.
11. HARMER, S. F.—“Sur l'embryogénie des Bryozoaires ectoproctes,” ‘Arch. Zool. Expt.’ ser. 2, vol. v, pp. 443-458, pls. xxvii, xxviii, 1887.

12. HENNEGUY, L. T.—“Le corps vitellin de Balbiani dans l'œuf des vertébrés,” ‘Journ. l'Anat. Physiol.’ vol. xxix, pp. 1-39, 1893.
13. HINCKS, THOMAS.—“Notes on British Zoophytes, with Descriptions of some New Species,” ‘Ann. Mag. Nat. Hist.’ ser. 2, vol. vii, pp. 353-362, pl. xiv, November, 1851.
14. JOLIET, L.—“Contributions a l'histoire naturelle des Bryozoaires des cotes de France,” ‘Arch. Zool. Expt.’ ser. 1, vol. vi, pp. 193-304, pls. vi-xiii, 1877.
15. JORDAN, E. O.—“The Habits and Development of the Newt (*Diemyctilus viridescens*),” ‘Journ. Morphol.’ vol. viii, pp. 269-366, pls. xiv-xviii, May, 1893.
16. KORSCHULT, E., and HEIDER, K.—“Lehrbuch der vergleichenden Entwicklungsgeschichte der wirbellosen Thiere,” “Allgemeiner Theil,” pt. i, Jena, 1902.
17. KRAEPELIN, KARL.—“Die Deutschen Süßwasser Bryozoen” II, “Entwicklungsgeschichtlicher Theil,” ‘Abhandl. Naturw. Ver., Hamburg,’ vol. xii, 1892.
18. MERTENS, H.—“Recherches sur la signification du corps vitellin de Balbiani dans l'ovule des mammifères et des oiseaux,” ‘Arch. Biol.’ vol. xiii, pp. 389-422, pl. xiv, 1895.
19. OSTROUMOFF, A.—“Zur Entwicklungsgeschichte der cyclostomen Seebryozoen,” ‘Mith. Zool. Stat. Neapel,’ vol. vii, pp. 177-190, pl. vi, April, 1887.
20. PROUHO, H.—“Recherches sur la larve de la *Flustrella hispida* (Gray),” ‘Arch. Zool. Expt.’ ser. 2, vol. viii, pp. 409-459, pls. xxii-xxiv, 1890.
21. PROUHO, H.—“Contribution à l'histoire des Bryozoaires,” ‘Arch. Zool. Expt.’ ser. 2, vol. x, pp. 557-656, pls. xxiii-xxx, 1892.
22. REDFERN, PETER.—“*Flustrella hispida* and its Development,” ‘Quart. Journ. Micr. Sci.’ ser. 1, vol. vi, pp. 96-102, 1858.
23. REPIACHOFF, W.—“Ueber die ersten embryonalen Entwicklungsvorgänge bei *Tendra zostericola*,” ‘Zeitschr. wiss. Zool.’ vol. xxx, suppl., pp. 411-423, pl. xix, May, 1878.
24. VAN DER STRICHT, O.—“La structure de l'œuf des Mammifères. Ire. Partie. l'Oocyte au stade de l'accroissement,” ‘Arch. Biol.’ vol. xxi, pp. 1-101, pls. i-iii, December, 1904.
25. VIGELIUS, W. J.—“Zur Ontogenie der marinen Bryozoen,” ‘Mith. Zool. Stat. Neapel,’ vol. vi, pp. 499-541, pls. xxvi-xxviii, June, 1886.

26. WALLACE, W.—“Observations on Ovarian Ova and Follicles in Certain Teleostean and Elasmobranch Fishes,” ‘Quart. Journ. Micr. Sci.,’ n. s., vol. 47, pp. 161-213, pls. 15-17, 1903.
27. WILSON, E. B.—“On Protoplasmic Structure in the Eggs of Echinoderms and Other Animals,” ‘Journ. Morphol.,’ vol. xv, suppl., pp. 1-25, pls. i, ii, December, 1899.

Postscript.—Since the above account of the yolk nucleus in the egg of *Flustrella hispida* was written, a paper (24) has appeared by O. van der Stricht on the yolk nucleus in the eggs of mammals. The account of the yolk nucleus given by this author appears in the main to confirm Bambeke's observations and views.

EXPLANATION OF PLATES 22—25,

Illustrating Mrs. R. M. Pace's paper on “The Early Stages in the Development of *Flustrella hispida* (Fabricius).”

REFERENCE LETTERS:

A. Aboral surface of larva. *A.Ec.* Aboral ectoderm. *Bl.* Blastopore. *C.* Ciliated crown, corona, or “couronne.” *Ca.* Aboral organ or “calotte.” *Ci.* “Fente ciliée.” *cr.* Crystalloid bodies. *C.T.* “Papille de plumet vibratile,” ciliated tuft. *Ec.* Ectoderm. *Ed.* Endoderm, or mesendoderm. *Fo.* Follicle cells. *Fs.* “Fossette supérieure.” *Fu.* Funicle. *G.* Chromatin granules. *g.* Globules in cells of pharynx and internal sac. *I.* Intestine. *I.S.* Internal sac. *M.* Muscle fibres. *M.C.* Aboral groove or mantle cavity. *Mc.* Mesenchyme lining of zoëcium. *N.* Nucleus. *n.* Nucleolus. *N.M.* Neuro-muscular cord. *O.* Oral surface of larva. *Oe.* Œsophagus. *O.Ec.* Oral ectoderm. *Ol.* Oil globules. *Ov.* Ovary. *P.B.* Polar bodies. *Ph.* Pharynx. *Py.* Pyriform organ. *R.* Rectum. *S.* Shell. *S.C.* Segmentation cavity. *S.G.* “Système glandulaire supérieure.” *S.G.* “Système glandulaire inférieure.” *St.* Stomach. *T.* Testes. *T.S.* Tentacle sheath. *vc.* Vacuoles in the yolk nucleus. *V.M.* Vitelline membrane. *z.* Space or clear zone surrounding the yolk nucleus. *Y.* Yolk spherules. *Y.N.* Yolk nucleus. *Z.* Zoëcial cavity. Those tissue tracts which are possibly to be regarded as being destined to give rise to the mesoderm are marked by a small asterisk.

In the figures of segmenting ova, the first four cells formed have been lettered, merely for convenience of reference, "A," "B," "C," "D," and the daughter cells arising from these latter are distinguished by index numbers, a negative index indicating the generation to which a particular cell belongs, and a positive index its place in that generation; thus $A\frac{5}{4}$ denotes the fourth cell derived from A in the fifth generation (see also p. 452).

All the figures, both of sections and of entire larvæ, have been drawn by the aid of the Zeiss camera lucida.

PLATE 22.

FIG. 1.—Transverse section of a young colony of *Flustrella hispida*, collected in March. The section is taken close to the apex of the colony, and shows the position of the testes on the lateral walls in the front of one zoecium, and the ovary lying on the funicle at the back of a neighbouring zoecium. $\times 65$.

FIG. 2.—Section of a young ovary lying on the funicle, showing the follicle cells commencing to grow in among the young ova. $\times 225$.

FIG. 3.—Section of an older ovary; the follicle cells are now seen to have increased in number. $\times 225$.

FIG. 4.—Section of a young ovary showing four young ova, the walls of which are still unformed. The yolk nucleus is present in the form of small dark granules. $\times 400$.

FIG. 5.—Section of a somewhat older ovary showing four ova around which the follicle cells have not yet developed. The dark granules which represent the yolk nucleus have increased in number, and in many cases are seen to lie in close contact with the membrane of the germinal vesicle. $\times 400$.

FIG. 6.—Section of a young ovum more advanced than those shown in Fig. 5. The granules of the yolk nucleus are seen to lie in four groups, each containing two granules, and three of these groups are surrounded by clear spaces (x). $\times 400$.

FIG. 7.—Section of a young ovum of the same age as that shown in Fig. 6. The granules of the yolk nucleus are grouped together and lie within a clear space. $\times 400$.

FIG. 8.—Section of a young ovum somewhat more advanced than that shown in the preceding figure. The majority of the dark granules have become fused to form a single large yolk nucleus lying within a clear space (x) separated from the germinal vesicle. Two vacuoles have already appeared in the yolk nucleus. $\times 400$.

FIGS. 9—12.—A series of four sections through a slightly older egg, to show the difference in the appearance of the egg according to the point at

which the section is taken. Fig. 9.—A section of the egg showing the commencement of the clear region in which the yolk-nucleus generally lies. In Fig. 10 the section passes through the nucleus and nucleolus, and the clear space has assumed a hemispherical shape. In Fig. 11 the yolk-nucleus is shown as a well-developed crescentic body lying in the clear space visible in the preceding figures, and in close contact with the germinal vesicle; the section passes through only the upper part of the nucleus. Fig. 12.—The yolk nucleus is seen lying in a clear space. The germinal vesicle is no longer visible, but lies underneath the cap formed by the yolk nucleus. $\times 400$.

FIG. 13.—A section of an ovum of about the same age as that illustrated in the preceding figures. The yolk nucleus has become rather more crescentic in form, and three large vacuoles are present and contain crystalloid bodies (*cr.*). $\times 400$.

FIG. 14.—A section of an egg of the same age as that shown in fig. 10. The yolk nucleus is more markedly hemispherical in form, and the number of vacuoles is larger than at previous stages. $\times 400$.

FIG. 15.—Section of an older egg. The yolk nucleus has now assumed the form of a ring surrounding, and in close contact with the germinal vesicle. An indication of approaching degeneration is seen in the reticulate structure of the yolk nucleus. $\times 400$.

FIG. 16.—A section of a still older egg in which the degeneration of the yolk nucleus has commenced. The yolk nucleus has lost its regular outline, and shows a markedly reticulate structure, the meshes of the network staining more deeply than the interlying substance. The outlines of the surrounding space have also become somewhat irregular. $\times 400$.

FIG. 17.—A section of an egg at a slightly later stage. The degeneration of the yolk nucleus has advanced considerably and is very marked. The body has completely lost its regular outline, and the boundary of the surrounding space is very irregular. $\times 400$.

FIG. 18.—Section of an older egg, showing the complete disintegration of the yolk nucleus, which has now assumed the form of numerous minute, darkly-staining granules which lie in a loose ring around the germinal vesicle $\times 400$.

FIG. 19.—Section of an egg of about the same age as that shown in fig. 18, but which exhibits a somewhat different method of fragmentation of the yolk nucleus. In this case the products of disintegration have assumed the form of dark, irregular patches lying within clear spaces, and forming an open ring around the germinal vesicle. $\times 400$.

FIGS. 20, 21.—Sections of eggs showing still other methods of fragmentation of the yolk nucleus. $\times 400$.

FIGS. 22, 23.—Sections of slightly older eggs in which the products of disintegration of the yolk nucleus have retreated towards the periphery of the egg, there forming an open ring of deeply staining patches, each of which lies within a clear space. $\times 400$.

PLATE 23.

FIG. 24.—Section of an egg showing the first appearance of the yolk in the form of minute globules scattered in the protoplasm. All trace of the yolk nucleus has vanished. $\times 400$.

FIG. 25.—Section of an egg in which the yolk is fully developed. The section was stained with iron hæmatoxylin, and the centres of many of the yolk spherules remained stained even after prolonged washing. $\times 400$.

FIGS. 26, 27.—Sections of eggs of about the same age as that illustrated in figs. 14 and 15, and which have been treated with osmic acid. The yolk nucleus is in immediate contact with the germinal vesicle, and the clear space in which it usually lies is not present; oil globules (*Ol.*) are present both in the yolk nucleus and in the surrounding protoplasm. $\times 250$.

FIG. 28.—Section of an egg stained with osmic acid, in which yolk-formation is occurring. All trace of the yolk nucleus has vanished; oil-drops are scattered among the developing yolk, and are especially abundant towards the periphery of the egg; they may be distinguished by their darker colour. $\times 250$.

FIG. 29.—Section through the germinal vesicle of a young egg. The chromatic network is well developed, and darkly staining nodules are present at its nodes. Lying at the edge of the germinal vesicle is a dark nodule (*Y.N.*) similar to those which occur at the nodes of the chromatin reticulum, and which is apparently in process of passing out through the membrane of the germinal vesicle to become one of the granules which will coalesce and form the yolk nucleus. $\times 650$.

FIG. 30.—Section of the germinal vesicle showing the gradual attenuation of the chromatin network as maturation proceeds. $\times 650$.

FIGS. 31, 32.—Sections of the germinal vesicle in somewhat older eggs, showing the increased size of the germinal vesicle and the attenuation of the chromatin network. $\times 650$.

FIG. 33.—Section of the germinal vesicle showing the thickening of the chromatin network which occurs in later stages. $\times 650$.

FIG. 34.—Section of the germinal vesicle of an egg in which the formation of the polar bodies will shortly take place. The chromatin network is much thickened, and the substance between its meshes stains more darkly than at earlier stages. The nuclear membrane has become irregular. $\times 650$.

FIG. 35.—Section of the germinal vesicle at a stage slightly later than that shown in fig. 34. The nucleolus has become relatively very large, the chromatin network is thicker, and the substance between the meshes stains still more deeply than at previous stages. $\times 650$.

FIG. 36.—Section of the germinal vesicle in a mature egg before the formation of the polar bodies has taken place. The germinal vesicle has decreased in relative size and has assumed an amoeboid form. All trace of the chromatic network has vanished. $\times 650$.

FIGS. 37-42.—Series illustrating the primitive cleavage of the egg. $\times 65$.

FIG. 43.—The segmenting egg at the four-cell stage. $\times 90$.

FIG. 44.—The segmenting egg at the eight-cell stage. Lateral view. $\times 90$.

FIG. 45.—The segmenting egg at the twelve-cell stage. $\times 90$.

(a) Lateral view. (b) Aboral view.

FIG. 46.—Oral view in optical section of the larva at the twelve-cell stage, showing the formation of spindles in the four oral cells prior to the division which gives rise to eight oral cells. $\times 400$.

FIG. 47.—Larva at the sixteen-cell stage. $\times 90$.

(a) Oral view. (b) Lateral view. (c) Aboral view.

FIG. 48.—Oral view of the larva at the sixteen-cell stage, showing the formation of spindles in the four central oral cells prior to their division to form four new oral cells of the sixth generation. $\times 180$.

FIG. 49.—Larva at the twenty-cell stage. $\times 90$.

(a) Oral view. (b) Lateral view. (c) Aboral view. The four large oral cells are of the sixth generation; of the eight surrounding cells, four belong to the fifth and four to the sixth generation.

FIG. 50.—Larva at the thirty-two cell stage. $\times 90$.

(a) Oral view. (b) Lateral view. (c) Aboral view.

PLATE 24.

FIG. 51.—Section of an egg in which the first polar body has been formed and the second is in process of formation; the first polar body lies outside the egg. $\times 250$.

FIG. 52.—Section of a larva at the four-cell stage showing the formation of the nuclear spindles prior to the division to form the eight-cell stage. $\times 225$.

FIG. 53.—Longitudinal section of the larva at the twenty-cell stage. $\times 225$.

FIG. 54 a.—Transverse section of the larva at the twenty-cell stage, showing the formation of nuclear spindles in the aboral cells A_3^2 , C_3^2 , prior to division. The four oral cells belong to the sixth generation. $\times 225$.

FIG. 54 *b*.—Transverse section of the larva at the twenty-cell stage, showing the formation of nuclear spindles in the aboral cells $A_3^1-C_3^1$, and in the oral cell A_3^2 , prior to division to form cells of the sixth generation. $\times 225$.

FIG. 55 *a, b*.—Transverse sections of the larva at the twenty-cell stage, but at a slightly later period than that illustrated in Figs. 54 *a, b*, the division of two of the aboral cells being now completed. $\times 400$.

FIG. 56.—Longitudinal section of the larva at the thirty-two cell stage. $\times 225$.

FIG. 57.—Longitudinal section of the larva showing the formation of the primitive mesendoderm cells by the division of the four large central oral cells. $\times 225$.

FIG. 58.—Section of the larva showing the enclosure of the four large central oral cells within the primitive segmentation cavity, forming, together with the four cells previously enclosed, in all eight mesendoderm cells; a blastopore (*Bl.*) is represented. $\times 225$.

FIG. 59.—Section of the larva at a slightly later stage than that shown in the previous figure. The difference in size between the cells of the aboral and oral ectoderm is noticeable; the eight primitive mesendoderm cells have divided, forming a mesendodermic mass which projects from the oral face into the segmentation cavity; the blastopore is still open. $\times 225$.

FIG. 60.—Section of the larva at a stage subsequent to the closure of the blastopore. *M.C.* marks the position of a slight groove which is to be seen on the exterior of the larva, and the slight protuberance visible below this in the external view is due to the difference in size of the cells of the oral and aboral ectoderm, which meet along this line. The mesendoderm almost completely fills the segmentation cavity. $\times 225$.

PLATE 25.

FIG. 61.—Section of an older larva in which the formation of the mesendoderm is completed, but in which the larval organs have not yet begun to form. The difference in size between the cells of the oral and aboral ectoderm is now very marked; the groove *M.C.* has deepened, and the oral cells below it are slightly protruded, giving rise to a raised ring on the external surface of the larva; this ring is, however, quite unconnected with the ciliated ring which develops later. The mesendodermic mass has by this time completely obliterated the segmentation cavity. $\times 225$.

FIG. 62.—Longitudinal section of a young larva at the period when the larval organs are beginning to form. Three oral invaginations are observable; these will later develop into the internal sac, pharynx, and pyriform organ, respectively. Globules (*g.*) are seen to be exuding from the cells of the internal sac, and similar globules are also present within the cells of the internal sac

and pharynx. The neuro-muscular cord (*N.M.*) is in process of development. The mesendoderm forms a solid mass in the posterior part of the larva. Viewed from the left side. $\times 225$.

FIG. 63 *a*.—Longitudinal section through the larva at a slightly later stage than that illustrated in Fig. 62, viewed from the left side. The mesendodermic mass has now become hollowed out to form the stomach. Viewed from the left side. $\times 225$.

FIG. 63 *b*.—Transverse section through the region of the pharynx of a larva of the same age as that shown in Fig. 63 *a*. Two bands of tissue (*), which may possibly represent the true mesoderm, are budding off from the mesendodermic mass. $\times 225$.

FIG. 64.—Transverse section of a larva older than that which is represented in Fig. 63; the plane of section passing through the stomach, internal sac, and pharynx. $\times 225$.

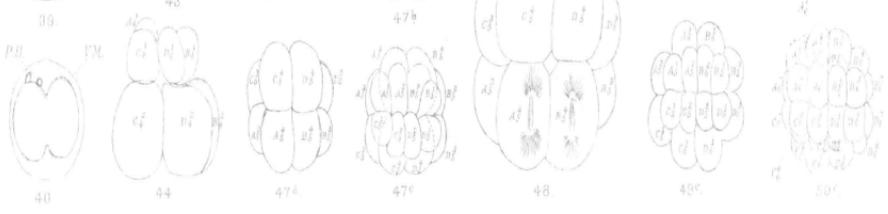
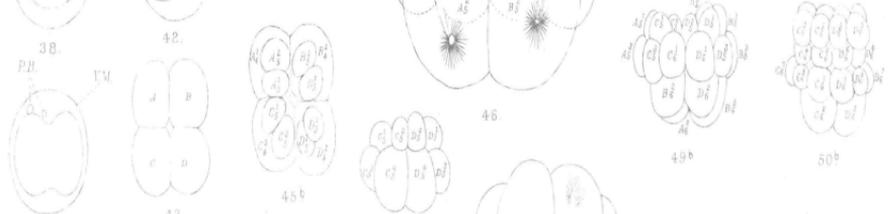
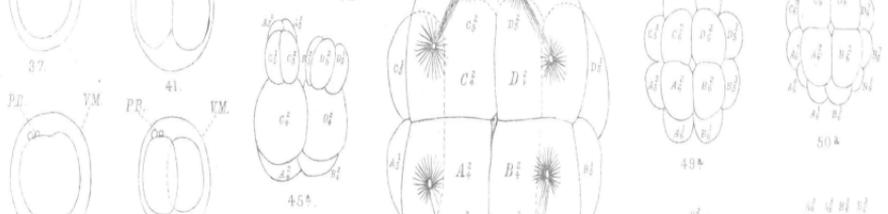
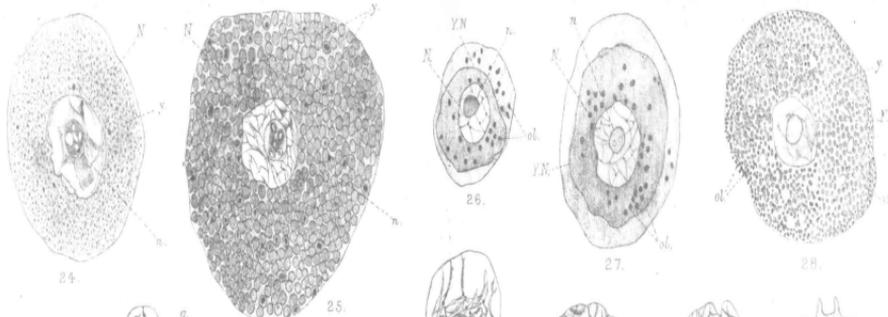
FIG. 65 *a-c*.—Sections of mature larvæ.

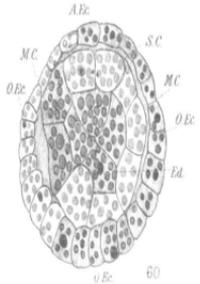
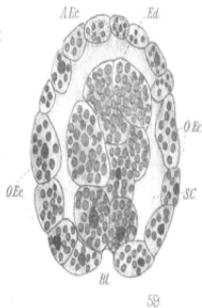
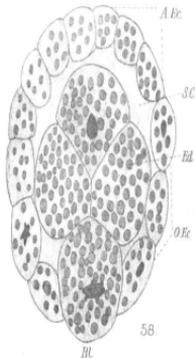
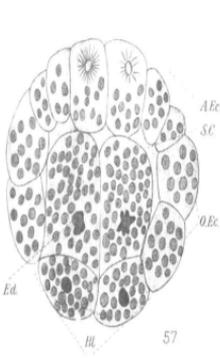
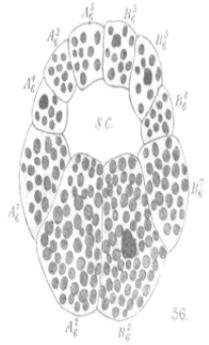
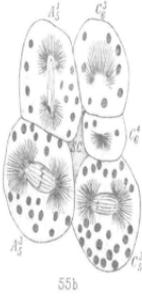
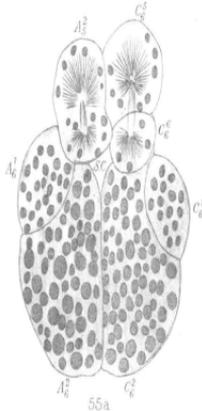
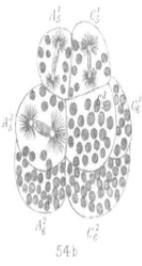
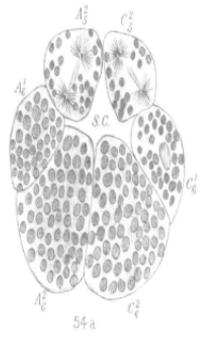
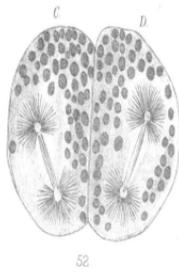
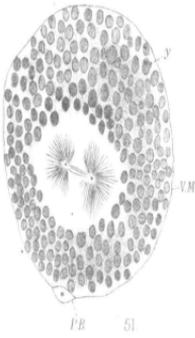
a. Longitudinal section of a larva, viewed from the right side, in which the larval organs are all fully developed. The pharynx and internal sac have lost the globules which were present in earlier stages; the pyriform organ now consists of the "système glandulaire supérieure" (*S.G.*') with the "fossette supérieure" (*F.s.*) and the "système glandulaire inférieure," (*S.G.*'') only the right side of which is visible: the "fente ciliée" is not shown. The stomach has become very elongated. The ciliated crown (*C.*) is now visible. (From a drawing by Dr. S. F. Harmer.)

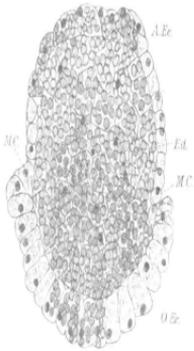
b. Transverse section of the larva in a plane passing through the stomach and internal sac. The two bands of tissue which appear to be budding off from the mesendoderm are again noticeable. $\times 225$.

c. Transverse section passing through the stomach and pharynx of the larva. $\times 225$.

FIG. 66.—Transverse section of a larva in which degeneration has commenced. The walls of the internal sac have become much thickened and the protoplasm near the opening to the exterior has assumed a granular appearance. The ciliated crown now consists of a single row of large cells. The stomach has vanished, and the mesendoderm is represented only by scattered nuclei and yolk spherules, among which lie numerous muscle-fibres. $\times 225$.



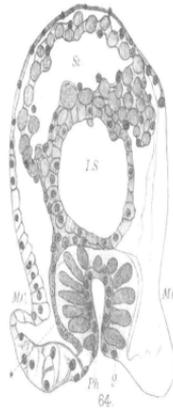




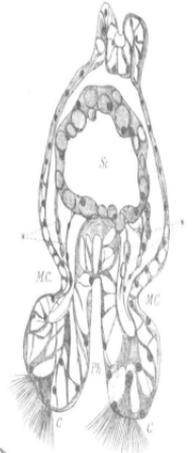
61.



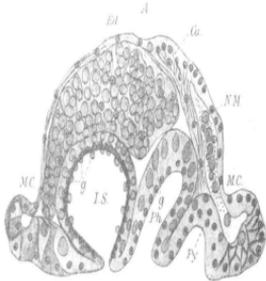
63b



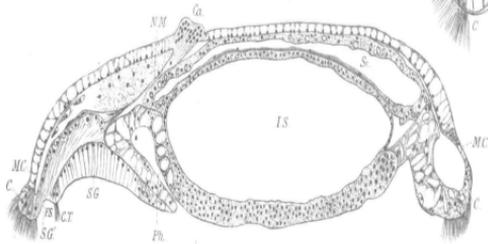
64.



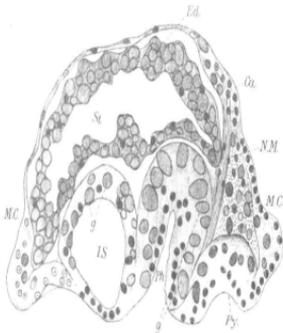
65c



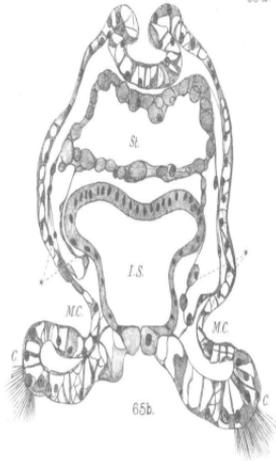
62.



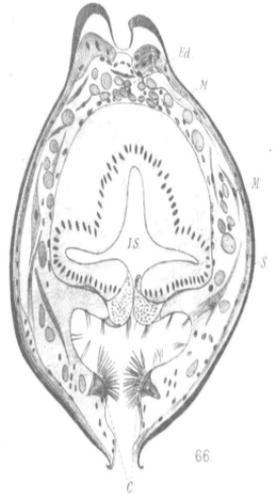
65a.



63a.



65b.



66.