No. 11. — *Studies from the Newport Marine Zoölogical Laboratory. Communicated by* ALEXANDER AGASSIZ.

XV.

On the Development of Agalma. BY J. WALTER FEWKES.

THE genus *Agalma*, in its growth from an egg into the adult, passes through three larval stages which can be readily distinguished from each other. These larvæ, from the nature of the growth of the *Agalma* colony, are not separated from one another by clearly marked distinctions, but temporary organs found in one stage are often carried over to the next in the course of the passage of one larva into that next following. The peculiarities, however, of each are strongly enough defined to justify the division of the embryological history of *Agalma* into the three stages to which reference is made.

These three characteristic larvæ about which the young of the genus *Agalma* group themselves have been given the following names: 1. The primitive larva; 2. The Athorybia stage; 3. The larva like the adult in general features, although possessing together with organs of the adult certain provisional structures bequeathed to it from the earlier conditions of growth through which it has passed.

The last two of these larval stages are not considered in this paper, except incidentally to record observations on certain appendages of the Athorybia stage, showing the fate of organs of permanent and others of provisional nature which play an important part in the appearance of the youngest or primitive larval condition. The present contribution deals with the outward changes in the growth of the egg from fertilization to the primitive larva. In that epoch many important organs, some of which persist into the adult, originate; and, more significant still, at that time first arise the three layers out of which every organ of the whole colony is developed. The development of the first of the three larval stages of *Agalma* may consequently be looked upon as a key to the phylogeny of the Oceanic Hydrozoa. It is therefore at all events necessary, before we can trace the relationships of different genera widely or closely related to *Agalma*, to know accurately the changes in external form which the ovum passes through in these genera. Upon such knowledge we can hang our speculations regarding the possible descent of the members of the Siphonophora one from another, or from a common ancestor.

The species of *Agalma* which has been studied is the only *Agalma* thus far recorded from New England waters. It is called *Agalma elegans,* and was first described by the author. When this animal was first taken, in 1876, I regarded it as the same as the "form (b)" of *Agalmopsis elegans* Sars, or closely related to it.

Early Changes of the Egg before Segmentation.

The earliest changes in the egg take place, in all cases observed, while it is enclosed in the female gonophore.* These go on with great rapidity, as will be shown by the following statements. Specimens of *Agalma* captured on August 6, at noon, were found four hours later to have dropped their gonophores, from which had come ova segmented in the 4-cell stage. It must be mentioned, however, that by transferring the *Agalma* from the sea into aquaria they were placed in unnatural conditions, so that changes in temperature and other causes may have accelerated or retarded their rate of growth. There is nothing to show that there are not other kinds of segmentation besides that which is here described.

* In a popular article on the development of *Agalma elegans*, published in the American Naturalist for March, 1881, certain changes in the germinative vesicle which were mistaken for segmentation were spoken of. This interpretation was erroneous, and the true segmentation was not described. On p. 188, *op. cit.*, the egg is spoken of as cast into the water and there impregnated. There is nothing to prove that this is the case in *Agalma.* It has, however, excellent support in the history of observation. Gegenbaur, Beiträge zur näheren Kenntniss der Schwimmpolypen (Siphonophoren), p. 49. Writing of the genera *Agalmopsis, Forskalia, Physophora, Hippopodius*, and *Diphyes*, he says: "Die Befruchtung erfolgt erst nach dem Austritte der Eier aus der Eikapsel; denn niemals fand ich Samenfäden in letzere eingedrungen, eben ausgetretene Eier dagegen stets von ihnen umschwärmt. Sie sassen dann strahlenartig mit dem Köpfchen an der Peripherie des Eies an, mit dem Fadentheile selbst in zitternder Bewegung." I have not been able to observe a similar condition in *Agalma*, nor was a free egg with nucleus and nucleolus found floating in the water. In one instance these bodies were observed to vanish while yet the ovum was in its gonophore, while the stalk of the same was attached to the parent. All eggs found free from the gonophore are destitute of these structures.

Four hours after an *Agalma* was placed in the aquarium, eggs in the 4-cell stage were picked out of the water in which it was confined. I have traced one and the same egg from the 2-cell to the 4-cell stage, and find that it takes 2 h. 10 m. for the necessary changes to be perfected in this growth. On another egg it was determined that it takes 45 m. to develop an egg in the 2-cell stage from an egg in which the germinative vesicle, or "nucleus," had disappeared. By this observation it will be seen that it requires a little over an hour to pass from the egg just fertilized into the stage which exhibits the first sign of a primitive cleavage, plus the interval of time which elapses after the 2-cell stage is formed and before it begins to form the secondary furrow, or origin of the second cleavage-plane. This last interval is probably not more than 30 m.; consequently the interval which elapses after fertilization before the formation of the primary furrow is about half an hour.

Impregnation probably takes place in the gonophore. I have not been able to fecundate the *Agalma* egg artificially, nor was it seen to take place naturally. I have repeatedly tried to fertilize ova with sperm from the same colony, but have always failed. This fact led me, in 1880, to state that the animal cannot be impregnated by spermatozoa from its own male bells. Last summer (1884), however, to obtain some information on this point, an isolated *Agalma* was kept in a glass jar, and it dropped eggs which became segmented and later developed into primitive larvæ. The water in which it was confined was not changed meanwhile, nor new liquid added. Of course this experiment does not absolutely demonstrate that the spermatozoa from the same colony can or cannot unite with an unfecundated ovum of the same, for sperm may have been in the water before the animal was placed there. Experimentation on the subject has many difficulties; but it must be confessed, that, as far as I have thus far gone in my studies, it looks as if the male bells of an *Agalma* may sometimes fertilize ova from the same axis. The great difficulty in the artificial fecundation of the *Agalma* egg was pointed out by Metschnikoff.* The ovum in the gonophore is enclosed in what he calls an "Umhüllung," from the walls of which the tender egg cannot be extracted without harm to its contents.

The first naturalist to fertilize artificially the Siphonophore egg was Gegenbaur.† Metschnikoff ‡ was equally unsuccessful with myself with

* Studien über die Entwickelung der Medusen und Siphonophoren. *Zeit. f. Wiss. Zool.*, XXIV. p. 49.

† Beiträge zur näheren Kentniss der Schwimpolypen (Siphonophoren), p. 49.

‡ *Op. cit.*, p. 49.

the egg of *Agalma*. Haeckel * says that he made attempts to fertilize artificially the ova of the genera *Praya*, *Diphyes*, *Abyla*, *Hippopodius*, *Athorybia*, *Agalmopsis*, *Halistemma*, *Forskalia*, *Crystallodes*, and *Physophora*. "Die Mehrzahl der Versuche schlug fehl, und in vielen Fällen gingen die befruchteten und sich entwickelnden Eier zu Grunde. Ehe sie noch über die ersten bereits von Gegenbaur beschriebenen Entwickelungstudien hinaus die Entwickelungsvorgänge zu verfolgen, gelang mir nur bei drei Physophoriden-Gattungen, nämlich bei *Physophora* (bis zum XXVIII[sten] Tage), *Crystallodes* (bis zum XXVII[sten] Tage), und bei *Athorybia* (bis zum VII[ten] Tage)." He does not state with sufficient exactness in the case of *Crystallodes*, the nearest ally of these three genera to *Agalma*, whether he artificially impregnated the ovum or not.

The natural ovulation in *Agalma* was the only means of getting material for the study of the embryology; and as this happened seldom, even in instances when I had in confinement a large number of large and sexually mature specimens, the amount of material at my control was small. The youngest larvæ are very hardy, needing, for early stages at least, no change of water, provided decaying matter from the adult animal be not allowed to pollute it.

Each ovum is carried in a bell-shaped structure called the female gonophore. The female gonophores (Pl. I. fig. 1) are found in botryoidal bunches at the base of the polypites, and generally adhere to the neighboring hydrophyllium when it is broken from its attachment to the stem. The gonophore is fastened to the axis by its apex, through which a small tube communicates between a system of vessels called the radial tubes and the cavity of the stem. No marginal tube or marginal appendages of any kind were detected on the bell. The course of the radial tubes in the bell of the female gonophore (Pl. I. fig. 3) is very irregular, and varies very greatly in different individual gonophores. In a form of gonophore which was common, the following arrangement in the disposition of the tubes was observed. Two radial tubes arise from a common point under the apex of the bell, at the junction of the same with the tube of the apex. These lie in opposite hemispheres on the walls of the bell cavity. Consider the course of one of these radial tubes. After extending from under the apex of the bell about half-way down the sides of the bell on its inner surface, it bifurcates, each division passing at right angles to the course of the undivided tube. Each of the bifur-

* Zur Entwickelungsgeschichte der Siphonophoren. Eine von der Utrechter Gesellschaft für Kunst und Wissenschaft gekrönte Preisschrift. Utrecht, 1869. p. 10.

cations passes around the bell parallel with the margin, and joins a corresponding bifurcation from the undivided tube of the hemisphere opposite that in which the bifurcation first described takes place. Before these bifurcations join, however, each sends a loop downward, which approaches the neighborhood of the bell margin, but eventually returns to the bifurcation.

The single ovum lies in a thin-walled sac,* which hangs from a point directly under the apex, and when ripe fills the whole bell cavity, sometimes projecting a little through the opening. The free gonophore is propelled in the water by violent contractions of the walls of the bell.

The male gonophore, like the female, is often found free in the water in which the *Agalma* is confined. When attached to the stem it is found in clusters at or near the base of a taster midway between two adjacent polypites. In many live specimens of *Agalma* some of the attached male gonophores will be found to have milk-white contents. Like the female, the male gonophore is commonly transparent.

The bell of the male gonophore is more elongated and larger than the female. It measures 2.5 mm. in length and .4 mm. in greatest diameter. At the apex of the bell there is a short peduncle by which it is attached to the adult. Through this peduncle there extends a tube, — the peduncular tube. There are are four thread-like simple radial tubes which have a direct course in the bell walls and unite with a circular marginal vessel. Each male gonophore has a narrow, thin velum. The bell walls are capable of quick contractions when free from the axis.

* Whether the egg in the gonophore is surrounded by a membrane by which it is held there, or not, no one has clearly proved. I think such is the case. In the first place, the homology of the gonophore with the gonophores of other genera which have an ovisac would seem to point to such a condition in *Agalma*. In my figure of the egg just escaping from the gonophore a structure was observed in the bell cavity which called to mind the ruptured walls of such a sac. After the ovum was cast, there is reason to suppose that the "sac" is retained in the gonophore. Metschnikoff speaks of the egg of *Agalma* as "membranlos." Haeckel says the egg-cell of *Crystallodes*, "wie bei den übrigen Siphonophoren ist ganz nackt," and that of *Physophora*, "wie die Eier aller übrigen Siphonophoren sind dieselben durchaus hüllenlos." "*Hippopodius gleba*," writes Metschnikoff (*op. cit.*, p. 46), "ist die einzige mir bekannte Siphonophore, deren Eier mit einer freilich äusserst dünnen Membran überzogen sind." *Hippopodius* and *Vogtia*, according to Kölliker, have ovisacs in which, when in the gonophore, *numerous* ova are contained. I have also observed in a *Eudoxia* which resembles *E. Lessonii*, that here also we have *numerous* ova in an ovisac in the female gonophore, and there are many other similar observations on record.

The larger part of the cavity of the male bell is taken up by an ovate, slightly opaque mass, which is a sac inflated with spermatozoa. This sac, like the sac which carries the ovum, fills almost the whole cavity of the bell. The distal pole of the sac is closed.

Free spermatozoa are obtained in great quantities by simply pressing the body of the sac of the male gonophore, when they escape through the ruptures in the walls. The spermatozoa are the ordinary tailed variety with rounded, often pyriform heads, which are sometimes prolonged into a pointed end opposite the tail.

In the smaller female gonophores (Pl. I. fig. 1), and also in some others of larger size, we recognize in the contained egg a transparent cell, germinative vesicle, in which is a dot, and sometimes within the last are one or more granules. The mass of the egg, however, is formed of a clear substance, through which there extends a protoplasmic network, imparting the appearance of a complex spongy mass of polygonal cells to the egg contents. This network has not been figured or specially described by others in the egg of *Agalma*, although it has been seen by Metschnikoff and figured by him in *Epibulia*, *Stephanomia*, and *Halistemma*. Although he neither figures nor specially describes this network in *Agalma*, Metschnikoff* may have referred to it when he says: "Die vollkommern reifen membran- und kernlosen Eier [of *Agalma*] zeigen eine ähnliche Zusammensetzung wie die oben beschreibenen Eier der *Epibulia aurantiaca* und des *Hippopodius gleba*, unterscheiden † sich aber von ihnen durch ihre feinen röthlichgelbe Färbung, welches sie dem Vorhandensein eines diffusen Pigmentes verdanken." I shall return to these "cells" later, in my account of the progress of the growth of the egg.

Precisely how the spermatozoön comes in contact with the ovum, if the latter is placed in a closed sac, is somewhat of a puzzle. The germinative dot and vesicle disappear before this sac is ruptured. At about this time one or two globules (*p g.*) were observed on the egg. In my figure the nucleus and nucleolus have not disappeared. These changes go on so fast, that I am not confident that both are found together, and the globules may have appeared after the disappearance of dot and vesicle. These globules seem to be the same as the "deformed spermatozoa" described in another genus by P. E. Müller. If the disappearance

* *Loc. cit.*, p. 49.

† The statement of Metschnikoff, p. 46 (quoted above), that the eggs of *Hippopodius gleba* "mit einer freilich äusserst dünnen Membran überzogen sind," would seem to be another difference.

of the clear cells denotes that fecundation has occurred, how have the spermatozoa effected an entrance into the egg? The germinative vesicle and dot disappear probably before the gonophore is detached from the axis of the adult, and, without doubt, before the egg leaves its gonophore. In the immature gonophore in which the body *pg.* was seen, the opening into the bell cavity of the gonophore had not formed. Whatever the cell *pg.* may be, spermatozoön or polar globule, both germinative dot and vesicle disappear before the ovum leaves its gonophore. If this event is a result of an impregnation, there seems to remain but one conclusion, — namely, that the fertilization of the ovum takes place in the gonophore. We are led to suppose that the spermatozoa either penetrated the sac walls of the ovum and gonophore, or passed through the apical canal, which is not in free communication with the surface of the ovum. It seems more natural to adopt the latter supposition, unless we suppose that nucleus and nucleolus vanish before impregnation. The cell, with its enclosed cellular body, which we have called the nucleus and nucleolus, disappears and leaves the egg of homogeneous appearance, with the contents made up of the protoplasmic network of cells already mentioned. The next change is that by which the egg separates itself from the sac in the gonophore in which it is contained.

Several authors have commented upon the peculiar sinuses which are sometimes found at this time in the female bell about the egg. These sinuses are of many shapes, and lie between the egg and its membranous sac (Pl. I. fig. 2). They have the appearance of spaces left here after preliminary movements of the ovum before escape from the gonophore, or by a shrinkage of the walls. A single gonophore (fig. 4) was observed in which the ovum was in the act of escape; and in that gonophore the folded remnant of a structure, which may be the sac which formerly enclosed the egg, was seen just under the apex of the bell in its cavity. The diameter of the opening into the cavity of the bell was in this instance observed to be smaller than that of the egg, so that the egg in some instances suffers a considerable compression before it escapes from the cavity of the gonophore. After the egg leaves the gonophore it assumes a spherical form, with a diameter of .45 mm. (Pl. I. fig. 6). One pole is ruby in color, the other transparent. The network of protoplasm which extends through the entire contents imparts to it a cellular appearance, while a thin layer, probably of protoplasm, is found over its entire surface.

Cleavage.

First Cleavage Furrow. — Gegenbaur,* who says that he observed the segmentation of the ovum of the genera *Agalmopsis*, *Physophora*, *Forskalia*, *Hippopodius*, and *Diphyes*, states that the whole process of segmentation is finished in from twenty-four to thirty-six hours. Haeckel† says that in *Physophora*, *Crystallodes*, and *Athorybia* the segmentation is finished at the end of the second day. Metschnikoff does not state the exact limit of time when the segmentation is finished, although from the age of the youngest larva of *Agalma* which he figures I should judge that the segmentation was accomplished in the second day. All recorded observations on Siphonophore eggs point to the conclusion that the cleavage is wholly completed before the beginning of the third day after fecundation.

My first specimen of *Agalma* was captured on August 6th, at noon, and before the morning of August 8th it had laid eggs which were in the same stage as that figured by Metschnikoff on the fourth day. In other words, a little over a day and a half after the *Agalmata* were placed in the aquarium, eggs from them had segmented and had formed the two layers described by Metschnikoff in the changes of the fourth day. My observations are thus at variance with those of Gegenbaur, Haeckel, and Metschnikoff. What is the meaning of the discrepancy? Looking over my notes in vain to find an error in this particular, it has seemed possible that errors of observation have crept in for the reason that individual eggs have not been followed through their consequent stages. An *Agalma* in captivity will mature its eggs at different times, so that at the end of the fifth day segmented eggs in company with those which are far along in the development of the primitive hydrophyllium may be picked out of the same water. From the nature of the case, unless individual eggs are isolated and the time of their fecundation recorded, it is impossible to know the age of any specified stage.

The first change which takes place in the spherical egg after it has left the gonophore is the formation of the primary cleavage furrow, *pr.* At one pole of the ovum (Pl. I. fig. 7) an indentation appears in the form of a furrow on the surface of the egg. Although I have not observed at the outset the exact relationship of this furrow to the rosy pole, I have seen that later, after the first plane of cleavage has been

* *Op. cit.*, p. 50.

† *Op. cit.*, for *Physophora*, p. 19; for *Crystallodes*, p. 51; for *Athorybia*, p. 89.

completed and the egg is in the 2-cell stage, this plane passes through a rosy pole. While this gap in observation is too important to be overlooked in studying the relation of the primitive plane of cleavage to the poles of the egg or the axis of the adult animal, enough has been observed to show that the first plane of cleavage passes through the pole of the egg adjacent to that part of the sac which is attached to the gonophore, if the rosy pole of the egg in the 2-cell stage and that of the egg in the gonophore are the same. We are able to identify a rosy pole in the egg, even into those post-segmented stages when the embryo begins to push out the two layers of the primitive hydrophyllium on the surface of the yolk; and while we have not traced the continuity of this pigment in an egg in this stage with the segmented egg older than the 8-cell stage, the presumption is that the poles are the same in both cases.

The primary furrow, *pr.*, bending into the ovum on one side of the *Agalma* egg, causes many obscure or sharply defined folds on each side. Similar plications are also mentioned and figured by Metschnikoff * in *Epibulia*. The egg at this time as shown by Metschnikoff in the latter genus resembles the ova of *Geryonia* and the Ctenophora.

As the groove on the animal pole deepens, changes in the external contour of the egg follow with great rapidity. I have timed the duration of a few of these variations, and give camera drawings to illustrate their appearance at intervals of time.

At 8 h. 45 m. in the morning the indentation which marks the appearance of the primary cleavage furrow has just begun to appear. The egg at this stage is smaller than that just laid, but whether this diminution in size is due to the changes which result from the formation of the primary furrow or individual variation, we have no data by which to determine. The diameter of *this* egg in the plane connecting the pole where the furrow has taken place with the opposite is .30 mm.; the longer diameter is .35 mm. The profile of the egg, looking at it in a plane at right angles to the primary furrow, is oval or slightly notched at one pole.

Fifteen minutes later, at nine o'clock A. M. (Pl. I. fig. 7), the profile of the same egg in the same position has become still more heart-shaped, and the primary furrow has deepened to an amount greater than the radius of the egg. The depression forming the primary furrow almost girts the egg, extending over the surface for more than two thirds its circumference.

* *Op. cit.*, p. 40.

At 9 h. 10 m. A. M. (Pl. I. fig. 8) the primary furrow, *pr.*, has deepened still more, and the constriction has encroached more than before on the whole circumference, so that now the two hemispheres of the egg are connected by a narrow band or "bridge" of protoplasm, the breadth of which is about .05 mm. The longer diameter of the egg is .35 mm.; the shorter, .25 mm.

At 9 h. 20 m. A. M. (Pl. I. fig. 9) the constriction has grown wholly around the egg and the primary furrow has deepened so much that a small protoplasmic band .02 mm. in diameter is all that now connects the two cells. The other dimensions are about the same as the corresponding diameters of the egg at 9 h. 15 m. A. M. (fig. 9), although it was noticed that one hemisphere of the 2-cell stage was slightly smaller than the other.

At 9 h. 25 m. A. M. (Pl. I. fig. 11) the cell which was the smaller has grown in size so that now both cells of the 2-cell egg are of uniform size. At 9 h. 30 m. A. M. (fig. 12) the two cells have been pressed closely together, and the first plane of cleavage (1 *cl. pl.*) has been fully formed, although the undivided part of the egg still remains in the form of a slight bridge connecting the two cells which form the egg. No nuclei were observed in either of the cells.

It will thus be seen that the development of the 2-celled ovum from the time the primary furrow first appears up to that when the first cleavage plane is well formed is forty-five minutes. For a long time after the formation of the first cleavage plane has been effected, both hemispheres of the egg exhibit abnormal changes by which the egg is made to assume curious, often grotesque forms. Here and there over the surface of the egg rise pseudopodic elevations, which sometimes take the form of long rhizopodal threads. Later, these extensions sink back into the substance of the egg and new combinations arise. The two spheres, or hemispheres, now draw away from each other, or become squeezed together. They lose their globular, symmetrical form, and their profiles become more angular, or sometimes the angles are pushed out into conical projections. These changes often foretell the immediate death of the egg, but full as often take place in healthy ova which reach a good old age.

A considerable length of time may elapse before the initial changes leading to the formation of the second plane of cleavage can be detected. We are not in my judgment justified in supposing that the vital forces of the egg are "resting" at that time until we know more accurately the state of the interior and the changes which are going on there.

This is the nearest approach which we have in the *Agalma* egg to a "resting stage."

Second Cleavage Furrow. — How much time intervenes after the formation of the first cleavage plane before signs of a second furrow appear, has not been accurately observed. It is thought to be about thirty minutes. In a stage of segmentation, not raised from that just described, but like it also in the 2-cell stage, it was possible to follow the whole progress of the growth of the second furrow. This egg was not raised from those formerly described, but was picked out of the water, and was observed in the 2-cell stage on the fourth day after the *Agalma* was captured. At 1 P. M. (fig. 13) it showed the first trace of the second cleavage furrow, and an hour later the egg had passed into the 4-cell stage. The changes of that hour are as follows as far as external form goes.

If we suppose this egg to be placed in such a position (fig. 13) that the first plane of cleavage (1 *cl. pl.*) is vertical, there will be observed on one side of this plane, viz. in the left-hand cell, a slight depression or furrow (*se.*) indicated at first by a variation from a straight line which the plane seen in profile seems to have. This depression is caused by the infolding of the surface of the egg at that point, and is the beginning of the second cleavage furrow. The furrow is at first at right angles to the primary furrow, and in its earliest condition one cell only of the 2-cell stage is modified.

At 1 h. 10 m. P. M. (*se.*, Pl. I. fig. 14) the growth of the furrow is very slight. The depression has deepened, the chasm widened, and folds similar to those described in the walls of the primary furrow have been developed.

At 1 h. 15 m. P. M. (Pl. I. fig. 15) the second cleavage furrow (*se.*), while extending itself and deepening in the left-hand cell, has appeared also in the right-hand as well. It is now no longer placed at right angles to the primary cleavage plane, but lies across it at an angle of from 60° to 65°. A slight predominance in size of the left-hand end of the furrow is shown in the figure. The diameter of the egg at right angles to the first plane of cleavage is now about .60 mm.; the shorter diameter, about .45 mm.

The growth of the egg in the next three minutes is important. At 1 h. 18 m. P. M. (fig. 16) the second furrow has lengthened and deepened, growing in such a way as to produce a certain twisting in the first plane of cleavage. A contortion of the first cleavage plane, 1 *cl. pl.*, is brought about by the growth of the second furrow. In an egg seen in the same plane as in former instances, the line indicating the first plane of cleavage, which in them was unbroken, is bent at right angles at the point where the secondary furrow has appeared. The second cleavage furrow is at this time a little over .15 mm. long. The longer diameter of the egg is .60 mm.; the shorter, about .45 mm.

The general appearance of the egg two minutes later than the last, or at 1 h. 20 m. P. M. (fig. 17), although in most respects similar to it, has several marked differences, the result of the progressive growth. One of the most striking of these differences is the still greater increase in the amount of the deviation from a straight line which now separates the lower end of the upper line from the upper end of the lower vertical, both being the profile of the first cleavage plane, 1 *cl. pl.* The length of the second cleavage furrow, *se.*, has now increased to .25 mm., its breadth remaining about the same, and in its sides are frequent plications running parallel with the first cleavage plane, much more sharply defined than in any which has preceded it. Up to the present time (fig. 17), twenty minutes after the first visible changes by which we pass from an egg with two cells into one with four, the secondary furrow has been limited in its extension. It now slowly deepens, and at the same time grows along the surface of the ovum toward the equator, although at 1 h. 20 m. it has not yet extended far enough to reach the periphery of the egg as seen in profile. There is as yet no indentation marking the limit of the second cleavage groove on the equator of the egg.

In the same egg five minutes later, at 1 h. 25 m. P. M. (fig. 18), the second furrow, *se.*, is found extending across the whole hemisphere, and is represented in the figure by the large horizontally placed furrow. The size and depth of this indentation may be estimated by the depression at either extremity of this furrow. In profile it is seen to equal in depth the radius of the egg. Like the primary groove, *pr.*, this likewise eventually extends almost through the egg, dividing it into two symmetrical hemispheres connected by an undivided "bridge." The walls of the furrow, still grooved with cleavage folds, have not yet begun to approximate. In this stage (fig. 18), although we seem to have four segmentation spheres, the second plane of cleavage does not extend more than two thirds across the diameter of the egg as seen from the original surface of infolding. On the side of the egg away from the observer, the 2-cell stage was slightly grooved by the second furrow. The sides of the second cleavage furrow have not yet begun to draw together. At this time in the growth of the ovum the walls of the second furrow on each side, and especially at the peripheral extremities, or that part most distant from the primary plane of cleavage, exhibit rhizopodal elevations similar to those which accompany the formation of the primary furrow, and which we shall later see are found to form especially in later stages of growth, wherever a new plane is about to appear. Similar rhizopodal phenomena are also premonitory of death in the cells of the egg.

At 1 h. 30 m. P. M. (fig. 19), half an hour after the secondary cleavage furrow began to appear, the secondary groove (*se.*) shows signs of closing, and the walls draw together to form the second cleavage plane (2 *cl.pl.*). The closure of the secondary furrow takes place in substantially the same manner as the primary, and begins at the junction with the primary, working gradually to the periphery. All the time that the growing together of the sides of the furrow is going on, as the movement of closure advances towards the equator it is accompanied by the formation of new folds and the pushing out of pseudopodia in the line of its advance. In my figure representing the egg at 1 h. 30 m. P. M. these folds can be seen in the left hand of the figure, where the furrow is only partly closed.

By the closure of the second furrow, combined with the contortion which is thus caused in the primary plane of cleavage, the profile of the first plane (primary), *pr.*, appears zigzag, or the line which was formerly vertical is now not straight from one pole to the opposite, but is broken midway in its course. As this vertical marks the direction of the primary cleavage plane (1 *cl. pl.*), we have indications that the primary cleavage plane, once intact, is now broken or bent. That modification in this plane can be recognized in later stages of development, being seen as late as the 8-cell stage. The diameter of the egg on the primary cleavage-plane is about .60 mm.; on a plane at right angles, .45 mm. The segmentation spheres have no visible nuclei. The great mass of the ovum is transparent, and the part surrounding the upper end of the vertical line, which is the primary plane of cleavage, is of a rosy color.

The next stage of cleavage, 1 h. 35 m. P. M. (fig. 20), thirty-five minutes after the beginning of the modification of the 2-cell stage, differs very slightly from that just described. The second cleavage furrow (2 *cl. pl.*) is now closed almost to its very periphery, although protoplasmic elevations are seen at intervals along the furrow, a sure sign that the process is not yet completed. Remnants of the unclosed furrow are seen at each end of the horizontal furrow (2 *cl. pl.*).

At 1 h. 45 m. P. M. (fig. 21) the protoplasmic forces are still active in sending out the rhizopodia, and the secondary cleavage plane (2 *cl. pl.*) is not wholly formed, and at 1 h. 55 m. P. M. (fig. 22) the 4-cell stage is practically complete, although here and there, as at the left of the figure, a slight protoplasmic elevation can be seen. The second plane of cleavage is practically formed.

An hour and ten minutes, 2 h. 10 m. P. M. (fig. 23), after the 2-cell stage we have an egg divided into four cells by two planes at right angles to each other. None of these cells have a nucleus, and all are still penetrated by the network of "cells" which we have already described in the unsegmented ovum. The vertical plane passes through a rosy region of the egg; the opposite pole is more transparent. The diameter of the egg on the first cleavage plane is a little less than .50 mm.; on the opposite plane, about .45 mm. Although on the face of the egg which is before us the ovum is divided into the 4-cell condition, I have not been able to observe the opposite pole. Subsequent stages seem to indicate that the secondary plane does not extend wholly through it, but that at the opposite side there still remains an undivided surface. Later changes in the general outlines of the ovum lead me to suspect that the undivided part, either by growth or protoplasmic extension, is of considerable size after the formation of the 4-cell stage.

Third Cleavage Furrow. — The appearance of another cleavage furrow on the same egg, the third which has been traced, was first noticed at 3 h. 15 m. P. M. (Pl. II. fig. 3), two hours and thirty minutes after the 2-cell stage. In the mean time certain changes in the contour of the egg which are not fully understood had taken place. At 3 h. P. M. (Pl. II. fig. 1) the primary (1 *cl. pl.*) and secondary (2 *cl. pl.*) planes of cleavage, represented by the vertical and horizontal planes, occupy the same relative position as formerly, and the right-hand cells are in the main the same in contour. On the side of the left-hand cells, as figured, away from the observer, has appeared a large undivided lobe (*et.*), a little smaller than the original left-hand cell of the 2-cell stage. The egg has probably been slightly rolled on its axis, by which the large undivided lobe is turned into sight, whereas formerly it was concealed behind the two left-hand cells of the 4-cell stage. I was not able to observe satisfactorily the origin of this large lobe. The only explanation which can at present be given to account for its existence is one suggested above, that it is the bridge or connecting band which has not been divided by the second cleavage furrow. If, however, its fate resembles that of the protoplasmic bridge of the primary furrow, my explanation is probably erroneous.

The large lobe is the point of origin of a new cleavage furrow, which I have called the tertiary or third cleavage furrow (3 *cl. pl.*). In the general structure and mode of origin the third cleavage furrow bears a striking likeness to the primary and secondary. It forms at right angles to the direction of the second furrow and parallel with a part of the first furrow in the large undivided lobe on the left-hand side. At 3 h. 5 m. P. M. (Pl. II. fig. 2) the tertiary furrow had not begun to appear; but ten minutes after, at 3 h. 15 m. P. M. (Pl. II. fig. 3), it had reached a considerable size. Like the primary and secondary furrows, the walls of the tertiary are formed by an infolding of the surface of the ovum, and have the characteristic sharply defined folds and plications already mentioned.

Figures of the egg at 3 h. 20 m. P. M. (Pl. II. fig. 4), and at 3 h. 25 m. P. M. (fig. 5), are introduced in order to show the progress of the growth of the tertiary furrow in the division of the large undivided lobe on the left-hand side of the egg. At 3 h. 30 m. P. M. (fig. 6) two hours and a half after the formation of the first cleavage furrow, the tertiary furrow has divided this lobe horizontally into two smaller cells. The portion of the tertiary plane which bisects the large lobe is, like the primary and secondary, perpendicular to the plane of the paper on which the egg is figured. The two axes of the egg, a vertical, which is the original cleavage plane, and the horizontal, the secondary plane, are easily distinguished, and at one end of the tertiary furrow, now almost completely closed in, there is figured a marked protoplasmic elevation. This stage is a 6-cell stage, composed of the four cells which have already been mentioned and the two additional which have just formed. The tertiary furrow was the third furrow observed, but I suspect that between the secondary and tertiary (by my nomenclature) the large lobe which I have represented as divided by this furrow was constricted from the two left-hand cells by another, whose growth was not observed.

Morula.

The complications in the growth of the ovum after the stage last mentioned make it very difficult to follow the birth of new segment spheres or cleavage planes. The last stage of the egg in which the course of the original cleavage can be traced with any certainty is at 3 h. 45 m. P. M. (Pl. II. fig. 7), or two hours and three quarters after the formation of the first cleavage, when we have an 8-cell stage. From this we pass into morula stages, in which additional cleavage planes were not successfully traced as they originate, and in which the primary and secondary planes could not be recognized as such.

Before leaving the stage (fig. 7) in which the egg was found at 3 h. 45 m. P. M., let me mention an appearance in the egg which was not understood, but which may have a significance in the embryology of these animals. At the point in the egg adjacent to the break which has taken place in the direction of the primary furrow, a depression is formed which resembles an opening leading into the interior of the ovum. From the arrangement of the cell walls in the immediate vicinity, it seemed as if this opening was formed by the drawing apart of the walls of the cells, but whether it is the result of decay or not cannot be at present stated. The single egg in which it was observed, however, afterwards died before passing into advanced larval conditions.

It is at about this time in the development of the *Agalma* egg that some of the most extraordinary examples of protoplasmic elevation from its surface were observed. The resulting changes in external form often baffle all attempts to observe accurately the normal outlines of the cells of the segmented egg. These rhizopodal prominences are most clearly marked in those eggs which have been in long captivity, and seem wholly different in different ova.

Before closing our account of the segmentation, let us compare our observations with those of other naturalists on the same or closely allied genera. The poverty of our knowledge of the segmentation of the egg of the genus *Agalma* is so great, that I find few descriptions in the writings of others available for comparisons. Metschnikoff, although not figuring the segmentation of the egg, evidently observed it, as the following mention indicates. He says,* "Die Dotter zerklüftung, resp. Larvenbildung findet auf dieselbe Weise statt, wie ich oben für *Epibulia aurantiaca* angedeutet habe und wie sie bei allen von mir beobachteten Siphonophoren als Regel gilt. Was aber die Vorgänge der Organbildung betrifft," he continues, "so finde ich die meiste Analogie mit den von Haeckel untersuchten *Crystallodes rigidum* und *Athorybia rosacea*, obwohl auch in dieser Beziehung *Agalma Sarsii* manches Eigenthümliche darbietet." Turning for further information to his account of the segmentation in *Epibulia* we find him devoting a few significant paragraphs to this interesting process. He says,† "Die bald auf das freie Ablegen (es gelang mir nie künstlich aus dem Schlauche befreite Eier

* *Op. cit.*, p. 49.

† *Op. cit.*, pp. 40, 41.

zur Entwickelung zu bringen) folgende Eizerklüftung beginnt nur an einem Pole, in einer Weise, wie ich oben für *Geryonia* angegeben habe. Es bildet sich an dem besagtem Ort eine Furche deren Wände durch eigenthümliche Falten ausgezeichnet werden, welche ein deutliches Zeugniss von der Festigkeit der peripherischen Protoplasmaschicht abgeben. Die besagte Furche vertieft sich in Meridianaler Rechtung gegen den anderen Pol zu, das ganze Ei in zwei Hälften zertheilend, die nur durch eine Brücke zusammengehalten werden. Schliesslich zerfällt das Ei in zwei gleich grosse sog. Furchungskugeln, ohne dass an ihnen irgend eine Spur der originalen Entstehungsweise erhalten bleibt. Das zweikugelige Ei zerfällt auf eine ähnliche Weise in vier Theile, welche sich wieder vermehren, und der sog. regelmässige Zerklüftungsprocess setzt sich weiter fort, bis das Ei in Eine mehrzellige vermittelst der Flimmerhaare freischwimmende Larve verwandelt wird." This account of the segmentation process in *Epibulia* is certainly the best which we have of this period in the development of any Siphonophore. It is, however, the history of the growth of the egg of a Calycophore, while *Agalma* is a Physophore. The value of a comparison of the two is of greatest importance in phylogenetic studies of the respective groups; as showing how close this process is in widely different genera. The segmentation of the Siphonophore egg, as followed by Gegenbaur and Haeckel, differs considerably from that of *Agalma*. The description of the former naturalist is short, but concise. Segmentation was observed by him in several genera. He says: * "Num folgt rasch die Theilung des Dotters, die mit dem Auftreten einer ringförmigen Furche um den Aequator des Eies sich einleitet. Dies wiederholt sich dann an jedem Theilungsproducte, bis das ganze Ei aus einer Masse gleichartiger Furchungskugeln besteht, die ihm das bekannte 'Maulbeerförmige' Aussehen verleihen. In 24–36 Stunden ist der ganze Process vollendet. Ein hier besonders genau zu verfolgender Umstand ist die jedesmalige Theilung des Keimbläschens, welche der Theilung des Dotters vorausgeht; in gleicher Weise verhalten sich dann auch die Theilungsproducte des Keimbläschens zu der Bildung neuer Dotterkugeln." The division of the "Keimbläschen," which was not observed in *Agalma*, is thus reported in at least one genus by Haeckel. In *Physophora* he says: † "Ich kann diese positive Beobachtung Gegenbaur's, welche für die theoretisch wichtige Frage von der Continuität der Zellengenerationen von hoher Bedeutung ist, durch mehrfache eigene Beobachtungen bestätigen.

* *Op. cit.*, pp. 49, 50.

† *Op. cit.* for *Physophora*, p. 18; for *Crystallodes*, p. 51; for *Athorybia*, p. 89.

Der ersten Halbirung des Eidotters geht die Halbirung des Keimbläschens, und dieser wiederum die Halbirung des Keimfleckes voraus." The segmentation of *Crystallodes*, he says, "ist nicht wesentlich von demjenigen der *Physophora*-Eier verscheiden, welchen wir oben bereits geschildert haben." And later, "Der Furchungsprocess des Eies weicht bei *Athorybia* nicht von der oben geschilderten Eifurchung von *Crystallodes* und *Physophora* ab."

Development of the Primitive Covering-Scale.

Epiblast, Hypoblast. — The morula (Pl. II. fig. 8) now becomes covered with a granular layer of ciliated cells whose origin was not observed. This layer is thickest at one pole, where its walls have a reddish color. It has well-marked granular nuclei, which with acetic acid (Pl. III. fig. 2) are found most abundant at the rosy pole. The rosy pole of the segmented egg with its investing layer is supposed to be the same as the rosy pole of the first cleavage plane, and will be spoken of as the "germinative pole," or the "area germinativa." In *Crystallodes*, according to Haeckel,* it is "ein kreisrunder dunklerer Fleck, und zwar an derjenigen Stelle der Oberfläche welche dem späteren aboralen oder proximalen Pole der Längsaxe entspricht. Dieser Fleck, der Fruchthof (*area germinativa*) genannt werden kann, ist bedingt durch eine rasche Vermehrung der Zellen an dieser Stelle der Oberfläche." Metschnikoff † says in his account of the development of *Agalma:* "Die erste embryologische Erscheinung bei der freischwimmenden vier Tagen alten Larve besteht in der Ablagerung einer peripherischen Ectodermschicht, welche jedoch auf einer Hälfte des kugeligen Körpers (die ich fortan als die obere bezeichnen werde) viel dicker als auf der anderen ist. Am folgenden Tage kommt auch das Entoderm zum Vorschein, sich unmittelbar unter der verdickten Stelle der äusseren Schicht concentrirend."

The earliest appearance of the superficial layer in *Agalma elegans* was not observed to be confined to one pole, but in the youngest stages observed the layer completely surrounds the egg; it is only later, *dm* (Pl. III. fig. 1), that it thickens at the pole known as the germinative pole. There is a noteworthy fact in the growth of *Agalma*, that, whenever a new organ is formed on the surface of the *Agalma* egg, we have a concentration of the reddish pigment at that place, while the color, when present, is more diffused on other parts of the egg. This law holds good

* *Op. cit.*, p. 53. † *Op. cit.*, pp. 49, 50.

in the formation of that polar elevation which marks the origin of the primitive hydrophyllium, the first-formed organ of the larva. The various designations which have been used in the nomenclature of the two poles of the egg in this and following stages admit of misinterpretations. If we call the pole at which the increase of the thickness in the surface layer takes place the upper pole, we convey a wrong impression as to its natural position in the water; for if we observe the position in which the egg floats in stages a little older, it will be seen that the so-called upper ("obere") pole is always downward, as it naturally would be brought in equilibrium by the increase in weight resulting from the growing organ. Not less misleading are the terms oral and aboral. When the mouth of the first-formed polypite appears, it is in a position 90° from that pole (the *area germinativa*) at which the primitive hydrophyllium first forms. The aboral pole is therefore 90° from the position assigned to it, if the terms have anything more than an arbitrary significance. The rosy color seen at one pole of the unsegmented egg dates from the time when the ovum was in the sac within the gonophore. At that early stage the pole of the ovum opposite the attachment of the sac is rosy in color, and through all stages of cleavage up to one with eight cells that same rosy pole has been recognized. Here (8-celled stage) the relations to the axis were lost; but a rosy region was still to be seen, and it seems legitimate to conclude that the rosy pole is identical in these cases, rather than that the color has migrated from one region of the ovum to another in unseen stages intermediate between those submitted to exact observation. Moreover, going a step farther, can we not also regard that pole where the single layer is beginning to thicken, and which has the same reddish color, as identical with those which we have studied? I think we can suppose that the rosy color in this stage indicates the same pole, which is marked out by it at the very beginning, — the same, in fact, through which the first cleavage plane was observed to pass. Although I have spoken of this pole as the germinative pole, its axis is not the same as the axis of the adult animal. The investing layer spread over the surface of the egg is thickest at the germinative pole, and diminishes in thickness gradually to the opposite pole. The thinning out of this layer is a regular diminution on all sides; and up to the present time there are no right and left sides to the layers which cap the germinative pole.

In the next stage (Pl. III. fig. 3) following the last, the ovum, instead of being spherical, has become more elongated, assuming the form of a prolate sphere, and the portion directly under the germinative pole has been raised by a slight constriction, forming a swelling on the external surface. At this time we can distinguish two layers, *eb.*, *hb.*, in the undivided single layer of the former stage, while between them, as they lie one above the other, there is a slight thin crescent-formed space, which later increases in size, and is filled with a third layer. The elevation, apparently three-layered, with the part of the yolk immediately below it, forms a disk-shaped body with concave surface resting upon the spherical egg. This disk hangs downward as the egg floats in the water. In another egg (Pl. III. fig. 4) of about the same age, the shallow constriction which marks off the disk from the remainder of the egg is somewhat magnified. Although the general outlines of this embryo are distorted (the constriction being too deep), the stage is an interesting one as showing on one side a slight notch which has appeared in the outer layer, *eb.* The existence of this notch enables us to determine certain primary axes, formerly not distinguishable, on the surface of this larva, which have relations to the axis of the adult *Agalma.* Before passing to this point, let me say that the outer of the two layers is the epiblast, the inner the hypoblast, and the layer of the intermediate chamber the middle layer (*mb.*), later constituting the gelatinous mass of the hydrophyllium. The custom of looking at the float as a starting-point for reference of organs, and using the terms proximal and distal in reference to this structure, has been adopted in the writings of some naturalists. This nomenclature can as well be followed here in the larva as in the adult. The float, although in *Agalma* it is not the first structure to appear, can be regarded in the young, as in the adult, as situated at a fixed point or pole for reference when studying other organs, since in all genera it is the first permanent structure which appears.

It will be found in the subsequent history of our larva, that the float develops near by a region of the disk opposite to that in which the notch in the outer of the two layers lies. We can approximately say that in Pl. III. fig. 4 it will appear just below the indentation on the left hand, as the figure is drawn. The whole of the disk-shaped elevation which has formed on the egg and destroyed its sphericity lies, therefore, on one side of the future float. That side may be called the germinative side, for on it appear one by one all the remaining organs of the *Agalma* body. They have, however, at first no regularity in the position in which they form. Using the nomenclature which has been suggested, the notch is on the distal side of the disk, as it is most distal from that pole of the ovum later to be occupied by the float. The hemisphere of the ovum which faces the observer may be called the right side, as referred to an axis passing through float and distal rim of the elevation, and that opposite the left, for reasons which will soon appear.

The larva is now a little over two days old. The many cleavage planes, *cl. pl.*, forming the polygonal segmentation spheres in the yolk are clearly defined. The protoplasmic network, *vt. c.*, throughout the ovum, is likewise still well marked. The outer of the two layers, or the epiblast, is ciliated externally. At the elevation on the germinative pole it has a reddish color. The layer beneath the epiblast, or the hypoblast, is thinner than the more superficial. A horizontal diameter of the egg is .45 mm.; the longest axis at right angles to it, and passing through the germinative pole, is .55 mm. Both epiblast and hypoblast together at the thickest point are not more than .01 mm. in thickness.

In a slightly older larva (Pl. III. fig. 5) the significance of the notch at the distal rim of the primitive elevation of the germinative area becomes more apparent. The epiblast and hypoblast, formerly of about the same thickness, have in this stage somewhat changed their relative dimensions, and when seen in profile are observed to have assumed folds which are of significance in the shape of the future covering-scale. The epiblast on the distal side of the disk-like elevation has thickened, and two well-marked angles appear on its exterior. Its surface on the distal side rises by a smaller angle from the yolk surface than on the proximal, and slopes away more gradually to the opposite side. The hypoblast hugs the yolk cells at all points except at one place (*c. p. l.*), where it rises from them, leaving a recess which is later the cavity of the primitive larva. Near by this cavity the hypoblast is slightly separated from its enveloping layer, the epiblast, by a middle or third layer. The two angles found on the surface of the epiblast at the distal rim of the forming disk have grown more prominent, as shown in the two following sketches (figs. 6, 7), and the two layers have separated more and more from each other.

When looking at the egg in its present stage of development, we notice at once how sharp the difference is between the proximal and distal portions of the rim of the disk-like elevation. They differ very much in shape from each other; and this difference is magnified as we follow the course of the development into older larvæ. In the light of what is known of the existence of bilateral symmetry in the adult *Agalma*, we may regard this difference in the two borders of the scale as among the earliest expressions of that condition. The forming disk possesses a proximal and distal border, and therefore a right and left side, as referred to a line passing through these regions. This line lies in the same plane as the axis of the adult *Agalma*, although it is not clear that the right and left sides of the disk-like elevation correspond with the right and left sides of the appendages later found on the adult *Agalma* axis. The general appearance of the yolk and the size of the egg is approximately the same as in the preceding stages. The right and left sides used for figures up to Pl. III. fig. 4 have not the same significance as here interpreted.

The next oldest larva (fig. 9) differs primarily from the last in the greater elevation and prominence of the layers formed on the yolk. The epiblast and hypoblast are much thicker; the former has a reddish, the latter a yellowish color. The constriction around the elevated disk between its edges and the surface of the ovum has deepened o the distal side of the elevation as seen in profile, but the indentation is very slight on the proximal side.

Within the disk a gelatinous layer, so transparent as to be invisible, has formed by a separation of the epiblast and hypoblast. The thickness of this layer is greatest near the distal end of the disk. Yellow and reddish pigment is found in the epiblast on the surface of the yolk sac. It was also noticed that the epiblast at *pn. cy.*, near the proximal end of the elevated disk, is much thicker than that near the distal side, and that there was a tendency to form a slight epiblastic elevation at that point. If the reader will compare the figure of this stage with one of about the same age by Metschnikoff, he will find a great difference in external shape between the two. My larva is approximately the same as Pl. VIII. fig. 5 in the oft-quoted work by that author, who says that his larva is five days old. My adult *Agalma* was put in the aquaria on August 6, and the stage represented in fig. 8 was found free in the water on August 8, or two days later. I likewise picked out of the same water three days after, or five days after the adults were put there, larvæ of the same age, while with these were still others much farther advanced, and some which were just passing through the early stages of segmentation of the egg.

I find a discrepancy, which may be a generic difference, in the rate of growth day by day recorded in Haeckel's observations on the development of *Crystallodes*, and Metschnikoff's of *Agalma*. In larvæ of *Crystallodes* four days old the float was as far advanced as in the *Agalma* six days old of Metschnikoff, while on the second day both the *Agalma* and *Crystallodes* larvæ were still in a morula stage. These discrepancies arise from the difference in the mode of growth of the float in the genera, or from the fact that different clusters of eggs, or different members even of the same cluster, mature at different times. We must not suppose, in studying the development of *Agalma* eggs, that the ova found free in the water were all cast at the same time. The only trustworthy method of observation is to trace individual eggs into larvæ and time their development, which is a most difficult thing to accomplish successfully with these tender creatures. Even if we follow and time with care the rate of growth in our glasses, it is a question whether we should not make an allowance for retardation or acceleration of this time brought about by changes in the temperature of the water in which they are placed in our aquaria.

The disk formed at one pole of the egg by the epiblast, hypoblast, and an intermediate transparent layer, may be called the primitive hydrophyllium or covering-scale, to distinguish it from others which are later formed. In a stage following the last this body has assumed an elevation upon the surface of the egg greater than formerly. As far as its general outlines go, no great change has taken place in the larva with advancing age; but near the rim of the disk another minute elevation in the walls of the epiblast has pushed itself up, which is destined later to play an important part in the structure of the adult. This elevation (*pn. cy.*), which at this time cannot be distinguished from a simple bud such as any other organ of the *Agalma* body at first has, is the beginning of the future float. It is a true bud, as already pointed out by Metschnikoff.

The accounts which Metschnikoff and Haeckel give of the origin of the float in genera so nearly related as *Agalma* and *Crystallodes* are radically different. In *Crystallodes*, according to Haeckel, the air-sac originates from the primitive cavity as a bud. Speaking of changes on the sixth day, he says:* "Die wichtigste Veränderung aber, welche am sechsten Tage eintritt, ist die vollständige *Abschnürung des Luftsackes* von dem Centralraum der Primitivhöhle. Das Entoderm, welches die Wand des Luftsackes bildet, und welches bisher an seiner Einmündung in die Centralhöhle unmittelbar überging nach oben in das Entoderm des Deckstück-Nährcanals, nach vorn in das Entoderm des Polypiten, wächset nun vollständig an dieser Stelle zusammen. Der Larvenkörper enthält also nunmehr zwei vollständig getrennte und geschlossene, mit Flüssigkeit erfüllte Höhlen: die einfach rundliche oder längliche runde Luftsackhöhle, und die Centralhöhle, welche in vier Canäle sich verzweigt, in die Canäle der beiden Knospen, des Deckstücks und des Polypiten. Das Entoderm, welches alle diese Höhlräume auskleidet,

* *Op. cit.*, p. 58.

ist eine einschichtige Lage von Flimmerepithel. Dasselbe erscheint bei durchfallendem Lichte bräunlichgelb, bei auffallendem Lichte spangrün gefärbt. Der Luftsack selbst ist rings von den hellen Zellen des Nahrungsdotters umgeben, und steht nur an seinem proximalen Ende (der Abschnürungsstelle) in Berührung mit der Wand der Polypitenbasis, welche daselbst in das Deckstück übergeht."

In the genus *Agalma* Metschnikoff thus describes the appearance of the float on the fifth day. He says: * "Zu gleicher Zeit bemerken wir dicht unterhalb des Deckstückes, auf der Fläche, die ich als Rückenfläche bezeichne, eine locale Ectodermverdickung, welche als erste Spur des Luftapparates angedeutet werden muss. Am sechsten Tage hat sie die Form eines halbkugeligen Körpers angenommen, der unter der äusseren Ectodermbedeckung und in der Nähe des einstweilen noch localen Entoderms seine Lage findet." The origin of the float in *Agalma elegans* resembles more closely that of *Agalma Sarsii* than that of *Crystallodes*. It arises as a simple epiblastic elevation of the yolk surface, not far from the proximal side of the hydrophyllium. That elevation is primarily of epiblast, but later the hypoblast may also enter into its formation. As the float grows older, the bud diminishes in size, thickening inward, and a separation of the hypoblast from the epiblast takes place, which is filled by an intermediate body, either thickened epiblast or the intermediate or middle layer. The subsequent growth of the float will be seen in descriptions of later stages of the primitive larva.

In Fig. 13 we find that the primitive hydrophyllium has increased very much in size, while in the progress of that growth the distinction between the proximal and distal edges of the disk which we have earlier detected are still maintained. The great body of the scale is gelatinous, the mass of which is formed by an enormous growth of a middle layer (*mb.*), which lies between epiblast and hypoblast. The relative thickness of the epiblast has greatly diminished. It is still ciliated and easily distinguished from the other layers when seen in profile and along the rim of the hydrophyllium, while scattered over the surface of the scale appear the small epiblastic structures or nuclei (?). The forming covering-scales called serrated hydrophyllia (*ser. hyph.*) have a slightly red color.

The primitive cavity (*c. p. l.*) lined with hypoblastic cells which have a distinct yellow color has risen with the growth of the bell, and extends towards the distal rim of the hydrophyllium. The edges of the disk are free, the hydrophyllium fitting over the egg like a helmet, the visor

* *Op. cit.*, p. 50.

being represented by the distal border. In the proximal region of the primitive hydrophyllium we find that the epiblast and hypoblast have separated from each other, and that between them has formed a layer or cellular mass representing the great gelatinous mass of the medusa bell. Outside of it is the epiblast, while lining the cavity is the hypoblast. The latter layer can be traced from the lining of the primitive cavity for some distance over the surface of the yolk cells under the epiblast. The epiblast can also be traced from the superficial position on the yolk over the surface of the hydrophyllium. I find by a comparison of this figure with those by Metschnikoff representing the first appearance of the float, that it most closely approaches his Fig. 6, Pl. VIII. In his figure, however, we miss a representation of the inner hypoblast between the bud which forms the float and the yolk cells which were seen in the stages here figured. Comparing, however, his Fig. 5 of the same plate with his Fig. 6, we find in the latter an ectodermic bud but no hypoblast, while in the former a layer continuous with the lining of the primitive cavity lies under the epiblast where the float is developed. His Fig. 6 represents the origin of the float as far as the epiblast goes like mine, but we miss in it a deeper layer of hypoblast which is probably present. The epiblast at this stage probably divides into a superficial and a deeper portion. It is suggested that the latter is the same as the middle or gelatinous layer of the medusa bell.

Under the visor at the distal rim of the helmet-shaped hydrophyllium of Fig. 13 the layer of epiblast is thicker than in most other regions, and has a reddish color. Its surface is rough by reason of elevations, which are probably superficial, uprising from the epiblast. At this point, or near by, the serrated hydrophyllia (*ser. hyph.*) characteristic of the second larval stage of *Agalma* first appear. The diameter of the primitive hydrophyllium, from distal to proximal border is .40 mm.; its elevation above the yolk, .15 mm. The diameter of the egg is .47 mm. These larvæ were picked out of the water in which the *Agalmata* were confined at six o'clock, August 8th. The hydrophyllium naturally floats downward in the water, the yolk being apparently lighter.

Primitive Larva. — The maximum development of the primitive or larval hydrophyllium is reached in the next stage, represented in Fig. 14. In this larva the yolk of the egg is still spherical, and little reduced in size, notwithstanding the enormous growth of the scale from it. The helmet-shaped hydrophyllinm almost completely invests the ovum. The bounding planes of the irregular polygonal cells of segmentation are clearly to be seen through the side of the hydrophyllium, and the enveloping layers of the yolk are traceable over its whole surface. Within the segmented yolk cells appears the protoplasmic network (*vt. c.*) which dates back to the original ovum in the gonophore. The primitive hydrophyllium is seen fitting over the ovum like a helmet, which, although fastened to it at the germinative pole, is free on the sides. Its border and sides cover about two thirds of the yolk which is here represented through the transparent lateral walls.

The primitive hydrophyllium is transparent, slightly reddish in certain regions, its great bulk being gelatinous. The following distinction between the distal and proximal rim can be easily seen when in profile. The wall of the distal edge, which corresponds to the visor of the helmet of our former comparisons, is much thicker than the opposite, and more rounded. The proximal rim ends in a sharp angle, and its walls are very thin. In a figure of this stage we have the larva represented as if we were looking at it from the left-hand side as defined above. Over the surface of the primitive hydrophyllium is spread a single layer of thin polygonal cells of the epiblast, which are seen in profile around the rim of the bell and on its bounding lines, even on the inner surface adjoining the yolk sac. Over the external surface the prominent nuclei of the same cells can be readily traced, dotting it at intervals, and in places well-defined cell-walls can be faintly seen. The layer from which these cells came, or the epiblast, was one of the first layers to form, and throughout the growth it has been gradually becoming relatively thinner and thinner. Although the bodies called nuclei of these cells are very well marked in *Agalma elegans*, I do not find them represented in the figures which have been published of other species of the genus *Agalma*, or *Crystallodes*. The remnant of that cavity, which has been called the primitive cavity, is now a tubular body with thickened hypoblastic walls of yellow color, and extends from the base of the float towards the distal portion of the hydrophyllium.

At this stage in the development of the primitive hydrophyllium it was observed that from the nuclei of several of the epiblastic cells, situated on the surface of the hydrophyllium above the fundus of the cavity, there were thread-like extensions, probably protoplasmic, which connect the surface of the larva with the hypoblast of the cavity. At times the surface of the hydrophyllium from which these threads arise is depressed as if forcibly drawn back by them. In a few instances the threads end blindly in the gelatinous layer at a point not more than half-way from the epiblast to the cavity. These threads sometimes have a close resemblance to certain similarly placed threads in worm larvæ, as in the well-known *Tornaria*, where they extend from the cavity of the larva to an apical cluster of modified epiblastic cells through an intermediate gelatinous layer. I was unable to observe these threads closely enough to detect any tubular structure in them. Later in the growth of the larva there are two filiform bodies connecting the cavity of a hydrophyllium with its surface, which may possibly be the same as the thread-like extensions of which we have already spoken. In the development of *Agalma Sarsii* as figured by Metschnikoff, we have no representative of these threads in the primitive larva, or in stages of later growth. Hæckel figures certain structures in the hydrophyllium of *Physophora* which have been found by me in *Agalma*, which in *Physophora* have the form of small tubes extending from the cavity to the surface. These call to mind the protoplasmic bodies in the primitive hydrophyllium of *Agalma*, although they are different in many respects. Hæckel gives them a morphological significance in *Physophora*, and regards them as comparable with certain parts of the chymiferous radial tube system of hydroid gonophores. He does not represent them in the younger forms, at least, of the primitive larva of *Crystallodes*. Cilia were not observed on the outer surface of the primitive covering-scale, but were seen on the epiblast covering the yolk at this age.

Of the remaining structures found in the primitive larva the most important in the future history is a spherical organ (*pn. cy.*) adjacent to the end of the primitive cavity. This body is the future float, and at this stage lies inside the egg, or between the yolk cells and the superficial covering, although no marked external elevation could be seen. The float is enclosed by a layer of cells which was traced continuously into the hypoblast of the primitive cavity, and also into the hypoblast which covers the yolk sac. Within the hypoblast the contents of the float and the hypoblastic layer were slightly separated. A continuation of the same layer, epiblast, reflexed from the inner surface of the covering-scale, extends over the float and is continued over the surface of the egg.

A second appendage, which assumed the form of a slight projection from the surface of the yolk on the left-hand side of the cavity of the primitive hydrophyllium, is also present in this stage of the primitive larva. In profile this structure (*ser. hyph.*) is arch-shaped, and has a slightly reddish color. It is the beginning of a covering-scale which, although provisional in nature, has given the name of "Athorybia stage" to a larval condition of *Agalma* which follows the first or primitive larva. On the right-hand side of the cavity of the primitive hydrophyllium is a cluster of cells of red color, which is the beginning of a second similar serrated hydrophyllium. This latter cluster, however, has not raised itself any considerable amount above the surface of the yolk. The longest diameter of the primitive hydrophyllium in Fig. 14 is .75 mm.; the thickness at the distal side, .17 mm. The length of the primitive cavity is .25 mm.; its breadth is .10 mm. The float is almost .10 mm. in diameter. It will be seen from these measurements that the scale has now reached a very great size as compared with its dimensions in earlier larvæ. It has now the maximum size to which it ever attains.

Fate of the Primitive Hydrophyllium.

It is known that this primitive hydrophyllium is a temporary or embryonic structure; but its fate, whether it is simply thrown off or absorbed, is not at present definitely made out. Both Hæckel and Metschnikoff have pointed out that it is a provisional structure, but neither has traced it far enough in the last phases of its history to satisfactorily show whether it is simply discarded, absorbed, or passes with external changes of outline into some other structure. The most definite statement which we have is as follows. Metschnikoff says, "das erstgebildete kappenförmige Deckstück abgeworfen wird" in the genus *Agalma*.

The primitive hydrophyllium of *Agalma elegans* suffers many modifications in external form in some of the older stages; but whether these modifications were abnormal, resulting from the fact that the animal is in confinement, was not determined. It seems to me more natural to suppose, that, instead of being thrown off in the subsequent stages, the primitive covering-scale passes by a few modifications in its external contour into some other organ, probably a differently formed covering-scale.

Fig. 15 represents the larva of *Agalma* on August 10, four days after the capture of the parent. This larva was picked out of the water, in which it was freely swimming below the surface. The figure represents the larva as seen from that pole which is opposite the germinative pole, so that the various organs which have appeared near that region are seen through the yolk contents. This position, assumed while the egg is floating, is that which is best adapted to exhibit the newly forming organs in their relation to the cavity of the primitive hydrophyllium.

We recognize in this stage many organs which have already been described, and one or two new ones lately formed. In the first place, the yolk — a prominent spherical mass of polygonal segmentation spheres with the internal protoplasmic network — should be mentioned. This occupies most of the middle portion of the figure. Around it in profile the epiblastic and hypoblastic layers, of which the former is ciliated, may be seen. The larger, more transparent body, seen above and on either side of the yolk, is the projecting primitive hydrophyllium. This distal portion of this scale is represented at the top of the figure, the proximal at the lower part, while the right hand of the figure is the left of the scale, following the nomenclature of previous descriptions. The nuclei of the epiblast and the polygonal outlines of the epiblastic cells are easily seen here and there over the surface of the scale. The cavity (*c. p. l.*) of the primitive larva has two or more thread-like structures (*fil.*) extending from its hypoblastic lining to the nuclei of epiblastic cells. The hypoblast of the primitive cavity has a yellow color, especially well marked at its distal end, where its walls are likewise covered with small pigment dots, black, or nearly so, in color. At the opposite extremity of the primitive cavity, near the float, it ends in a closed cone-like termination, which is hidden by the float in the figure.

It is perhaps needless to say, that the spherical body near the middle of the figure is the float, seen through the yolk contents; and that on the right and left sides of the primitive cavity are two buds, which later develop into the serrated hydrophyllia characteristic of the Athorybia stage. In both of these can be recognized a very thick outer layer, which is probably the middle gelatinous layer, over which is spread a thin layer of epiblast, and an inner thinner layer, which is hypoblast. Within this last layer in each case we have a cavity which is the beginning of the future tube which penetrates the scales. A considerable quantity of reddish pigment is found in the yolk in the immediate neighborhood of the last-mentioned organs. It is very difficult for me to formulate any law for the relative position in which the successively appearing buds of the larva of the *Agalma* develop. We know that in the adult *Agalma* those nectocalyces which are nearest the float are the youngest, and that the newly formed organs of this name always develop between those already formed and the float.

Fig. 2, Pl. IV. represents a very instructive stage in the development of the primitive larva, which was taken on August 9 at noon, or on the third day after the capture of the adult. It is seen in a little different plane from the preceding, but in such a way that the organs already mentioned can be easily distinguished. We have in this stage an addition of most important character, for at this time first appears the beginning of the polypite. The larva is shown in such a way that the embryo is twisted somewhat as compared with former stages, and the hydrophyllium has its proximal edge so turned into view as almost completely to cover the yolk. By this new position of the larva the conical end of the primitive cavity near the float is well shown, while the two buds which later form the serrated hydrophyllia are thrown to one side. The most developed of these last-mentioned organs has a spatulate form, and shows the three layers, epiblast, middle layer, and hypoblast, as well as a cavity which occupies most of the interior of the organ. The other hydrophyllium is not as well formed, and is more highly colored.

In addition to the buds which have been mentioned as already formed, we have represented in this stage a significant thickening (*pyt.*) at one pole of the two layers which surround the yolk of the egg. This pole is situated in a point at right angles to that where the bud which forms the float first appears. The elevation of these two layers takes the form of a simple bud comparable with other buds of the primitive larva, and ultimately forms the first or primitive polypite of the *Agalma.* The elevation of the primitive polypite is reddish yellow and ciliated, with the lower layer slightly separated from the cells of the yolk. Although the point at which the polypite in *Agalma* develops is very different from that at which the same organ of *Crystallodes*, as recorded by Hæckel, arises, these differences are not too great to have a similar morphological interpretation. Like all organs or parts of the *Agalma* body, the polypite originates as a three-layered bud from the surface of the yolk. Like them also it separates from the yolk-cells, leaving a cavity between the hypoblast and vitelline cells. A part of those walls of the yolk which enclose the yolk-cells becomes the outer wall of the float; another part is modified into new buds, which develop into tasters, hydrophyllia, and tentacles; and still another part forms the walls of the first-formed polypite. Can we not consider that the yolk-sac in this case, as in *Crystallodes*, is not changed into the polypite, as in *Physophora* and some other genera?

From Fig. 16, Pl. III., taken five days after the capture of the *Agalma*, we may obtain a somewhat better idea of the relationship between the buds which form the float, the serrated hydrophyllia, and the first-formed polypite. In the view of the larva as here seen, we are looking at the larva from the side opposite that on which the primitive hydrophyllium is attached. The primitive cavity is thus thrown behind the yolk, and is concealed by the buds which have already appeared, one of which is shown in profile. The larva is placed in what is considered its normal position comparable with the natural position of the adult. The float is well developed, and resembles closely that of the adult. Below it there is a well-marked red pigment-spot on the external walls of the ovum, which forms a convenient point for the orientation of other organs, and which itself forms in later stages a well-known organ (embryonic tentacle); and at the pole of the egg opposite the float we find the partly formed polypite. The lower part of the large transparent body behind the yolk is the distal rim of the hydrophyllium; the upper part is the proximal border. The axis of the future *Agalma* is thought to pass lengthwise through the float, and to cut also that pole of the yolk at which the polypite is forming.

The axis of the larva, as thus indicated, does not coincide with that which originally passes through the egg from the point at which the first elevation of epiblastic and hypoblastic layers took place to the opposite pole. It is apparently at right angles to this. If I am right in regard to the relationship, or, to use a stronger word still, the coincidence, of the former axis with the first plane of cleavage in the unsegmented ovum, the axis of the adult *Agalma* is at right angles to the first plane of cleavage. It may be mentioned at this point, that in the gonophore, as the egg first forms, the axis of the ovum passing through the red pole and the point of attachment of the gonophore is normally at right angles to the axis of the *Agalma*. The horizontal diameter of the larva at this stage is .70 mm. The vertical diameter is .75 mm. The longer axis of the ovum is .45 mm.; the shorter, .35 mm.

Fig. 1, Pl. IV. is taken from a larva a little older than the last, but still five days old. It resembles the young *Agalma Sarsii* at the close of the second week. The axis is placed vertical in the same position as that of the adult as usually represented. The separation of the hypoblast from the yolk-cells has left a cavity of relatively considerable size at the point where the polypite has begun to form. This cavity recalls a similar cavity in the larva of *Crystallodes* as figured by Hæckel. There is as yet no apparent diminution in the size of the primitive hydrophyllium, and the outlines of the epiblastic cells upon it can be easily traced. The yolk-cells still enclose the protoplasmic network, and have the same polygonal shape as earlier in their history. The float is more elongated and lies on one side of the yolk. It is filled even in this larva with air or gas. Below it is a mass of reddish pigment concentrated in a cluster. The size of this larva is about the same as that of the last.

On August 13, seven days after the *Agalma* had been placed in the water, I was surprised to see, on looking for my larvæ through the walls of the glass vessel in which they were confined, that they had very much decreased in numbers. This led to the discovery that, whereas up to about this date they were found at all depths in the water, the larvæ are now to be seen only upon the surface. They often cluster together there, and the size of the float imparts to them a silvery color, like a small bubble of air resting on the water. The reason why the larvæ seek the surface at this phase of their development probably is, that the float has grown so large, or that the size of the primitive hydrophyllium has diminished. Whatever may be the cause which led the *Agalmata* to come to the surface, an effect which can probably be ascribed to the two causes mentioned above combined, we find that the size and general outlines of the first-formed covering-scale have undergone several modifications. Fig. 5, Pl. IV. shows a larval stage taken August 13th, in which the size of the scale is much smaller than in the larvæ already described. It is found at this time in the life of the larva that the border of the covering-scale has a tendency to draw together, and its surface becomes grooved or furrowed. In Fig. 6 we see a continuation of the same process, and in Fig. 7 still more reduction in the size of this body. One or two structural features have led me to regard the flat angular body on the yolk of these larvæ as the primitive hydrophyllium reduced in size. The tube which is found in the primitive scale, especially at the marginal termination, has a yellow color with black dots. These figments were found in the tube of the more reduced scale in its present condition. The small nuclei spread over the surface of the primitive hydrophyllium, called in our above description the nuclei of the epiblast, are easily recognized on the surface of the modified scale. With the reduction in external form of the plump walls of the first-formed scale, or primitive hydrophyllium, there has taken place also a change of form in its internal cavity. At the distal border of a scale represented in Pl. IV. fig. 8, the tube of the scale has bifurcated and extends in two divisions to the bell rim, where both end in the neighborhood of clusters of large nematocysts or lasso-cells. A yellow color was observed at these points, although the tube of the scale throughout most of its course is not as markedly colored. The small cell-like spots which appear on the surface of the scale and resemble the nuclei of the epiblast as already described, are well marked on the ridges of the scale.

I have been unable to identify a scale of this kind with any of those figured in the larval stages of *Agalma* as described by Metschnikoff. Hæckel, however, figures a similar scale with divided tube in *Crystallodes*, but from his descriptions it does not follow that he regards it as the modified primitive scale. In *Physophora*, however, we find an approximation in shape to this scale in the primitive hydrophyllium, and moreover in this genus, as in mine, there is a smaller tube extending from the cavity of the scale to the surface, and ending in or near clusters of lasso-cells superficially placed. If the first-formed scales (primitive hydrophyllia) in both *Physophora* and *Agalma* are homologous, we may find the smaller bifurcations connecting the cavity of the scale in *Agalma* with its surface to be the same as the similar structures described by Hæckel in the young *Physophora*, provided, of course, that the flat scale of Fig. 8 is the modified primitive covering-scale of Pl. III. fig. 14. The flat scale (fig. 8) is certainly different in the contour and course of the central tube from the serrated hydrophyllia, and no other structure is thought of to which to refer it except the primitive hydrophyllium, that large covering-scale whose origin dates back into the youngest stages of the larva. What has already been here written of the modifications in form which the first-formed covering-scales go through, does not of course show that in the end it may not be simply cast off. My studies throw no light on this point. If it is ultimately dropped it undergoes modifications in outline before the consummation of that event.

CAMBRIDGE, July, 1885.

EXPLANATION OF THE PLATES.

br.	Bridge connecting two segmentation spheres.
cav.	Cavity.
cl. pl.	Cleavage plane.
1 *cl. pl.*	First cleavage plane.
2 *cl. pl.*	Second cleavage plane.
3 *cl. pl.*	Third cleavage plane.
c. p. l.	Cavity of primitive larva.
dm.	Thickening of the superficial layer.
cb.	Epiblast.
ct.	Undivided portion of ovum.
fil.	Filament.
goph.	Gonophore.
gt.	Oil globule.
hb.	Hypoblast.
hyph.	Hydrophyllium.
m.	Membrane.
mb.	Mesoblast.
n.	Nucleus.
nl.	Nucleolus.
om.	Ovum.
pg.	Polar globule ?
pig.	Pigment spot.
pr.	Primitive furrow.
pr. hyph.	Primitive hydrophyllium.
pn. cy. / *py. cy.*	Pneumatocyst.
pn. ph.	Pneumatophore.
pyt.	Polypite.
r. pol.	Rosy pole, when not indicated, upper pole of figure.
r. tb.	Radial tube.
sc.	Secondary furrow.
scr. hyph.	Serrated hydrophyllium.
1 *scr. hyph.*	First serrated hydrophyllium.
2 *scr. hyph.*	Second serrated hydrophyllium.
tb.	Tube.
ubr.	Umbrella.
vcl.	Velum.
vt.	Vitellus.

vt. c. Vitelline cells forming a "protoplasmic network" through the yolk contents. In many of the figures only a few of these cells are drawn. They are found throughout the whole contents of the egg.

y. Unknown body, possibly remnant of membrane which encloses the egg.

All the figures, with the exception of Pl. IV. figs. 3–7, were drawn by the author with an Oberhäuser camera, objective B. B., eye-piece 2, Zeiss. Size reduced one half in photography. All figures except Pl. II. fig. 2 were drawn from living eggs and larvæ. The last mentioned was treated with dilute acetic acid before drawing.

PLATE I.

Fig. 1. Immature female gonophore with egg in the interior. Nucleus and nucleolus shown through its walls. Attached to parent.

" 2. Egg in a small immature gonophore, with sinuses between ovum and bell walls of gonophore.

" 3. Female gonophore found free in water. Enclosed ovum .5 mm. in diameter.

" 4. The ovum in the act of escape from the gonophore.

" 5. Ovum removed from a gonophore (artificially).

" 6. Ovum just escaped from gonophore (naturally), .45 mm. in diameter.

" 7. Egg showing the formation of a primitive furrow at one pole.

" 8. The same, ten minutes older than last.

" 9. The same, fifteen minutes older.

" 10. The same, twenty minutes older.

" 11. The same, twenty-five minutes older.

" 12. The same in two-cell stage, thirty minutes older than Fig. 7. Diameter .35 mm. The egg traced from Figs. 7–12 is .1 mm. smaller than that from Fig. 13 on.

" 13. Two-cell stage with beginning of a secondary furrow (*sc.*), .60 mm. in long diameter, .43 mm. in least diameter.

" 14. Two-cell stage, ten minutes older than Fig. 13. .60 mm. in diameter.

" 15. The same, fifteen minutes older.

" 16. The same, eighteen minutes older.

" 17. The same, twenty minutes older.

" 18. The same, twenty-five minutes older, showing the formation of the secondary furrow, extending in a horizontal direction over the surface of the ovum. It also shows the deviation of the primary cleavage plane (1 *cl. pl.*) from a straight line when seen in profile.

" 19. Formation of a 4-cell stage by the closing in of the secondary furrow (*sc.*). The furrow is still open at each end. Thirty minutes older than Fig. 13, .50 mm. in diameter.

" 20. Four-cell stage, thirty-five minutes after Fig. 13.

" 21. The same, forty-five minutes after.

" 22. The same, fifty-five minutes after.

" 23. The same, one hour and ten minutes after.

PLATE II.

Fig. 1. Four-cell stage, two hours after Pl. I. fig. 13.
" 2. The same, two hours and five minutes after.
" 3. Segmented ovum showing the beginning of the tertiary furrow (3 *cl. pl.*), two hours and fifteen minutes older than that represented in Pl. I. fig. 13.
" 4. Four-cell stage, two hours and twenty minutes older.
" 5. The same, two hours and twenty-five minutes older.
" 6. Older stage, two hours and thirty minutes after Fig. 13. .50 mm. in diameter.
" 7. Segmented ovum, two hours and forty minutes older than Fig. 13.
" 8. Superficial granular layer (*dm.*) formed on the segmented ovum. Planula? .45 mm. in diameter.

PLATE III.

Fig. 1. Segmented egg with a marked increase of thickness of superficial layer at *dm.* .54 mm. in diameter.
" 2. The same, treated with acetic acid (two layers at pole, *eb.*, *hb.*).
" 3. An older egg, in which the thickness of the two layers is more marked. .60 mm. in greatest diameter.
" 4. The same, older.
" 5. The same, still older, showing the cavity of the primitive larva (*c. p. l.*).
" 6. A portion of the egg and the growing protuberance at its pole.
" 7. The same, older.
" 8. An older larva, with constriction between the scale and the surface of the ovum.
" 9. The same, older. The right side of this figure corresponds with the left of preceding and following figures, except in Fig. 12.
" 10. Embryo of about the same age as the last, reversed.
" 11. Embryo found in water, August 8 (adult put in August 6), a little older than the last.
" 12. Embryo still older.
" 13. The same, older, .47 mm. in diameter.
" 14. A primitive larva taken on the third day after the capture of the adult (72 hours old ?), .70 mm. in diameter.
" 15. A larva a little older than the last, seen from the pole opposite that on which the primitive hydrophyllium is formed. The larval appendages are therefore for the most part seen through the yolk.
" 16. A larva so placed that the axis of the future *Agalma* is almost vertical. The union of the primitive hydrophyllium and the yolk is on the side of the yolk turned away from the observer. Older than last.

PLATE IV.

In none of the figures are the vitelline cells and the nuclei of the thin epiblastic layer of the primitive hydrophyllium brought out with sufficient distinctness. There should be two layers instead of one at *pyt.* in Figs. 1, 2, and 3.

In Figs. 2 and 4 the wall of *c. p. l.* is too black, and does not show the thickness of the hypoblast. The rows of nematocysts on the surface of *hyph.*, Fig. 8, are not well shown. The clusters of nematocysts at the margin of *hyph.* after the bifurcation of *tb.* are faulty. The cilia on the surface of the ovum, well seen in Fig. 2 at *pyt.* in my drawing, are not found in the photographic reproduction.

Fig. 1. A larva (primitive larva) in about the same age as the last and in a like position, except that the primitive covering-scale or hydrophyllium is turned a little more to the plane of the observer.

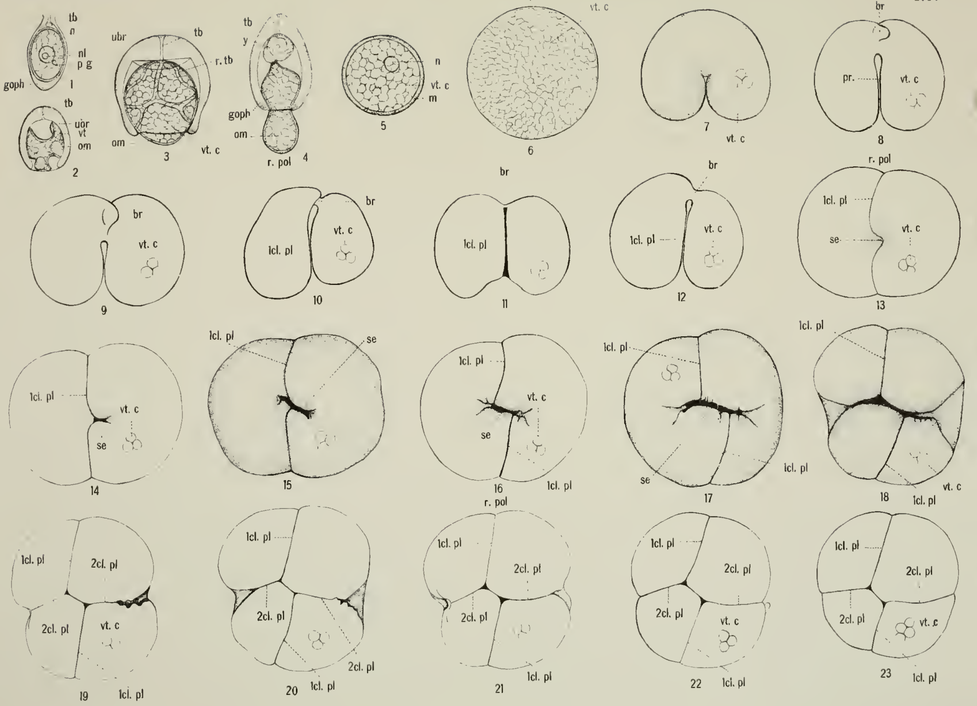
" 2. The same, looking through the primitive covering-scale upon the apex of the float, which lies in the geometric centre of the figure. Three days old.

" 3. Older larva, free-hand drawing, showing growth of serrated scale (*ser. hyph.*).

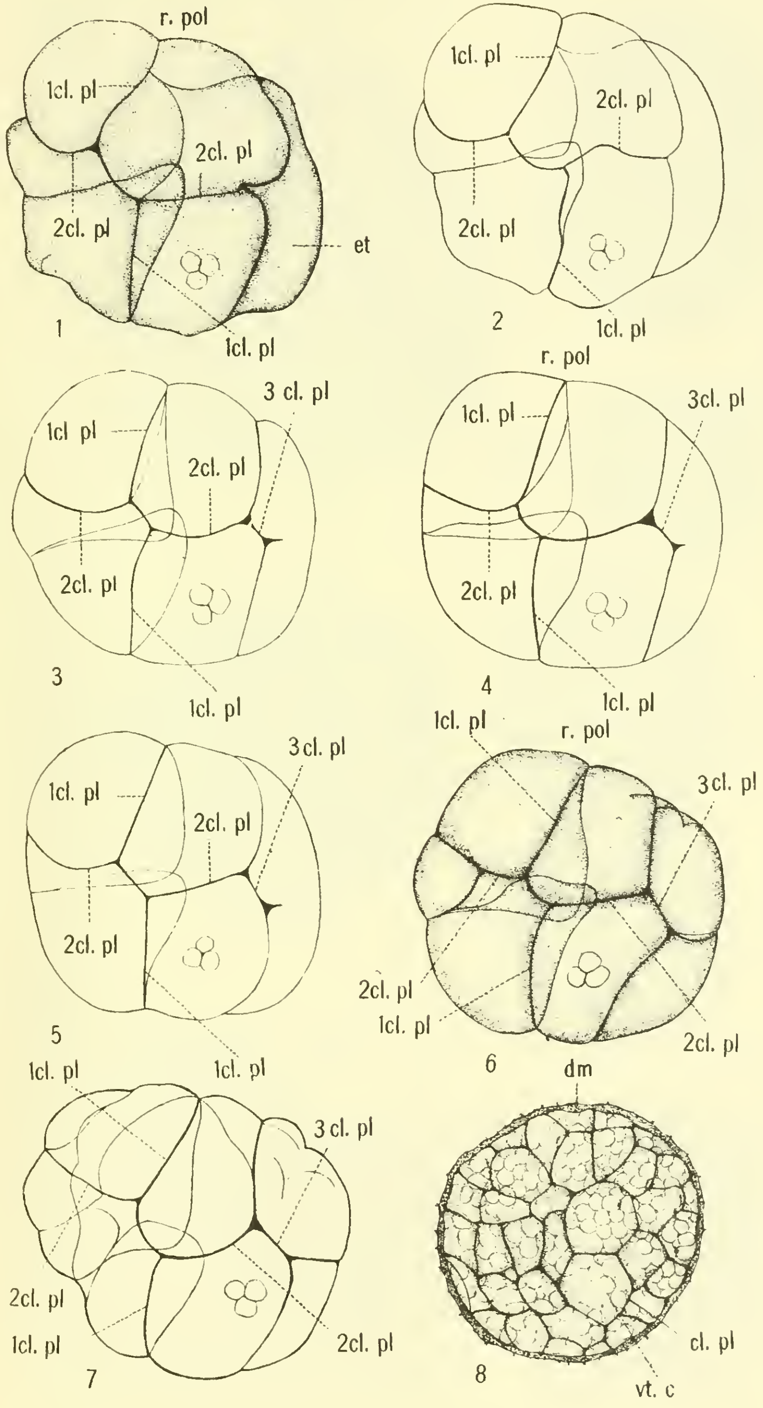
" 4. The same, lateral view.

" 5–7. Successive stages, in reduction in size of the primitive hydrophyllium, following its great development.

" 8. A larval *Agalma*, one week old, bearing a large flat hydrophyllium (remnant of the primitive covering-scale) through which runs a tube (*tb.*) which bifurcates and ends at the distal edge in clusters of nematocysts.



tb
n
nl
p g
goph
1
tb
uòr
vt
om
2
ubr
tb
r. tb
om
3
vt. c
tb
y
goph
om
r. pol
4
n
vt. c
m
5
r. pol
vt. c
6
7
vt. c
br
pr.
vt. c
8
br
vt. c
9
br
lcl. pl
vt. c
10
br
lcl. pl
11
br
lcl. pl
vt. c
12
r. pol
lcl. pl
se
vt. c
13
lcl. pl
vt. c
se
14
lcl. pl
se
15
lcl. pl
vt. c
se
16
lcl. pl
lcl. pl
se
lcl. pl
17
lcl. pl
18
vt. c
lcl. pl
lcl. pl
2cl. pl
2cl. pl
vt. c
19
lcl. pl
lcl. pl
2cl. pl
2cl. pl
20
lcl. pl
r. pol
lcl. pl
2cl. pl
2cl. pl
21
lcl. pl
lcl. pl
2cl. pl
2cl. pl
vt. c
22
lcl. pl
lcl. pl
2cl. pl
2cl. pl
vt. c
23
lcl. pl


r. pol
1cl. pl
2cl. pl
2cl. pl
et
1cl. pl
1
1cl. pl
2cl. pl
2cl. pl
1cl. pl
2
r. pol
1cl pl
3 cl. pl
2cl. pl
2cl. pl
1cl. pl
3
1cl. pl
3cl. pl
2cl. pl
1cl. pl
4
1cl. pl
r. pol
1cl. pl
3cl. pl
2cl. pl
2cl. pl
1cl. pl
5
3cl. pl
2cl. pl
1cl. pl
2cl. pl
6
1cl. pl
1cl. pl
3 cl. pl
2cl. pl
1cl. pl
2cl. pl
7
dm
cl. pl
vt. c
8

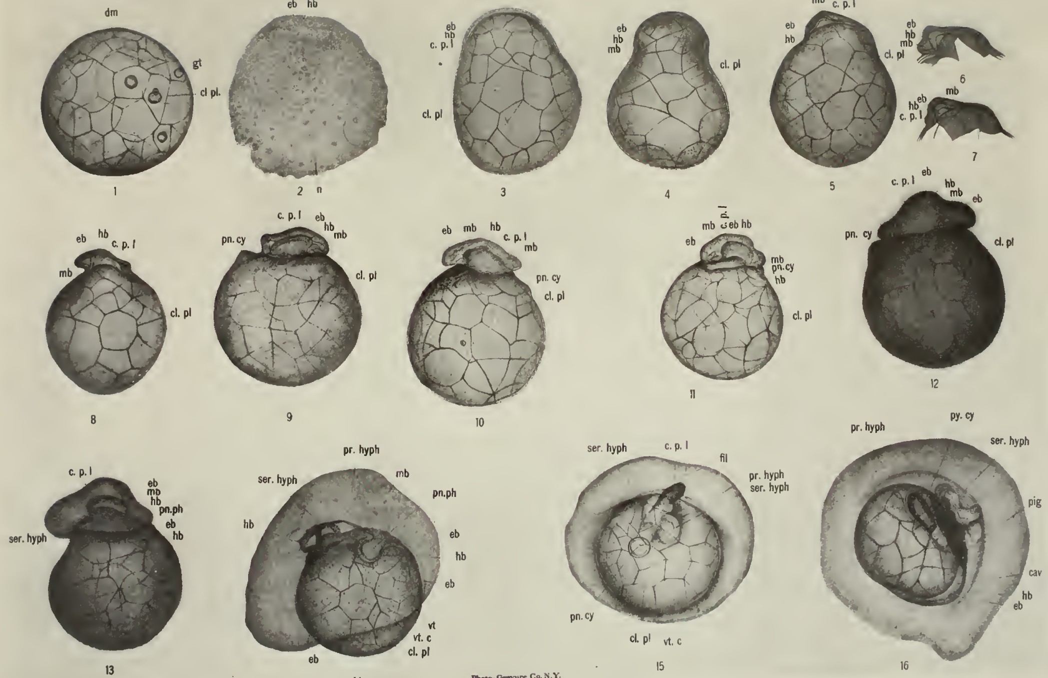


Photo-Gravure Co. N.Y.

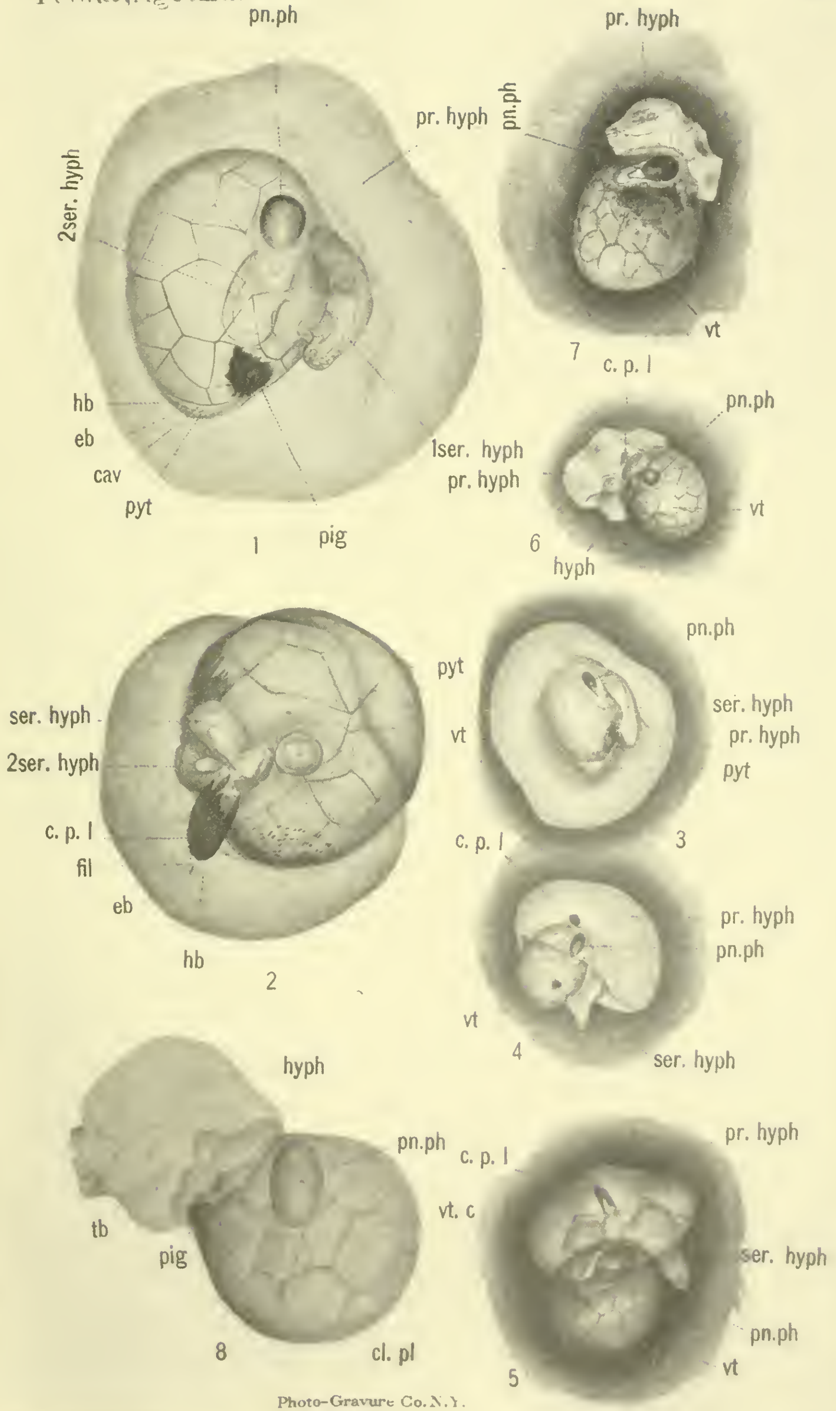


Photo-Gravure Co. N.Y.