No. 4. — *Studies from the Newport Marine Zoölogical Laboratory. Communicated by* ALEXANDER AGASSIZ.

XVII.

Preliminary Observations on the Development of Ophiopholis and Echinarachnius. BY J. WALTER FEWKES.

THE following paper considers the development of our common Ophiuran, *Ophiopholis aculeata*, Gray, and of the Clypeastroid, *Echinarachnius parma*, Gray. All the observations were made last summer, those on the former at Eastport, Maine, those on the latter at the Newport Laboratory, Newport, R. I.

OPHIOPHOLIS ACULEATA, GRAY.

Few observations have been published on the metamorphosis of our common Ophiopholis. The eggs of *O. aculeata* (*bellis*, Lym.), according to A. Agassiz,* are laid in bunches, and the young develop without passing through a free plutean stage. He gives two figures of the young Ophiopholis made by L. Agassiz in 1848.

In "Sea-Side Studies" the young Ophiopholis is said to be carried in a pouch, in which the first stages of development occur.† A figure, one of the two mentioned above, is doubtfully identified as a drawing of the young Ophiopholis in the second number of the Embryological Monographs.‡ Packard § states that in Ophiopholis the development is direct and without metamorphosis.

* Embryology of the Echinoderms. *Mem. Amer. Acad.*, Vol. IX., pp. 18 and 22. The pluteus referred to *Amphiura squamata* in the "Embryology of the Echinoderms," and doubtfully to Amphiura in "Embryological Monographs," may be a pluteus of Ophiopholis. *Amphiura squamata*, Sars, is viviparous, and has no free pluteus.

† Sea-Side Studies in Natural History. Marine Animals of Massachusetts Bay, p. 137.

‡ *Mem. Mus. Comp. Zoöl.*, Vol. IX. No. 2, Pl. III. Fig. 20.

§ Zoölogy for Students and General Readers, p. 110. As nothing is said of direct observation, it is probable that this statement is a compilation probably from those already quoted.

These are the most important references which have been found to the embryology of this, one of our most common Ophiurans. My observations differ radically from the statements quoted.

The eggs of Ophiopholis are cast free in the water, and the young pass through a metamorphosis, in which a larva commonly called the pluteus is formed. The mode of development of this pluteus is different from that of any Ophiuran which has yet been described. It is most closely allied to that of Ophiothrix, but in the mode of formation of a gastrula differs widely from the account of a species of Ophiothrix. "*O. versicolor*," traced by Apostolides.*

The formation of the gastrula in Ophiurans has been very unsatisfactorily studied. An invaginated gastrula has never been figured in this group. Balfour † in a short notice states that he has observed in Ophiothrix that the gastrula stomach is formed as in other Echinoderms by an invagination (of the blastoderm). The same mode had previously been suggested as probable by many embryologists, and had found its way into all the more important text books. It is not accepted by Apostolides, one of the latest students of the development of these animals.

Apostolides ‡ strongly combats the explanation of the method of formation of the gastrula by invagination, and brings forward new observations on Ophiothrix, the same genus studied by Balfour, to show that no invagination of the blastoderm occurs, and that the hypoblast of the stomach is formed from cells in the inside of the blastosphere. To these observations he brings as aids his studies of Amphiura to prove that in Ophiurans the normal method of invaginated gastrulæ does not exist.

The observations, therefore, which I have made, are thought to have a morphological importance as supporting the *a priori* views of most embryologists, and the direct observations of Balfour on another genus, of the method of formation of the stomach of the pluteus of Ophiurans by a primitive invagination of the blastoderm. I have never observed the gastrula of Ophiothrix, and can speak with confidence of Ophiopholis only, as far as this point is concerned. The differences between Apostolides'

* 1e Thèse. Anatomie et Développement des Ophiures. *Arch. d. Zool. Exp. et Gen.* X. Apostolides does not seem to have sufficiently studied the descriptions of the various species of Ophiothrix in the writings of Ljungman (Oph. Öf Kong. Akad. p. 625, 1871. Description of *O. Lusitanica*), and Lyman (*Bull. Mus. Comp. Zoöl.*, Vol. III. Part 10, pp. 240–249). The "*O. versicolor*," Apostolides, is probably, as has been suggested to me by Mr. Lyman, the same as *O. Lusitanica*, Ljn.

† A Treatise on Comparative Zoölogy.

‡ *Op. cit.*, pp. 192 and 207.

and Balfour's observations on Ophiothrix may be settled by those whose good fortune it may be to study the embryology of this genus, but since the archenteron is shown in the account of observations here published to be formed by an invagination in Ophiopholis, we may still adhere to our acceptance for some genera of brittle-stars of a general law of Echinoderm development, known to apply to the gastrulæ of some genera of the Holothurioidea, Echinoidea, and Asteroidea. While, however, my observations are believed to show that in at least this genus an invaginated gastrula occurs, they do not prove that the opening into the primitive infolding becomes the anus of the pluteus.

Our most accurate knowledge of the young stages of Ophiurans relates to a viviparous genus, Amphiura.

Although the development of Amphiura has been studied by several observers, we find in their accounts of the subject so many discrepancies, that a call is made for a new study of the first stages of this and related genera. Metschnikoff* supposes that in Amphiura the stomach, "Verdauungsapparat," is formed by invagination. According to Apostolides the endoderm is formed by delamination, and there is no such invagination, although he describes a primitive opening in the larva, which he considers the anus.† Why he should give this name to the opening in question does not appear, and if he has grounds for such an interpretation he does not make them evident in his account.

Another opening into the digestive tract, of the origin of which he is equally reticent, he calls the mouth. The endoderm or wall of the digestive cavity, according to this author, is formed in Amphiura by delamination, and not by invagination of the blastoderm.

As bearing upon the question of whether the primitive opening of the larva becomes a mouth or not in Ophiurans, an observation of Sir Wyville Thomson on *Ophiacantha vivipara*, Ljn., is important. He says: ‡ "Although I had not an opportunity of working the matter out

* Studien über die Entwickelung der Echinodermen und Nemertinen. *Mém. de l'Acad. Imp. des Sci. St. Pétersb.*, VII. Sér., XIV. 8, p. 14.

† Metschnikoff, in a later publication (*Zeit. f. Wiss. Zool.*, XXXVII., p. 307,) expresses an opinion against the idea of Apostolides that the endoderm is formed by delamination in Ophiothrix, and explains the error into which he supposes Apostolides has fallen, by the supposition that he (Apostolides) has confounded the mesoderm with the entoderm. In a note on the same page he takes occasion, however, to express his agreement with Apostolides that an intestine and anus is present in the embryo of *A. squamata.*

‡ Notice of Peculiarities in the Mode of Propagation of certain Echinoderms of the Southern Sea. *Journ. Linn. Soc.* XII., pp. 77 and 78. Here mentioned as

with the care and completeness I could have wished, I feel satisfied from the examination of several of the young, at a very early period, that in this case no provisional mouth, and no pseudembryonic appendages whatever are formed, and that the primary aperture of the gastrula remains as the common mouth and excretory opening of the mature form." In a larva of an unknown Ophiuran, Krohn * finds the first infolding, "Vertiefung," in the position later occupied by the mouth of the adult.

I am unable to quote any direct observations on the gastrula of Ophiurans to show that the primary opening or gastrula mouth becomes a plutean anus. An anus is wanting in the adult Ophiuran.

Although Apostolides criticises the explanation given by others of the method of formation of the openings into the internal cavity (stomach) of the gastrula by an invagination, he does not show how mouth or anus is in reality formed. As he does not show the old view to be erroneous, and suggests nothing better, we must at present adhere to the commonly accepted explanation. The interpretation of Metschnikoff, who regards the first formed opening as a mouth, seems more reasonable than to suppose with Apostolides that it is an anus. Whatever it may eventually become, Metschnikoff's suggestion, that it is formed by an invagination, conforms with what I have observed in the gastrula of Ophiopholis.

In a short notice of the development of Ophiophragma, Professor Nachtrieb † refers to a blastopore, and a stomach "enteron" in its gastrula. No infolding of the blastoderm to form this enteron is recorded, but the recognition of the primitive opening as a blastopore in another Ophiuran genus is worthy of notice.‡ I believe the gastrula stomach of Ophiophragma will be found to be formed by invagination as in Ophiopholis. Professor Nachtrieb also studied the development of Ophiothrix, but his mention is too short to give me any information as to how he regards the gastrula stomach as formed. From what he does give it is supposed that the stomach is developed in the same way as that of Ophiopholis.

Ophiocoma didelphys, Wyv. Th. See also general results of the voyage of the "Challenger," by the same author, p. 241 *et seq.*, Fig. 50.

* Ueber einen neuen Entwickelungmodus der Ophiuren. *Arch. f. Anat. Physiol. u. Wiss. Med.* 1857.

† Johns Hopkins University Circular, March, 1885.

‡ By a comparison of Apostolides' figures of Ophiothrix it will be seen that the pluteus has pushed out the lateral arms to double the diameter of the body before a mouth or any external opening into the cavity of the pluteus is formed.

Observations.

Plate I. Figs. 1-23.

The material upon which my observations were made was collected at Eastport, Maine, in the summer of 1885. Adult Ophiopholes were dredged off Friar's Head, Campobello. Great numbers were taken just below the line of low tide on Clarke's Ledge, near Eastport.

The following observations on the development of the egg were begun after its fertilization, and after it had been laid.

Ova were voluntarily cast by the female on the 17th of August. They were found in multitudes at the bottom of the glass dishes in which the adults were confined, forming a greenish or yellowish cloud discoloring the water. A white fluid of spermatozoa was also found in another dish containing males. As both elements are cast in the water it is probable that in this species fecundation occurs outside the body,* as is generally the case among Echinoderms. The ova of Ophiopholis, like the adults, appear to be very hardy, and very little care is necessary to keep them alive. The contact of sperm and ova was not observed. The white fluid containing spermatozoa was mingled with fluid containing ova, and it is thought that artificial fecundation was thus effected.† The ova began to develop soon after. Fecundated ova were also found in water in which many Ophiopholes were living.

Each egg, Pl. I. fig. 1, is enclosed in a transparent capsule .13 mm. in diameter. This capsule in the first stages observed was not thick as in the viviparous genus, Amphiura, but very thin. It is thought to be homologous with the outer layer, *m c.*, mentioned and figured in Metschnikoff's account ‡ of the development of *A. squamata.* Its thickness may have been greater in younger stages. The eggs are not laid in bunches, masses, nor were they observed to be cemented together. They were not observed to develop in pouches, although pouch-like parts of the genital glands, ovaries, are sometimes squeezed out through the genital slits as in the genus, Gorgonocephalus. When the ova were first examined segmentation had not begun, but no germinative nucleus was seen. Each egg in the youngest stage, Pl. I. fig. 1, has the yolk com-

* In Amphiura fecundation takes place in the body, *teste* Apostolides, Metschnikoff, and others.

† Metschnikoff (*Zeit. f. Wiss. Zoöl.*, XLII. p. 664), artificially fertilized *Ophiothrix fragilis.*

‡ *Op cit.*, p. 14. Plate III. Fig. 3.

posed of a slightly opaque greenish centre, *vt*, the diameter of which is two-thirds that of the egg-capsule, and a superficial transparent layer *t*, which may be a thickened envelope, part or the whole of which later becomes a vitelline membrane. Both these elements of the ova are affected by segmentation.

The transparent plasmic envelope of the vitellus of Ophiopholis is thought to be identical with a similar layer described by others in starfishes and sea-urchins. Selenka,* for example, has described a similar layer in *Toxopneustes variegatus*, and the question of the origin and fate of the vitelline membrane (?) has been discussed by Giard, † Fol, ‡ Perez § and Selenka. || The discussion of the intricate complications of the question which the origin and fate of the cortical layer of the Echinoderm egg necessitate, must be passed over at present, as most of the phenomena considered by the above-mentioned authors antedate a stage of the Echinoderm egg corresponding with the youngest Ophiopholis which I have studied. The observations here recorded are supposed to have a value in indicating the existence of the cortical transparent layer in the Ophiurans where, possibly with the exception of Amphiura, it has been overlooked by other embryologists.

The outer or superficial layer of the yolk is believed to be the same as the "Structurlosen geblichen Hülle," described by Metschnikoff (*op. cit.* p. 14), in Amphiura. The same layer is thought to be figured by him in Pl. III. figs. 3–6, *m v.* as the "Dotterhaut."

While the outer membrane of the Amphiura egg is conspicuous in Metschnikoff's figures of the youngest eggs, in older stages it suddenly disappears. The same thing occurs in the capsule of the Ophiopholis egg, which leads me to suspect that they are the same structures.

According to Apostolides ¶ a part of the protoplasm seems to condense

* Beobachtungen uber die Befruchtung und Theilung des Eies von *Toxopneustes variegatus. Sitzungsb. d. phys.-med. Gesells. z. Erlangen.* X. pp. 1–7. Zoologische Studien. I. Befruchtung des Eies von Toxopneustes variegatus. Leipzig, 1878.

† *Compt. Rend.* LXXXIV. 7 (Trans.) *Ann. Mag. Nat. Hist.* [4] XIX. 113, pp. 434–436.

‡ *Compt. Rend.* LXXXIV. No. 14; *id.* LXXXV. No. 4; *id.* No. 14.

§ *Op. cit.*

|| *Op. cit.* The external transparent region mentioned above may correspond with the "superficial hyaline membrane" described by Fol (*Ann. Mag. Nat. Hist.* [4] XX.) over the vitellus of the starfish ovum. See also Perez, Sur la Fécundation de l'Œuf chez l'Oursin. *Compt. Rend. Acad. Sci.*, LXXXIV. p. 620; LXXXV. p. 353. *Ann. Mag. Nat. Hist.* [4] XX. pp. 156–158.

¶ *Op. cit.*

at the centre of the yolk in the first condition of the egg. It there becomes more dense and divides. Of the two masses of unequal size, "Toujours," he says, "une des deux présente des proportions considérables par rapport à l'autre." In fig. 3 Pl. XI., to which he refers for this phenomenon, this difference in size of the two is not well shown, and the condensed central region is not separated from the superficial by as strong a line of demarkation as in Ophiopholis. It is supposed that the condensed central part which is referred to in his description is the same as the slightly opaque or greenish centre of the Ophiopholis egg, but such an interpretation is open to doubt. The transparent superficial layer is not homogeneous throughout. At one pole on the outer surface of the transparent layer of the yolk, still fastened to it or not separated from the yolk cells, a single globule was observed, Pl. I. fig. 2, *d*, possibly in the process of forming. This globule forms a slight elevation on the surface of the transparent layer, and a corresponding conical elevation was observed under it on the denser part of the vitellus.* Later in time a globule separated from the yolk was observed, and in later stages of development an additional globule is formed, Pl. II. fig. 3. The largest number of free polar globules observed was two. Polar globules are not figured or mentioned in Ophiothrix by Apostolides. He speaks of them, however, in Amphiura. The superficial layer of the yolk appears to surround both cells, and in the contiguous surfaces of the two cells this layer is undivided, corresponding in its position with the plane of the first cleavage, Pl. I. fig. 3. This coincidence causes the two cells of the 2-cell stage to appear separated by a transparent layer, which at the same time unites them,† Fig. 3, 1 *cl. pl.*

The formation of the 2-cell stage does not occur immediately after the sperm is added to the glass containing the ova. As in Echinarachnius a considerable time elapses after the mixture of the two elements before the formation of a 2-cell stage. The indications are that the first changes go on more rapidly in Ophiopholis than in Ophiothrix, as observed by

* This conical elevation may be connected with the "Dotterhügel" of Fol and others. It has, however, no existence on the outer surface of the plasmic or cortical layer. A more acute histological examination of the single globule on the surface of the latter above the conical elevation is necessary before it can be stated whether it is a polar globule or a spermatozoön. I regard it as the first polar globule.

† A characteristic connection of the two blastomeres is mentioned in the egg of Ophiophragma by Professor Nachtrieb. Whether it has a likeness near or remote to this condition in Ophiopholis, it is impossible for me to say. From his short description I am unable to compare the two genera in this regard.

Apostolides. No change was detected in the ovum of the latter seven hours after fecundation. On the seventh hour after the contact of ova and sperm, according to Apostolides, the first indications of the segmentation of the egg appear in Ophiothrix. Seven hours after the capture of Ophiopholis some of the ova were found in the 4-cell stage, and a little over three hours after the egg was dropped by the female they were found in the 2-cell stage. Is this discrepancy the result of a difference of temperature in the water? *

The second segmentation stage, the 4-cell stage, Pl. I. figs. 6, 7, is brought about by a formation of a second cleavage plane, 2 *cl pl*, at right angles to the first. As in the first the two cells of the 2-cell stage are separated by a transparent layer, in the same manner each of the two cells of the 4-cell stage are divided by a similar plane. A nucleus is observed in each of the spheres of the 4-cell stage. The division of the 2-cell stage into four cells is regular,† and all the cells are of the same size up to the 4-cell stage.

Cleavage.

The first external change in form of the spherical egg in its segmentation is the formation of the first cleavage-plane. A constriction or annular groove, destined to divide the egg into hemispheres, forms about the egg in the same way as in other Echinoderms. It was not observed whether a collar-like ‡ extension of the superficial portion of the yolk sinks into the denser central region, or whether the two cells form in some other way, but in a well-formed 2-cell stage the two cells are separated by a transparent wall. The transparent wall is identical with that which covers the denser part of the ovum in its undivided condition.

The 4-cell stage is formed from the 2-cell stage by a cleavage-plane, 2 *cl pl*, at right angles to the first. As in the 2-cell stage the two cells or blastomeres are separated by a transparent layer, so in the 4-cell stage

* Metschnikoff (*Zeit. f. Wiss. Zoöl.* XLII., p. 665) has recorded a great difference in the time occupied by the early development of the eggs of the same Echinoderm from two localities. These differences are probably due to temperature. The influence of temperature on the rate of development of the ova of Echinoderms is a subject which would repay an extended investigation.

† If any irregularity in size exists the difference is very small.

‡ It may be supposed that the superficial layer following a constriction of the denser part of the ovum, bisects the latter by a centripetal growth. This would correspond with the mode of formation of the 2-cell stage in other Echinoderms.

the cells are likewise separated by a transparent wall of the same general appearance. Each of the cells of the 4-cell stage has a nucleus.

The 8-cell stage, Figs. 8, 9, follows close upon the 4-cell, the additional cells forming by a subdivision of those already existing. This division is generally regular, all the cells being of like size. In some ova smaller cells were observed with larger in the 8-cell stage. The cells have a centrifugal tendency, and a central unoccupied cavity, *cav*, can be seen enclosed by them. This cavity, which is the cavity of the blastosphere, grows in size as the larva matures. There is no solid morula stage, but a segmentation-cavity can be recognized in eggs as young as the 4-cell stage. The transparent layer *t*, which envelops the 8-cell stage, and which is thought to be the same as the thin superficial layer of earliest stages, is somewhat diminished in thickness. It is seen to be spread over the surface of the cells, and to separate the spheres of segmentation from each other.* A day after the eggs were laid they had developed into free swimming spheres, Pl. I. fig. 10, covered externally with cilia. These larvæ were found in great numbers free in the jars. The egg has developed into a larval stage, which has burst the imprisonment of the capsule, and the blastomeres have arranged themselves on the periphery of a hollow sphere. The superficial layer of cells, still more transparent than the profound, bears long vibratile cilia; the larva moves readily from place to place.

At one pole of this larva the blastoderm, or that shell of cells which encloses the cavity, is slightly thickened and more deeply colored than the remainder. This pole is the pole where the invagination to form the archenteron takes place, and it may be said at once that this pole is the seat of the next important change in the growth of the larva. At this point, Pl. I. fig. 11, *ach.*, the blastoderm begins to fold inward, forming an invagination, which later becomes the stomach. The position where this infolding begins is the pole at which the mouth of the gastrula, *or*, is later situated. At this time in the career of the young Ophiopholis it begins to depart widely in form from that of the genus Ophiothrix, as figured and described by Apostolides. The segmentation of the egg is very similar in the two genera, but the form of the blastopheres is somewhat different. The blastodermic cells are very much

* This condition of the plasmic cortical layer in stages of cleavage as old as the 8-cell condition, is thought to indicate that the layer may be something more than a vitelline membrane, although the vitelline membrane may be formed from some portion of it. It is not possible for me to arrive at any good interpretation of the homology of this structure.

more elongated and conical in Ophiothrix than in Ophiopholis, and the thin superficial layer of cells bearing the cilia is not represented in Apostolides' figures. The cavity of both is hollow. In Apostolides' * figure of Ophiothrix we have in the middle, cell-like structures lettered, *es.* He does not explain the lettering, but from the fact that he speaks of the cavity as "creux," it is supposed that this region is a cavity, the segmentation cavity. In a copy of this figure in Embryological Monographs † A. Agassiz letters the cells of the blastoderm ; *e*, ectoderm, and *y* "yolk cells." The structures *y* are the same as *es.*

In a comparison of our figures of a blastosphere, Pl. I. figs. 10, 11, with that of Apostolides, we see in both a slight infolding of the blastoderm, which is here regarded as the beginning of the invagination in both cases. Apostolides does not so consider it in Ophiothrix, but he ascribes to Balfour the mistake of considering it an infolding. He says: "C'est peut-être ce point que M. Balfour, qui n'a pas poussé très loin ses observations, a pris pour un commencement *d'invagination.* Il n'en est pourtant rien, la suite prouvera que ce point n'est que le premier indice de la formation des bras du pluteus." It is a significant fact that just between this stage (his fig. 9) and the stage which he figures in fig. 10, when calcareous rods are developed, is the time when the process of invagination occurs. I find no stages of Ophiopholis which resemble in shape his figures 10 and 11 of Ophiothrix.

Apostolides says: "Peut-être M. Balfour a-t-il obtenu des fécondations de *l'Ophiotrix rosula*, qui est plus abondante en Angleterre, et chez laquelle les choses se passent peut-être autrement que dans l'espèce que nous avons soumise à l'observation." It would be an interesting fact if one species of Ophiothrix forms a gastrula stomach by infolding, and another in the way described by Apostolides.‡ Closely related starfishes, sometimes regarded as simply different species, however, have a wide difference in their development. *A. vulgaris* has a brachiolaria, while Leptasterias has young without nomadic stages. The gastrula of the latter may or may not develop as that of the former. There is nothing to show that it is exceptional.

The "plan général" of the development of the gastrula of Echinoderms is more widely spread among Echinoderms than the following quotation

* *Op. cit.*, Pl. XI. fig. 9.

† *Mem. Mus. Comp. Zoöl.*, Vol. IX. No. 2.

‡ We are here brought face to face with one serious defect in Apostolides' and Balfour's observations, namely, the difficulty of knowing exactly the species which both studied.

from Apostolides' paper would seem to indicate : " Le jugement de M. Balfour repose sur de simples probabilités de ressemblance avec le type Holothurie, dont l'embryologie lui sert comme plan général de tous les Echinodermes." Since we know that the formation of a gastrula has been observed also in starfishes and sea-urchins, it would have at least been more just to Balfour to have inserted these types after that of the Holothurians in the above quotation.

It was noticed at the close of the first day that the thickened blastoderm begins to fold inward at one pole, and at the same time that the blastoderm at that point becomes more densely pigmented. The larva, Pl. I. fig. 13, is now pear-shaped, slightly flattened on one side and truncated at the pigmented pole. The flattening on one side is the first indications of the ventral surface, and one of the first expressions of a bilateral symmetry which later becomes so well marked by the growth of mesoblastic cells. The internal surface of the cells at the truncated pole bulge somewhat into the cavity of the blastosphere, and from it mesoblastic or amœboid, spherical, and star-shaped cells, *a cl,* begin to bud. These cells form in two lateral * clusters, Pl. I. fig. 14, and indicate at once the position of the infolded archenteron. They are the beginning of a middle layer, and from them many important structures form. The least diameter of the larva is .11 mm. ; the greatest .13 mm.

The same irregular triangular form, and the clustering of pigment about the blastopore seems to be found in the gastrula of Ophiophragma. It is the presence of this pigment on each side of the gastrula mouth which has been of assistance in the identification of the lateral arms, *l,* in later stages as compared with the blastopore. A clustering of pigmented cells at the lower extremity of the stomach has rendered it extremely difficult for me to study the changes which go on in the formation of the water tubes and other structures in this region of the embryo. The walls of the stomach are yellow and green. Metschnikoff † found it very difficult to observe the " Mesoderm formation " in *Ophiothrix fragilis,* which he was able to artificially fertilize.

It is supposed that our embryo can be compared with that of Am-

* There is already a considerable literature on the question of whether in Echinoderms the " Mesodermkeim " or " Mesoderm cells " arise in a bilaterally symmetrical manner as regards a " spaltartige Rinne " of the gastrula, by which the symmetry is early indicated. Selenka and others hold that they do ; Metschnikoff, that they do not. My observations show such a symmetry in the mesoblastic cells of Ophiopholis.

† *Zeit. f. Wiss. Zoöl.*, XLII. p. 664.

phiura by Metschnikoff (*op. cit.* Pl. III. fig. 6),* and that the two structures *v.*, supposed by him to be water tubes, correspond in position to the clusters of cells on each side of the invagination. These clusters in Ophiopholis were quite dense, and the vesicles, if they existed here, would be difficult to see. Metschnikoff says that in Amphiura these bodies are also difficult to see through the "Cutiszellen" (mesoderm cells), and that later in normal development one is lost. He was able to observe that one of these bodies in Amphiura develops into the water tubes of the adult. It is not wholly certain that similar bodies do not exist in Ophiopholis, Pl. I. fig. 14, *a cl.*, where clusters of amœboid cells make observation on live material somewhat difficult at these points.

The bilateral arrangement of the budding cells in the cavity of the blastosphere and the shape of the larva give to it a marked bilateral symmetry even at this early stage. The pole of invagination may be called an anterior pole, while the cells on each side indicate the sides of the larva. One hemisphere of the gastrula is flattened; the opposite is more rounded. The former may be called the ventral, the latter the dorsal surface.

At seven o'clock on the day following the spawning the invagination, which forms the archenteron, has extended about half way down the cavity of the blastosphere, Pl. I. fig. 15. Almost the whole of the second twenty-four hours is occupied by the changes which accompany the infolding of the archenteron.†

The pole of the infolding slowly sinks into the cavity, carrying with it at this point the shell of cells, or that part of the blastoderm which is to form the wall of a digestive canal. The larva has become very much flattened on the ventral side, so that when seen from the pole of invagination the lateral diameter is twice that at right angles to it in the same plane. As we have arbitrarily called the longest diameter, when seen from the pole of the blastopore, the lateral, a name which seems appropriate, not only on account of the bilateral symmetry which the larva at this early age has, but also from the fact that from its extremities form the two calcareous rods and fleshy arms, known as the lateral arms, we may speak of the other diameter as the dorso-ventral. The dorso-ventral diameter connects the dorsal and ventral side of the larva, which are

* Fig. 6 is a little older. The mode of origin of these vesicles was not observed by Metschnikoff. Their position relatively to the mouth of the larva is somewhat exceptional.

† The time occupied to form the gastrula of Ophiophragma is about the same as in Ophiopholis. Cf. Nachtrieb, *op. cit.*

readily distinguished from each other. The ventral side is quite flat, the dorsal more convex.

Looking through the larva with its infolded outer layer of cells or hypoblast, from the ventral side, we notice that the infolding has proceeded about two thirds the axial length of the larva, and formed a funnel-like tube. This tube is the hypoblast, the primitive stomach, and at the pole of infolding is situated a mouth, *or*. The whole larva, Pl. I. fig. 16, is now in the gastrula stage.

At the pole of invagination in the region of the cœloma, between the infolded walls and the external crust of cells, epiblast and hypoblast, two masses of cells, *a cl*, are situated, one on each side, which are the mesoblastic cells already spoken of. These cells are spherical, stellate, branched, or elongate. The walls of the anterior pole of the gastrula are more densely pigmented than the remaining parts of the larva. The pigmentation is most dense on each side of the mouth. When the same gastrula is seen from one side, Pl. I. fig. 15, it is noticed that the infolded archenteron does not hang exactly in the longer axis of the larva, but that the closed end approaches the ventral side. Its extremity has a tendency from the very first to draw near the ventral wall. It approaches so near that it may be supposed to be met by a second infolding, through which an opening may be formed. I have not observed this second invagination, or this opening to be formed; although the general law of Echinoderm development would call for such an occurrence. I did not observe a second opening to be formed in the larvæ of Ophiopholis.*

On the second day, Pl. I. fig. 16, after the fecundation of the Ophiopholis, it was observed that the invaginated end of the stomach becomes somewhat inflated, Pl. I. fig. 16, *g a*, by an enlargement of the cavity. Although this inflation has not been traced farther, and water tubes were not seen to arise from it, as we know takes place in the course of Echinoderm development, up to this point the modifications in this region of the archenteron closely resemble similar formations observed by others in the echinoid pluteus. The origin of the water tubes from the primary invagination is yet to be observed in Ophiurans, notwithstanding from *a priori* grounds we suppose such to be the case. All embryologists, however, do not accept such an explanation. According to Apostolides,† who has written the last important work on the devel-

* The clustering of cells in the cavity of the larva made accurate observations in regard to the changes which occur at this point very difficult. Nachtrieb seems to have had a similar difficulty in the genus, Ophiophragma.

† *Op. cit.*, p. 199.

opment of Ophiothrix, " Ces deux masses cellulaires ne sont pas, comme l'a supposé M. Balfour, dues à des diverticulum de l'archentéron, semblables aux cavités vaso-péritonéales des Holothurius, mais elles sont des produits d'une formation directe, comme cela a lieu pour l'estomac."

Pl. I. fig. 18, shows a larva slightly older than the gastrula last described. If we look at this larva from the flat or ventral side, we notice on each side of a single opening * small pigmented protuberances. These prominences in the future growth of the larva become more and more extended, and even in their earliest form give evidence that they develop into the lateral arms of the pluteus. The larva is now three days old, and has begun to assume a form like the youngest Ophiuran pluteus described by others. The longitudinal axis is .18 mm.; the distance from the tip of one lateral prominence to the opposite is .16 mm. The anal lobe has grown more pointed than in the larva just described, the body of which is about spherical. The oral lobe is smaller than the anal, although similar to it in form. It is as yet undivided. A mouth leading into a cavity opens on the upper pole on the ventral side of the oral lobe, and a broad band of cilia extending along the lateral arms surround the mouth, the oral lobe, and the ventral region of the body. The opening thus surrounded by a ciliated band is easily seen. Its lips are richly ciliated.

The limestone rods have already been formed in the body, Pl. I. fig. 19. There are two centres of formation of these bodies; but these centres of calcification are at first not joined. The limestone rods, *sp*, originate as spicules with three prongs. One prong extends into the lateral rod, another in the direction of the anal lobe, and a third into the oral lobe. Later, a fourth process is formed from the common union of the three already mentioned, which extends to the middle line of the dorsal side.

The amœboid or mesoblastic cells are formed throughout the region of the larva, between the epiblast and hypoblast. They are spherical, sometimes branched, forming suspensoria connecting the wall of the

* Still further observation is necessary to show whether the mouth of the gastrula of Ophiurans becomes the mouth of the pluteus, or whether, as in Holothurians (Cucumaria,) Selenka, the Starfishes (*Asterias vulgaris*,) A. Agassiz, (Asterina,) Ludwig, and in some Echinoids (Strongylocentrotus) according to Krohn and A. Agassiz, the gastrula mouth becomes a vent. I have not observed an anus in the pluteus of Ophiopholis. The single opening is, therefore, supposed to be the gastrula mouth. Whether, as in some other Echinoderms, a new opening is formed, and the gastrula mouth becomes an anus or not in later stages, was not observed.

stomach and the outer wall of the pluteus. The oral ciliated band is not as transparent as the anal lobe of the pluteus. Stomach walls and oral ciliated belt have a yellowish-green color.

In the oldest plutei which we shall mention, figs. 21–23, the larva has assumed a triangular profile when seen from the ventral side and the two lateral arms, *ll*, have pushed out on each side. The anal lobe is slightly pointed; the oral, *ol*, well developed, undivided, and rounded. The whole external surface is ciliated. The oral band of cilia is indicated by a closer approximation of the cells of the middle layer. The distal ends of the posterior rods are pigmented. The body of the pluteus is surrounded by a superficial transparent layer of cells. The mesoblastic walls of the arms are crowded with granules.*

The rods which form the supports of the lateral arms have lengthened to keep pace with the growth of the arms. These rods are not latticed. The rods of the anal lobe are bow-shaped, and at the apex of the anal lobe they bifurcate, the larger division extending to the apex of the lobe.

The anterior rods are smooth, and extend half way down the lobe, or in some cases to the ciliated oral band. The mouth, œsophagus, and stomach are well differentiated from each other. The hypoblastic walls of the latter, *ga*, are green and yellow.

The oldest pluteus is a little more than three days old. On the fourth day I left Eastport, and all my plutei died from want of care. It is probable, however, that they are hardy, and can be easily raised, and the young Ophiopholis traced from them to its adult.

The following summary of the preceding observations may be made: —

1. *Ophiopholis aculeata* has a development with metamorphosis, passing through a larval stage called the pluteus.

2. The ova are laid in the surrounding water. The yolk has a central and a peripheral region, which is distinguished in the 8-cell and previous stages of segmentation. The cleavage is like that of other Echinoderms.

3. A gastrula is formed by an invagination of the blastoderm, and consequently the stomach of the pluteus is an infolded wall of the blastoderm, and not formed by delamination from the cells in the cavity.

4. The mesoderm cells originate in two lateral clusters.

* Metschnikoff accurately represents, *op. cit.*, Pl. V. fig. 2, an Ophiuran pluteus which has the cells "cutis" crowded in the lateral arms in the same way as in Ophiopholis.

ECHINARACHNIUS PARMA Gray.

General Notice.

Our knowledge of the development of Echinarachnius is small. Johannes Müller * long ago described a pluteus which he referred to Echinocyamus. From its likeness to the pluteus described by Müller, which is a very characteristic one, A. Agassiz suggested † that the common Newport pluteus is the young of Echinarachnius. The pluteus of Arbacia is known, that of Strongylocentrotus ‡ is characteristic, and Mr. Agassiz was led to refer a pluteus, which is neither of these, and which is found in great numbers in Narragansett Bay, to the young of Echinarachnius. No one has up to the present brought forward any observations bearing on this suggestion. I have raised the egg of Echinarachnius into a pluteus, which is closely allied to his, and have raised plutei which are identical into a young stage of Echinarachnius. The plutei described by A. Agassiz are not mature. A. Agassiz has also figured § the young stages of Echinarachnius after the absorption of the pluteus. In a paper on the embryology of the genus Arbacia, I have described || the peculiar pigmentation on the viscous covering of the egg of the Echinarachnius while in the ovary.

These contributions constitute the greater part of our knowledge of the development of Echinarachnius.

The development of the pluteus of the "sand-cake" or "sand-dollar," ¶ *E. parma*, resembles in many respects that of Arbacia.** The

* Ueber die Gattungen der Seeigellarven. Siebente Abhandlung über die Metamorphose der Echinodermen. *Abh. k. preus. Akad. d. Wiss. Berlin*, 1855.

† Revision of the Echini. *Mem. Mus. Comp. Zoöl.*, III. p. 730.

‡ The pluteus of Strongylocentrotus must be rare at Newport. I have not recognized it in my fishing there in several summers.

§ *Op. cit.*, Pl. XII. Embryological Monographs, No. 2. *Mem. Mus. Comp. Zoöl.*, Vol. IX. No. 2.

|| On the Development of the Pluteus of Arbacia. *Mem. Peabody, Acad. Sci.*, I. 6, 1881.

¶ Many genera of Clypeastroids, besides Echinarachnius, are also called sand-dollars from the shape of the adult. In the South Mellita bears that name. Echinarachnius is sometimes called the sand-cake, in New England coast towns.

** For a history of the development of Arbacia see A. Agassiz, Revision of the Echini, pp. 729, 733–735. E. Selenka, Keimblätter u. Organenlage der Echiniden. *Zeit. f. Wissensch. Zool.* XXXIII. Pl. VII., Figs. 34–37. J. Walter Fewkes, On the Development of the Pluteus of Arbacia. *Mem. Peabody Acad. Sci.* I.

adult pluteus is very different from that of either Arbacia or Strongylocentrotus, and most closely resembles the pluteus described by Müller as that of the genus Echinocyamus.

Artificial Fertilization.

The sexes of Echinarachnius are distinct, the male and female organs being found in different individuals. Although the colors of the adult of different specimens vary, and in some instances it was possible for me to tell the sex without dissection, this could not be done in all cases. The colors of the ripe glands, ovaries and spermaries, can easily be distinguished. The former are commonly dark-red or purple; the latter orange or yellow.

Derbes * was not able to distinguish the male from the female of *E. esculentus* by external characters. The sperm according to him has a milky white color, and the ova are orange or brown.

The males and females of *S. dröbachiensis*, according to A. Agassiz,† are distinguished by a "more vivid coloring of the spines of the latter, which are of a violet tinge, while those of the males are more yellowish-green." The ova and sperm of Strongylocentrotus, he says, resemble in color that of *E. esculentus* as described by Derbes.

My method of procedure in artificial fecundation is as follows: The apical portion of the aboral region is incised through the test by a ring-shaped cut, with a radius equal to that of the petaloid openings. This dissection is carried on with the sea-urchin under water. The incised part is turned over, and transferred to a glass dish with water, and the remainder of the animal is placed in pure sea-water.

Upon the inner surface of the incised part fragments of the ovaries will be found, if the specimen is a female, and spermaries if a male. In the former case, if the eggs are mature, small transparent globules will be found to float away from the glands, especially if the organ is slightly washed with a pipette. If a white fluid exudes from the glands the specimen, if alive, is probably a male, and the white fluid is colored by

No. 6. H. Garman and B. P. Colton, some Notes on the Development of *Arbacia punctulata* Lam. *Studies Biol. Lab. Johns Hopkins Univ.* II., pp. 247–255, and W. K. Brooks, Handbook of Invertebrate Zoölogy for Laboratories and Sea-Side Work, figs. 78–83.

* Observations sur le Mécanisme et les Phénomènes qui accompagnent la Formation de l'Embryon chez l'Oursin comestible. *Ann. Sci. Nat.* [3] VIII. 1847.

† Revision of the Echini, p. 708.

sperm. Many eggs can be washed out of the undissected sea-urchin with a pipette introduced through the aboral region. The floating eggs and the milky sperm are mixed by simply pouring the water from one jar to the other. A better method of artificial fecundation is to collect a watch glass crystal full of eggs, leaving enough water for them to float, and then to drop a few drops of water charged with sperm among them. The contents are then gently stirred, and after a short time evidences of the success of the process may be looked for. I have found that chopping up the two glands together, although in some cases to be recommended, in most instances, and especially in the case of Echinarachnius, the egg of which is delicate, leaves so much decaying matter that the ova are killed. It is well not to put too much water with the ova, as repeated dilution renders the collecting of the ova for study difficult. I took no precautions about the temperature of the water, and did not find it necessary to change the water until after segmentation was finished.* Artificial fecundation was accomplished from the middle of July to the end of August.

Each ovum, Pl. II. fig. 1, is visible to the naked eye. It is surrounded by a viscous ? layer in which are beautiful, spherical or sometimes angular, red pigment spots, *pig*, which are supposed to correspond to the "clouded areas," described by A. Agassiz † in the star-fish egg. The viscous layer of the egg of *E. esculentus* is described by Derbes. A. Agassiz describes a "thick homogeneous structureless shell" in Strongylocentrotus. The pigment spots are conspicuous on the outer surface of the viscid capsule of the egg of Echinarachnius. After fertilization the ova sometimes sink and sometimes remain floating. Their specific gravity is about that of the water.

The diameter of the yolk, *vt*, is .13 mm. The diameter of the viscid covering is from .22 to .25 mm. The yolk is yellow ; the envelop transparent. The yolk was not observed in the free egg to fill its capsule in any stage of segmentation.

A nucleus and nucleolus were observed in ovarian eggs. These structures were difficult to see in free eggs.

The spermatozoa immediately after the mingling of the two sexual

* Selenka and others have already pointed out refined ways of fertilizing sea-urchin eggs. See Selenka, "Keimblätter und Organenlage der Echinodermen." *Zeit. f. Wiss. Zool.*, XXXIII. p. 40.

† Similar pigment spots are found according to Nachtrieb in the egg of Mellita. These spots on the ovum of Echinarachnius were first described in my paper on the development of Arbacia. *Mem. Peabody Acad.*, I. 6.

elements were observed crowded upon the outer surface of the viscid layer, with heads partially buried in it. None were observed to penetrate to the yolk. The egg was seen to be slightly jerked about, possibly by the combined movement of the many spermatozoa on its surface. In no case was the movement very great. No polar globules were observed.*

Cleavage.

The segmentation of the egg of Echinarachnius is regular, and the first formed segment spheres are of the same size. After the formation of the 8-cell stage from the 4-cell an inequality in size of the blastomeres is noticed. As in Strongylocentrotus one of the first changes after the disappearance of the nucleus is the drawing away of the yolk from the shell. From an hour to two hours after the ova and sperm have been artificially brought together, the first cleavage furrow, *p*, is noticed encircling the egg.

In some eggs this furrow, Pl. III. fig. 1, is limited to one pole, and the indentation gradually deepens until the egg is divided into two hemispheres connected at the pole opposite that at which the furrow first appears. Folds which recall similar plications observed by Metschnikoff in the Epibulia egg, and by myself in the egg of Agalma, appear on each side of this primitive furrow, Pl. III. fig. 2. These wrinkles are supposed to be the "Faltenkranzen." This method of segmentation reminds me of what we have in the egg of the Siphonophore. It was not traced beyond the 2-cell stage.

In most cases the primitive furrow is not limited to one pole, but girts the ovum. Four cells were, however, observed in a 4-cell stage, in each of which the furrow, which is to form a new cleavage plane, is limited to one pole of the cell. Pl. III. figs. 6, 7.

In those ova in which the primitive furrow girts the egg, the constriction deepens uniformly on all sides, until the ovum is divided into two equal spheres, Pl. II. fig. 3, united by flat faces with each other. In each of the two cells a nucleus can be seen. The blastomeres of the 2-cell stage are never seen widely separated from each other.

* According to Nachtrieb no polar globules were observed by him in the closely allied genus Mellita. I suspect, as is well known in some other Echinoids, that the polar globules are formed while the egg is in the ovary.

† More than one method of cleavage has been observed in the Oyster by Brooks, and in Renilla by Wilson. It is not improbable that the segmentation of Echinarachnius mentioned above is a second kind of cleavage.

The cleavage plane, 1 *cl pl*, which divides the ovum into two segments, may be called a meridional plane. It is the first cleavage plane. In the formation of the 4-cell stage the two segments already formed are divided by a plane at right angles to this, and the mode of division in the two is identical. The division of the two cells which form the 2-cell stage begins by a slight constriction, girting the spheres which later bisects them, forming four smaller nucleated spheres or blastomeres, all of the same size. There is no 3-cell stage in this kind of cleavage. The second plane of cleavage divides both cells of the 2-cell stage.

The formation of the second cleavage plane will thus be seen to differ from that of Asterina, as described and figured by Ludwig.* In Asterina the two cells of the 2-cell stage are of unequal size. The smaller of these divides first, so that we have a 3-cell stage, fig. 2 (*op. cit.*). In Echinarachnius both the cells were observed to divide at the same time and form the 4-cell stage. The cleavage plane which forms the 4-cell stage (2nd cleavage plane) in Echinarachnius is at right angles to the first, and identical in its position in each cell of the 2-cell stage. Ludwig, p. 6, *op. cit.*, says of Asterina: "Die Theilungsebene der beiden Zellen II. (larger cell of 2-cell stage) ist aber nicht etwa die auf die Zelle II. übergreifende Theilungsebene der Zellen I. (smaller cell of the 2-cell stage), sondern bildet mit letzterer, so wie auch mit der Theilungsebene der beiden ersten Furchungskugeln einen rechten Winkel." Three cells were observed abnormally formed in the ovum of of Echinarachnius, and their mode of formation is traced below.

In Strongylocentrotus, according to A. Agassiz,† after the yolk separates from the inner wall of the outer envelope, it is slightly depressed on one side, and a similar change soon after occurs on the opposite pole. After these depressions in the poles of the yolk of Strongylocentrotus occur, a slit is formed, according to A. Agassiz, which divides the egg into two large elliptical masses.

In the egg of Echinarachnius in normal cases a constriction was observed, Pl. II. fig. 2, girting the yolk, similar to fig. 23, p. 709, of the work last mentioned.† This constriction deepens uniformly on all sides until the 2-cell stage is formed. In several eggs of Echinarachnius, Pl. III. figs. 1, 2, 3, the 2-cell stage is formed in another way.

* Entwickelungsgeschichte der Asterina gibbosa, Forbes. *Zeit. f. Wiss. Zool.*, XXXVII. pp. 6, 7.

† Revision of the Echini, p. 710.

A furrow appears at one pole. This furrow penetrates into the yolk, forming in profile a slit-like structure, which in this way divides the yolk into the 2-cell stage. In Strongylocentrotus, figured by A. Agassiz, we notice that a flattening of each cell of the 4-cell stage occurs preparatory to the passage into the 8-cell stage. This flattening occurs on one side at first (p. 710, fig. 27). Several eggs of Echinarachnius, Pl. III. fig. 6, were taken in a similar condition. In many others, however, each of the four cells of the 4-cell stage is divided from the very first by a constriction reaching wholly around the cell, Pl. II. figs. 4, 8.

In several ova the following modification of development was observed after the 4-cell stage. An egg was found in the 4-cell stage apparently normally formed, Pl. II. fig. 9. Immediately after two of the spheres begin to fuse, and the wall of the cleavage plane separating them is broken down. In this way we pass by retrogression from an egg with four, Pl. III. fig. 9, into one with three segment spheres, Pl. III. fig. 12. Whether the many eggs in a 3-cell stage which were observed were all formed in this manner or not, cannot be stated. It was not observed how the 4-cell stage in this abnormal mode of development is formed from the 2-cell stage. Segmented ova with three segmentation spheres are quite common in some trials for artificial fecundation.

An egg fertilized at noon was found in the 2-cell stage at 1.30 P. M., and passed into the 4-cell stage at 2 P. M. At 3 P. M. it was in the 8-cell stage. We can, therefore, roughly say that the formation of a fresh cleavage plane occupies approximately an hour's time. By a comparison with the rate of growth of the starfish it will be seen that the rate of development of Echinarachnius is more rapid. The water in which my eggs were kept was evidently warmer than that in which Strongylocentrotus was reared.

The mode of formation of the 8-cell stage from the 4-cell does not differ from that of the 4-cell from the 2-cell. The segments of the 4-cell stage are, however, not always bisected, and here appears the first indication of an unequal segmentation. The spheres of the egg even in the 8-cell stage have a peripheral tendency. In the 8-cell stage it will be noticed, Pl. II. fig. 11, that the eight spheres cannot be so brought together as to touch each other on adjacent sides. A recess, *cav*, is thus early left, which later forms in the interior of the ovum a "segmentation cavity." This cavity increases in size as the size of the segmentation spheres diminishes in the progress of segmentation. An egg in the 32-cell stage was found four hours after impregnation, Pl. II. fig. 12.

The whole process of cleavage occupies about ten hours.* A rotation of the spheres of segmentation according to A. Agassiz occurs in Strongylocentrotus. This was not observed in Echinarachnius. Throughout all the changes the egg is enclosed in the capsule, *cap*, which has been mentioned in the unsegmented egg.

Shortly after the end of the first half day after fecundation, the blastomeres arrange themselves superficially about the segmentation cavity, forming a hollow sphere, which is the blastosphere, Pl. II. fig. 14. Minute cilia, which are long and fine, appear over its surface, and the egg begins to rotate and fret against the sides of the envelope or egg capsule, which closes it in. There is no solid morula stage; but a true blastula is immediately formed. At this time a thickening of the blastoderm at one pole takes place, the outline becomes more pyriform, Pl. II. fig. 15, and at the truncated pole a collection of pigment of deeper color than in the remainder of the ovum congregates. This increase in thickness of the cells at one pole is indicative of the formation of a gastrula mouth at that pole. Immediately after the thickening of the blastoderm an infolding begins to take place at this pole, Pl. II. fig. 16. By this infolding, *ga*, the layer of cells which form the walls of the cavity, or the blastoderm, are infolded, and form the hypoblastic layer, or walls of a gastrula stomach. The infolding is at first very slight, but the increasing age of the embryo carries the walls deeper and deeper into the cavity.

With the first indication of an ingrowth of the gastrula stomach, or archenteron, we find budding off into the segmentation cavity certain cells, *a cl*, which from their form, position, and other characters, are called the amœboid or mesoblastic cells. They give rise to important structures, which later appear in the embryo, between epiblast and hypoblast, and which belong to the middle layer or mesoblast. Prouho † finds in Dorocidaris that these cells are not all the same. When his paper came into my hands it was too late to verify in Echinarachnius what he finds in Dorocidaris. At the time my observations were made all the so-called amœboid or mesoblastic cells were regarded as the same in character, and although I supposed that they did not all form the same structures, their differentiation in form was thought to take place much later than in the gastrula stage. These cells form on each side of an axis, passing through the gastrula mouth or primitive infolding. Their bilateral arrangement was not as marked as in Ophiopholis. They

* Rate of growth in water of unrecorded temperature.

† *Comp. Rendus*, ci. pp. 386–388.

form among other structures the calcareous rods and the suspensoria, filiform bodies which connect the hypoblast and epiblast. In *Echinus miliaris* and Toxopneustes, according to Selenka (*op. cit.* p. 46), they also form certain muscles of the stomach and intestine.* I cannot at present say whether these mesoblastic cells originate from the hypoblast alone, or from the epiblast as well, in the genus Echinarachnius. It seemed to me that they arose from a neutral zone on the region of the blastopore. This zone or region, from its position at this time, is either epiblast or hypoblast, or both. As, however, the hypoblast is formed of infolded cells, which elsewhere are later epiblast, we might say that cells originating from this neutral zone are strictly derived from the epiblast. The observations of several naturalists are at variance on this point, as far as the gastrula of other Echinoids is concerned. Selenka holds that in *Echinus microtuberculatus*, *Sphærechinus granularis* and *Arbacia pustulosa* the mesoderm cells spring from the hypoblast. Other naturalists, as Greef, Metschnikoff and Bergh, derive them from the epiblast as well, in these and other genera.

Ludwig, who has reviewed the different observations of the embryologists who have studied the question, concludes that in general the mesoderm cells arise from the hypoblast, but that "auch aus dem Ektoderm sich Zellen abschnüren und zu Mesodermzellen werden können."

No special observations were made on the character of the contents of the segmentation cavity, and the space between hypoblast and epiblast in the gastrula. Ludwig † regards it as filled with a liquid through which the mesoblastic cells can move in Asterina. This seems more natural than to regard it with Hensen ‡ as occupied by "Gallertkern" or any fixed gelatinous structure.

Gastrula.

As the infolded blastoderm or hypoblast pushes its way in the form of a pouch into the segmentation cavity, it changes its form from a simple infolding to a funnel-shaped tube, the parts of which are at first undifferentiated. The primitive opening, blastopore, or gastral mouth, Pl. IV. fig. 1, *gm*, would seem to serve as both mouth and anus, since there is no other communication with the outside water. Krohn says

* The question what structures in the Echinoderm pluteus these cells form is a complicated one, and has been variously answered.

† *Op. cit.* p. 14.

‡ *Arch. f. Naturg.* 1863.

that the gastral mouth serves for reception of food until the formation of the real mouth (second opening). Salenka* says of the view of Krohn: "Ich kann dieser Ansicht nicht beitreten die nach aussen schlagenden Geisselendes Urdarms scheinen den Eintritt von fremden Stoffen durchaus zu verhindern."

A. Agassiz says that in the starfish and Strongylocentrotus gastrula currents of water enter the mouth, pass into the stomach, and pass out through the same opening. The gastrula mouth in these instances certainly serves as both mouth and anus.

Food was not seen to enter the mouth of the gastrula of Echinarachnius, and no observations were made on currents of water. The opening of the blastopore has probably the same function as the homologous opening in Asterias and Strongylocentrotus.

We find that the infolded funnel now becomes enlarged at the base into a chamber, and is attached to the outer wall of the embryo by suspensoria or filamentous bodies derived from the mesoblastic cells. Exteriorly the larva is truncated, flat on one side, more rounded in the diametrically opposite region. It is ciliated with fine long cilia, those on the pole opposite the blastopore being prominent. These longer cilia may be the same as the tuft opposite the blastopore mentioned by Professor Nachtrieb in Mellita, and by Prouho † in the gastrula of *Dorocidaris papillata.* The morphological importance of these cilia has been magnified, although they may indicate one more likeness between the well known pilidium and the Echinoderm larva. The invaginated cells of the hypoblast are cylindrical, ciliated, and not yet differentiated into the walls of the œsophagus, stomach, and intestine.

In a gastrula one day old, Pl. IV. figs. 1, 2, we observe that the invaginated pouch has extended to the opposite pole of the larva, and as it lengthens in this direction its free end slowly approaches the flat side of the gastrula, which side is that known as the ventral. It now bends still more to this region, and is met by a corresponding infolding from the ventral surface. The walls of this infolding break away, and form the future anus, *v*, of the stages immediately following the gastrula, and probably the mouth of the pluteus.

In a gastrula in which the opening had not broken through, Pl. IV. fig. 2, it was observed that the gastrula stomach, *ach*, sends out two horn-shaped cœca, which are similar to structures in other genera known as "water-tubes," "Enterocœlen" or "laterale Scheiben." In

* *Op. cit.* p. 48.

† Sur la forme larvaire du Dorocidaris papillata. *Comp. Rend.* ci. pp. 386–388.

Pl. V. fig. 2, *vp*, one * of these "vaso-peritoneal vesicles," now constricted from the enteron, is seen as a closed sac on one side of the gastrula stomach. The beginnings of the formation of pouches, which probably form the water-tubes, were observed in Echinarachnius, but I have not traced them in their later stages of growth. I have observed only one of these constricted off from the gastrula stomach. The vesicle is separated from the enteron before the "mouth opening" is formed. The wall of the infolded pouch now begins to differentiate itself by constrictions into three regions, corresponding with the œsophagus, stomach, and intestine, Pl. IV. fig. 5, of the fully grown pluteus. At about the same time, also, the limestone rods or calcareous framework of the pluteus first appears, Pl. IV. fig. 3, *sp*.

The calcareous rods appear on each side of one of the openings into the gastrula stomach. In Echinus, according to Krohn, the primitive invagination, or the blastopore, becomes the vent of the pluteus. A. Agassiz says the same of the gastrula of Strongylocentrotus. I have no observation on this point in the gastrula of Echinarachnius, and nothing to show that there is any difference in this genus from what is recorded in Strongylocentrotus and other Echinoids.

The formation of the two limestone spicules which characterize the pluteus at this age, takes place in the cluster of mesoblastic or amœboid cells, *acl*, on each side of the opening, which henceforth serves as the mouth of the pluteus. The first appearance of the limestone rods was detected in a gastrula one day old, Pl. IV. fig. 3. As in Ophiopholis, these structures arise in the bilateral masses of mesoblastic cells,† one on each side of the blastopore or oral end of the stomach. They are at first disconnected, branched, or stellate, and trifid, resembling small sponge spicules. Around them are clusters of the amœboid cells, from which they form.† The neighboring epiblastic wall of the gastrula is reddish and yellow. It was also noticed that at the lowest point of the infolded pouch the same color is prominent. At the last mentioned position the aggregation of cells and pigment renders it very difficult to observe the formation of the external opening. In one specimen, Pl. V. fig. 2, *cl*, I noticed an infolding of the ventral wall opposite the lower end of the invaginated stomach of the gastrula,

* According to Selenka, a single vaso-peritoneal sac constricts from the stomach of the gastrula. This sac later divides into the right and left vesicles.

† A. Agassiz has already called attention to the fact that the limestone rods are first deposited in the midst of similar cells, to which he gives the name "yolk cells." Revision of the Echini, p. 712.

which was thought to be the infolding to form the proctodæum of the last stages of the gastrula. The epiblastic cells, although becoming thinner by the growth of the mesoblast, are still large and prominent, while the hypoblastic are still cylindrical in shape. Both are ciliated. The former layer furnishes the motor cilia of the body; the latter the ciliated lining of the stomach.

Immediately after the first stage in the formation of the calcareous spicules in the larva, which from now on ceases to be a gastrula, the anterior lobe, *al*, and the two prominences which form later the posterior arms, *pr*, begin to push out, and the region in which they form has a resemblance to the three legs of a tripod. The larva when seen from the oral or ventral side, Pl. V. fig. 5, has on each side of the mouth, in a plane in advance of this opening, a small prominence, *pr*, thickly pigmented, especially on its distal end, into which extends a rod from the stellate calcareous rod of each side. In the interval between these rods a large undivided lobe, *ol;* bearing the mouth, appears. This undivided lobe is the anterior or oral lobe, and on its ventral surface is a circular ciliated opening, *or*, the mouth. As this lobe grows, the mouth opening is carried up with it on its side. When seen from one side, so that the length of the two prominences, posterior arms, and oral lobe can be compared, it will be found that the anterior lobe is more prominent than the two posterior arms. In this stage the body of the larva is nearly spherical in form, and as it rests on a tripod formed of the two posterior arms and the single anterior lobe, the intervals between these prominences are easily seen. The anal pole of the larva is pigmented, and filled with numerous amœboid and mesoblastic cells. When seen from the oral pole, we notice that the ventro-dorsal diameter is about the same as the lateral. The mesoblast is much thicker than either the hypoblast or the epiblast. A broad band of cilia surrounds the mouth.

In a pluteus two days old, Pl. V. fig. 6, raised from the egg, we have the two posterior rods still more developed than before, while the anterior lobe is still undivided. Seen from the ventral side the distal ends of the two posterior rods diverge in a V-shape, while the posterior face of the anterior lobe appears rectangular. The opening of mouth and anus are well seen.

In the interior of the pluteus we notice that the calcareous rods which support the posterior arms are double, and have not joined to become latticed. From a point in the body of the pluteus on a level with the anus these rods join the system of rods of the body. One division extends to the apex of the anal lobe on the posterior side. At the apex of this lobe it subdivides and interdigitates with other calcareous arms. At about this time or a little later a strong muscular band, well seen in adult plutei, connects the anal rods near the anal pole on the dorsal side. A simple not latticed calcareous rod, *ar*, bends downward on each side from the common origin of the posterior rods and the most posterior anal body rod, and is continued into the oral lobe. Seen from one side the course of this rod is at first horizontal, until it is about opposite the junction of the œsophagus and stomach, where it sends into the anal lobe a stout anterior anal branch, which extends into the apex of the anal lobe. It there bifurcates, and the divisions interdigitate with the corresponding divisions of the other rods. The calcareous rod of the oral lobe, *or*, is smooth and solid. A straight rod arises from the union of the posterior anal rod with the posterior rod, and extends to the medial line of the dorsal side, ending near the anal opening. Orange and yellow pigment is found in patches at the extremity of the posterior rods, in the anal lobe, and in the anterior lobe. The larva was at times observed to rest on the glass standing on the two posterior arms and the anterior lobe.* At about this time a strong muscular band first appears, which later is very easily seen connecting the anal calcareous rods near the apex of the anal lobe. The object of this muscle is probably to move the two posterior arms, although the rigid union of these two structures by interdigitation would seem to make any considerable motion impossible. As the larva matures, the body becomes more and more helmet-shaped, approaching the form of the Echinocyamus larva figured by Müller.

We are struck, in considering the external form of a pluteus, Pl. V. fig. 11, following the last in age, with the size of a protuberance of the dorsal surface, and the inflation of that region upon which the anus is situated. The posterior arms, *pr*, are well formed, and the oral lobe is not yet differentiated into the two oral arms. In a larva four days old, Pl. V. fig. 7, we see that the anterior or oral lobe has become notched at each corner of the free side of the lobe, and two oral or anterior arms have begun to form. In the stage just before this it was observed that the posterior arms are longer than the oral lobe. Now, however, the oral lobe has increased in length by the growth of the anterior lobe, the length of which has about equalled that of the posterior rods. With the growth of the anterior lobe the form of the œsophagus, *oe*, has kept pace. The last mentioned organ is now flask-shaped.

* This attitude was probably taken on account of the shallowness of the water.

It is at about this time, Pl. V. fig. 12, in the growth of the pluteus that we see signs of the formation of two additional pairs of rods, one of which is known as the antero-lateral, *alr*, the other the antero-internal. These two pairs of rods have certain points in common, as far as their mode of formation goes. Neither of them arises from the primitive centres of calcification from which the rods already formed invariably spring, and to which they are fused. Of these two pairs of rods the antero-lateral arise from separated lateral centres, and are latticed, Pl. VI. fig. 2, *alr*. While the antero-internal, Pl. VI. fig. 4, *air*, originate on a medial line forming at a single centre. The rods of the two arms. *air*, are simple, smooth, sometimes with small lateral spurs or teeth and not latticed.

The antero-internal spicule or rod is well formed in the body of the pluteus before any projection on the surface at the point where it later appears can be noticed. It arises as a trifid spicule in the basal region of the oral lobe. As it grows it becomes crescentic, the convexity turned to the oral lobe, and the two horns extend about parallel with the anterior rods. A slight spur or median tooth arises from the convex side of the crescent. The first appearance of the antero-internal arms is marked on the surface of the larva by a projection on each side of the anterior lobe within the anterior rods. Into these projections, as they increase in size, the extremity of the two horns of the crescentic spicule extend.

The antero-lateral rods, *al*, Pl. VI. figs. 3, 4, Pl. VIII. fig. 5, originate in a different way from the single median centre of calcification of the antero-internal. They arise, not from a single centre of calcification, but from two lateral centres. Just above the anterior lobe, in the interval between it and the base of the posterior arms, a projection forms on the edge of the plutean body. This projection raises with it a section of the circumoral ciliated band, and in its interior there forms a pair of rods which become joined and form a latticed rod, resembling the posterior rod. The antero-lateral rods are not fused with the other rods, and as by the growth of the antero-lateral rods little by little the arm equals in length that of the posterior, *pari passu* the rod grows without joining the remaining spicules. It is this freedom of the two systems which renders it possible for these arms to be moved by the muscles of the pluteus. The antero-internal system of rods also does not join the other rods, and is likewise movable, while the muscular fibers which accomplish this are easily seen near its junction with the anterior arms.

The pluteus is now, Pl. VI. fig. 4, in about the same stage as that figured by A. Agassiz.* It is immature, and an important growth takes place before it acquires the adult form. The pluteus which A. Agassiz has figured is, according to my observations, about a week old. Eggs artificially fertilized on July 16 developed into the pluteus, with the antero-lateral and the antero-internal arms just beginning to form, on July 23. It was not easy to raise these plutei into older conditions, but in the month of September there was fished from the surface of the bay with the dip-net a complete series of plutei, which connects the pluteus figured by A. Agassiz with the adult as here described, in which all the four pairs of arms are of equal length. As the preceding plutei were obtained by artificial fecundation, it is not to be supposed that the fact that mature plutei are found in the middle of September, indicates that these plutei are a month old. When artificially fertilized, the eggs, however, were just ready to be laid. If, as A. Agassiz † says is the case of Strongylocentrotus, the female Echinarachnius lays her eggs, or the eggs can be fertilized at all seasons of the year, it would be very difficult to determine the age at which the adult pluteus is attained from nomadic larvæ fished at random from the sea.

A larval pluteus of Strongylocentrotus (Toxopneustes) fig. 52,† is very similar to the stage of a pluteus of Echinarachnius at this age. In Echinarachnius as in Strongylocentrotus, the antero-internal arms are just beginning to appear, and although the antero-internal crescentic spicules have already formed, the arms corresponding to these rods are still quite small. This larva which was raised from the egg of Strongylocentrotus is twenty-three days old according to A. Agassiz.‡ It would thus be about two weeks older than my Echinarachnius of similar form, also reared from the egg.

The adult pluteus, Pl. VII. figs. 1, 2, of Echinarachnius, first appeared in great numbers at Newport in 1885, on September 16. In former years they have been found earlier in the season. The older stages were captured with a dip-net on the surface of the water, both by night fishing and in the day-time. For a number of years I have kept a record of the dates when our marine larvæ first appear in numbers, and find that the adult pluteus of Echinarachnius is most common at

* Revision of the Echini, p. 727.

† Our common sea-urchin (*S. Dröbachiensis*) matures its genital organs in winter, according to A. Agassiz. (Revision of the Echini, p. 709.) February is the month when he ordinarily succeeded with artificial fecundation. "The sea-urchins spawn during the whole year." *Op. cit.* p. 719.

‡ *Op. cit.* p 719.

Newport, R. I., in the last of September. As the plutei undoubtedly develop from ova laid by adults, which live within a short distance of the laboratory, their date of appearance is not dependent upon those causes to which we very probably ascribe the marked periodicity in the times when Salpæ and those animals which live in the high seas revisit Narragansett Bay.

Although it is not known whether Echinarachnius lays its eggs in all months, or can be fertilized at all times of the year, it can be said that in the last five years in which I have kept my record of the times when marine larvæ appear at Newport, the greatest number of larval Echinarachnius appear in September. None were collected in June, in July they are sporadic, and the largest number usually came in September. Every one acquainted with pelagic fishing knows how much variation there is in the time when pelagic larvæ appear, and these statements indicate only approximation. I suggest that they point to the end of August as the probable time of ovulation at Newport of the Echinarachnius.

The body of the adult pluteus is elongated, rounded on its anal pole. On the opposite end it is continued into four pairs of arms, all of which have calcareous rods, while two pairs, *pr* and *alr*, are latticed. The latticed rods are the stoutest, and are known as the antero-lateral, *alr*, and the posterior, *pr*. The latter arise from the posterior side of the body, and are fused with the system of rods which extends through the body. A very prominent cluster of dark red pigment cells, *pig*, is found near the distal ends of all the rods. Pigmentation of the same color occurs in small granules along the length of the arms, and on the body walls. The arms are skirted by a ciliated band, on the edges of which are small granulations. The ciliated band connects the two posterior arms on the median ventral line. Laterally from these arms the same band passes to the antero-lateral rods.

No ciliated epaulettes were observed. In some specimens it was noticed that the ciliated band in the connection between the two posterior arms on the ventral side was so folded that we have a median and two lateral lobes in the region of the band placed between the two posterior arms. Something similar is figured in the pluteus of Echinocyamus by Müller, but as Müller says that ciliated epaulettes are not found in his pluteus of Echinocyamus, we may conclude that the last mentioned bodies are not epaulettes in the closely allied Echinarachnius. The antero-lateral arms, *alr*, closely resemble the posterior in pigmentation, latticed axes, ciliation, and size. The ciliated band continued on them from the posterior arms is carried thence to the edge of the anterior lobe, passing to the anterior arms. The four arms which arise from the anterior lobe are called the anterior, *ar*, and the antero-internal, *air*. Both are furnished with a solid non-latticed central axis or calcareous rod. The anterior rods are fastened to the general calcareous framework of the body; the antero-internal are free, united to the former by muscular attachments. The larva may be compared to the parts of a chair, the antero-internal rods forming the front legs, the anterior the hind legs, and the oral lobe the back by which these rods are joined to the body of the pluteus. The distal ends of the anterior rods are more widely separated than the antero-internal. The anterior rods are skirted by a ciliated band continued from the antero-lateral as mentioned above. They bear a red pigment spot at a short distance from their distal end.

The last pair of arms to be mentioned, the antero-internal arms, *air*, are joined together at one end by the ciliated band which passes upon their rim from the anterior arms, and is joined between them, just as the other end of the circuit is joined on the medial line between the posterior rods. One edge of the junction of the two antero-internal arms forms one of the so-called lips of the mouth, Pl. VII. fig. 2. The oral opening, *or*, is placed between this structure, and the ciliated band joining the two posterior rods or arms. The two antero-internal arms commonly want the prominent pigmentation of the distal ends of the other arms. In one or two specimens, however, pigmentation was observed on the distal end of the antero-internal arms. As the calcareous rods which support the antero-internal rods have a separate origin from the rods of the body system, they are capable of movement, and are not fused with the other rods. A number of muscular threads by which this movement is brought about can be seen in the inner angles formed by the rod and the neighboring anterior rods, Pl. VII. fig. 18. In the interior of the body we find that the larger part of the body of the pluteus is occupied by the stomach. Between the stomach and the epiblastic layer which covers the body of the pluteus many cells are found, some of which are yellow in color. Filaments or threads connect the hypoblastic wall of the stomach and superficial epiblast.*

* While studying the Embryology of Agalma similar threads were noticed passing from the epiblast of the primitive hydrophyllium to the hypoblastic lining of the same. Similar threads are known in many worm larvæ. If these structures are the same as the so-called suspensoria (Selenka) of the gastrula, we may reasonably doubt whether Selenka is right in supposing them derived from the mesoderm cells. As far as observation has gone there is no reason to doubt that

The apex of the anal lobe is densely pigmented, and the walls are supported by highly ramified divisions of the anal rods (posterior and anterior), which interdigitate and form an intricate network. An anus is present, and the intestine is elongate or flask-shaped. The mouth is commonly widely open, circular, with ciliated lips. The œsophagus is densely ciliated on the interior walls. The pluteus moves from place to place easily but not rapidly, and is just visible to the naked eye. The length is .85 mm. ; diameter of the body .20 mm.

One of the most striking differences between the adult pluteus of Echinarachnius and A. Agassiz's drawing of that of Strongylocentrotus of the same age, is the existence of large pigment spots near the distal end of each arm, while the anterior and antero-internal arms of Strongylocentrotus have no such spots, at least of the great prominence which we find in Echinarachnius. The pluteus of Strongylocentrotus, according to A. Agassiz, has ciliated epaulettes. These structures are not represented in the pluteus of Echinarachnius. Like the pluteus of Echinocyamus, as figured by Müller, Echinarachnius has no ciliated epaulettes. The resemblance of the pluteus of Echinarachnius to that ascribed to Echinocyamus is very great. If we compare the figures given by Müller and those of the Echinarachnius pluteus here figured, we notice one or two marked differences between them. The arms of the pluteus are much longer and larger in Echinarachnius than in Echinocyamus. The posterior and antero-lateral rods of both genera are latticed. The antero-lateral and antero-internal in both are not connected with the body system of rods. The anterior lobe before the origin of the antero-internal arms is longer in Echinocyamus than in Echinarachnius. The characteristic pigment spots of the ends of the arms of Echinarachnius are not found in Echinocyamus. The difference of the young from the adult sea-urchin formed from the pluteus of Echinocyamus has attracted the attention of Müller. The young Echinarachnius raised from the pluteus is somewhat different from Müller's figures of the young Echinocyamus.*

the suspensoria are mesodermic, as Selenka says. In Agalma these structures appear to be epiblastic. It may be said, however, that they originate from the epiblast, just as the mesoblastic cells themselves may originate as simple extensions and buds. The homology, therefore, of the suspensoria and the filament in the primitive hydrophyllium cannot be made out at present. It may be said that the likeness between the two is great. (For filaments in the primitive larva of Agalma, see Embryology of Agalma, *Bull. Mus. Comp. Zoöl.*, XI. No. 11.)

* It is taken for granted that the pluteus described by Müller is an Echinocyamus, although he did not raise it in the egg.

Formation of the Young Echinarachnius.

The growth of the young Echinarachnius from its pluteus is not easy to trace on account of the condensation of pigment upon its walls as it matures. This formation of pigment renders it very difficult to study the sequence of the appearance of the plates, and obscures the internal changes which accompany the maturation of the larva into the adult. The contour of the young sand-dollar after it absorbs the pluteus is very different from that of the adult. No one would recognize both as belonging to one and the same Echinoid. The whole of the pluteus is absorbed into the growing Echinarachnius.

A vesicle, the vaso-peritoneal vesicle, on the left hand side of the stomach (see figures) appears in the very earliest stages of the growth of the sea-urchin from the pluteus to enlarge, and was observed to have the form of a retort-shaped structure, with an external opening on the dorsal side of the body, near the posterior arms, Pl. VII. fig. 3. It was not possible for me to determine whether the left " water-tube " sends out a prolongation which forces its way to the surface, opening through a dorsal pore, as A. Agassiz has described in Strongylocentrotus, or not. In the earliest stage in which I began to study the growth of the young sand-dollar, the dorsal opening had already formed, communicating through a tubular body with the water-tube. Consequently, the growth of the tube through the body was not observed or studied. In the pluteus in which this external opening had formed, the arms of the pluteus were all of the same length, and consequently the pluteus was regarded as adult. In the pluteus of Strongylocentrotus, according to A. Agassiz, the young sea-urchin first appears in a young or immature pluteus, in which the arms are not of the same length, judging from his fig. 52, in " Revision of the Echini, Embryology," p. 717. In this figure the antero-internal arms had not begun to push out from the oral lobe, and the antero-lateral rods were just formed. This pluteus appears to be immature as far as the appendages go, since they are not fully formed. The beginning of the young Echinarachnius on the left water-tube was not traced in a pluteus as young as this pluteus of Strongylocentrotus.

Balfour * in his account of this figure (fig. 52) gives an interpretation to the structure, *t*, different from A. Agassiz. The latter author says, " On the left water-tube we notice a very prominent loop, *t*, which, from

* *Op. cit.* pp. 472, 473.

its resemblance to the tentacular loops of Brachiolaria, and from its position on the water-tube connecting with the water pore, I have no hesitation in considering to be the first tentacular loop formed." Balfour considers this structure an invagination of the external surface of the larva, an infolding which later is to form the ventral region of the Echinoderm. Metschnikoff * ascribes to A. Agassiz the discovery of an invagination of the outer skin of the pluteus to form the body of the future echinus. I have not been able to find in A. Agassiz's works, quoted by Metschnikoff, that he has made such a " discovery," and certainly he does not give to the lettering of his figures the same interpretation which Balfour does, when he says that the structure in question, *t*, is a tentacular loop.

Pl. VII. fig. 3, represents the young of Echinarachnius formed on a pluteus of the same general form as that figured in Pl. VII. fig. 2. The left water-tube has here formed the " rosette " of five radial tubes, which are seen in profile in the figure. The whole body of the pluteus is not represented, but a portion of the edge of the stomach of the pluteus is seen on the right-hand side of the figure. The figure is a representation from the dorsal side.

The five radial bodies of the " rosette," one of which is lettered, *wt*, are the water-tubes or the ambulacral divisions which, when seen from one side, would form a five-rayed water system derived from the left water vesicle. The system communicates with a tube which passes through the mesodermic layer of the plutean body, and opens externally by an opening, *ma*, which is supposed to be the madreporic opening. This opening is at first situated near the base or proximal end of the posterior rods. Its communication with the water-tubes has a retort form, especially in older stages. The retort-shaped vesicle was observed in a stage a little older, Pl. VII. fig. 4, than the last to be in direct communication with the rosette-shaped system of five ambulacral vessels. Each of the five radiating ambulacral tubes which form the rosette extends outward from a central region, which is in direct communication with the retort-shaped body. These radial ambulacral tubes are at first simple, without lateral branches, cœca-like folds or loops, † with transparent walls, the outlines of which can be plainly seen

* *Op. cit.* p. 41.

† These five loops are supposed to be homologous with the " odd ocular tentacles " of Arbacia and Strongylocentrotus. See A. Agassiz, Report on the " Challenger " Echinoidea, p. 8. They are supposed to be the same as the " Fühler," F. in Asterina. (See Ludwig, *op. cit.*, Pl. VII. figs. 96, 97.)

through the wall of the pluteus. The line of the edge of the future Echinarachnius can be traced on the dorsal side of the stomach of the pluteus, while the "dorsal pore," *ma*, lies near the edge of the dish turned in the direction of the oral region of the pluteus. The growth of this opening is a migration from the vicinity of the posterior arm towards the middle line of the dorsal side of the body. As it grows in this direction it works at the same time to the anal apex of the pluteus, never, however, reaching that position. Unlike the figure of a Spatangoid pluteus, Pl. VIII. fig. 13, by Metschnikoff, the retort-shaped vesicle before division into the rosette does not extend so that the dorsal pore lies in the median line. In the figures which we have of the young Spatangoid, the line bounding the wall of the growing Clypeastroid is always recognized on the dorsal surface of the body in stages as old as fig. 8, Pl. VIII., of Metschnikoff's paper on the development of Echinoderms. I have given a series of figures to illustrate the relative changes in position of the dorsal pore, *ma*, from very early conditions, up to a stage when the deposit of pigments renders observation impossible. In the progress of this migration of the madreporic body or dorsal pore it will be observed that the length of the ambulacral tubes increases, and additional feet form as diverticula, while interesting calcareous deposits occur, Pl. VII. fig. 9. It was not observed whether these feet bud from the five primary tubes or not. There is no reason to doubt that they do. The appearance of pigment spots on the body of the forming sea-urchin takes place at about the same time as that of the trifid rods which they later obscure. The first limestone formation which was observed is a trifid spicule in the wall of the body of the growing sea-urchin. In its very first form this trifid spicule is spherical in contour. Later, it assumes a trifid shape, and seems to be enclosed in a transparent sac, the outer wall of which is believed to be formed of epiblast, the calcareous body being formed possibly in mesoblast. This transparent sac and its enclosed calcareous body of tripod shape resembles the structures, *cc*, in *E. lividus*, as figured by Metschnikoff.* If these bodies are morphologically the same in Schizaster and Echinarachnius, we have a likeness hitherto unrecorded between the young Spatangoid and the immature Clypeastroid.

Metschnikoff figures, Pl. VI. (fig. 10, *op. cit.*,) in the Ophiuran pluteus a similar calcareous body, to which he gives the name of "Hohlkehlen," already used by Müller.

Ludwig has already remarked on the resemblance of similar cal-

* *Op. cit.*, Pl. VIII. fig. 9.

careous bodies (fig. 100, *op. cit.* pp. 67, 68) to the "Basalplatte," of the so-called "Stühlchen," in the skin of the Holothurians, and to the "Rädchen" of the Chirodotæ. He considers that the "Chirodotarädchen den Basalplatten der Seesternstachel gleichzusetzen sind, gewissermassen rudimentäre Stachel darstellen, bei denen sich die ganze Ausbildung auf die Entwicklung einer Basalplatte reducirt hat."

A. Agassiz has called my attention to the resemblance of similar bodies in Echinarachnius to the calcareous wheels in the Holothurians. It seems probable that the stellate bodies in the young Echinarachnius are the same as the "Basalplatte" of the spine of Asterina.* It was not observed that these bodies, as they first appear in Echinarachnius, bear a definite relation either to the ambulacral tubes or the intermediate intervals which we may suppose are the interambulacral regions. Although a large number of plutei were examined, the number of these bodies was not found to be uniform. Some plutei have five, some one, others three, and many more than five, of these trifid calcareous bodies. As the echinus grows older the ends of the three spurs of the trifid spicule became divided or bifurcated, and even subdivided, while in some cases these bodies were again subdivided. In all these cases they are still enclosed in a transparent cyst or cell, similar to that figured by Metschnikoff for the "Hohlkehlen." This sheath or capsule is supposed to be the outer enveloping layer, epiblast, of the spine. It was of course my first impression that these rods were the beginnings either of ocular or genital plates, and I turned to A. Agassiz's memoir on the development of the starfish, where similar calcareous bodies are found, to see if it were not possible to homologize them with the plates which first appear in the Asteroidea. It was not possible to satisfactorily compare these structures, and I was then led to inquire whether these structures are the beginnings of plates or of other parts of the echinoid body. My observations at present have not gone far enough to answer these questions satisfactorily. If these trifid bodies are the beginnings of plates it cannot be stated at present whether they are ocular or genital plates, and there is a doubt in my mind whether they are plates of the test or spines of the same.

Pl. VII. fig. 16, is an instructive stage in the development of the sea-urchin within the body of the pluteus. On the right-hand side of the figure we see the ambulacral feet, *am*, of which there are more than five, the additional having probably formed by lateral budding. On

* Compare fig. 10, p. 69 (Ludwig *op. cit.*) with the trifid bodies of Echinarachnius. See also fig. 100, *op. cit.*, *a*, *b*, *c*.

the left-hand side we see the plates of the test of the future sea-urchin. In the middle of the figure, a little to one side (left), we notice a central plate, "centrale," *c pl*, of pentagonal outline, around which are arranged a ring of five plates, *pl*, closely fitting to the central plate. Outside, or peripherally to these, we see other pentagonal bodies, three below in the right-hand lower corner, and one above adjoining the upper letters, *pl*.

In all the peripheral system of plates we have a reticulation of calcareous nature.

In the five plates which surround the central plate we have two kinds of calcification, one of which forms plates of the test, the other probably the spines of the plate. The calcareous deposit of the plate forms an irregular network or reticulation of no regular form, while the calcareous deposit of the spine has a circular wheel-shaped or stellate form, from the rim of which there spring prolongations, in our figure drawn in a fan shape. The circular portion is the base of the spine; the fan-shaped continuations or extensions, the beginning of the shaft. In some of the plates which are more peripherally arranged as regards the five plates described, we find fan-shaped calcareous formations, and no reticulated or lace-formed calcifications corresponding to them.

In the stage which we are considering, the centre of calcification which is supposed to form the plate of the test (reticulated calcification) and that which later forms the spine (stellate calcification) are not joined.

The development of the spine in sea-urchins and starfishes has been traced by A. Agassiz. He says: * "The shell of a sea-urchin is made up of an irregular network of limestone cells, which makes its appearance in the early pluteus stage; with increasing size this network becomes closed at certain points, and sends off upright shanks, which little by little form very irregular fan-shaped spines. In our common sea-urchins these spines are immovable, forming at that stage part of the test itself. As the spines grow they become more pointed, but are still immovable. In somewhat more advanced stages a slight constriction is formed at the base of the spine, and very soon after that, below the constriction, a tubercle is formed, upon which the spine is articulated, and is then capable of a certain amount of motion, etc." (I have omitted his reference to plates and figures.) It would seem, then, look-

* Revision of the Echini, pp. 667–669.

ing at fig. 16, Pl. VII., if the stellate bodies in it are spines, and the reticulated network plates of the test of Echinarachnius, and if this genus resembles that described by A. Agassiz in the way the spines are formed, that the stellate cells must have arisen from the reticulations, and been constricted from them. There is nothing to show that in Echinarachnius stellate and reticulated rods arise one from the other; for these two centres of calcification are distinct in early stages, and we sometimes have stellate rods without the corresponding lace-work rods. This then would throw a doubt on the interpretation which I have given to the stellate calcification as immature spines, unless Echinarachnius is very different * from the sea-urchins described in Agassiz's account, as far as the growth of the first-formed spines is concerned.

The question whether the stellate bodies are spines or pedicellariæ is a very difficult one. If the spines of the genus Echinarachnius form, like those of Strongylocentrotus, from the reticulated plate of the test, as recorded by A. Agassiz, we cannot regard the stellate bodies as true spines. According to Metschnikoff,† the pedicellaria is one of the first structures to appear in the echinus of *E. lividus*. Pl. VII. fig. 7, of the last-mentioned work, shows a young pedicellaria, which has a very close likeness to some of the stellate calcareous bodies of the young Echinarachnius. The growth of the stellate calcareous body was not traced into a pedicellaria in Echinarachnius. The homology of the five plates surrounding the central plate in Pl. VII. fig. 16, has not been satisfactorily made out. It may be conjectured that they correspond with either the genital or ocular plates of the adult, but they were not traced to these plates, and such an interpretation would be conjectural. The formation of new plates, according to A. Agassiz, takes place in Strongylocentrotus in a spiral manner. The new plates of Echinarachnius are thought to form in the same way as those of other Echinoids, but I have been unable to trace them, on account of the great deposit of pigment, and the consequent opacity of the forming test. In stages older than Pl. VII. fig. 16, the sea-urchin was nearly opaque. In fig. 17, Pl. VII., the different plates which compose the test could not be

* The formation of the stellate bodies which have been identified as spines in Echinarachnius resemble in their growth the growth of the spines of Asterina, as figured by Ludwig (*Zeit. f. Wiss. Zool.* XXXVII. pp. 67–70, fig. 100), more closely than they do those of Asteroidea and Echinoidea figured and described by Agassiz. Ludwig makes no mention of A. Agassiz's accounts of the development of the spines in starfishes and sea-urchins.

† *Op. cit.*, Pl. VII. fig. 6.

identified, on account of pigmentation, although a single opening, *ma*, which is thought to be the madreporic opening, was clearly observed. This interpretation of the opening, *ma*, is conjectural; for, with the exception of the single fact that it occupies the same position as the opening, *ma*, of previous stages, there is nothing to show that it is the madreporic opening. Its communication with the water system could not be traced.

The oldest stages, Pl. VIII. figs. 15, 16, of the young Echinarachnius here considered, were taken by dredging in the shallow waters on a sandy bottom, where these Echinoderms live. The dredge brought up a large number of very small sand cakes which were free in its meshes, while many of the younger specimens were washed out of the sand and "roots" of Laminaria from the bottom. These young Echinarachnii are regarded as developed from plutei hatched the past summer. They were dredged near the end of September. A young Echinarachnius, older than that here (Pl. VIII. fig. 15) described, is figured by A. Agassiz.* My figures represent stages between that which he has given and the young Echinarachnius, just after it has absorbed the pluteus. The form of the young sea-urchin in this stage is spherical, elongated, plump, more like a Spatangoid or some "round sea-urchin" than a Clypeastroid. A. Agassiz has compared it to that of the genus Echinometra. The larger diameter is 1 mm.; the smaller .8 mm. The young are almost completely opaque, on account of the formation of spines, pigment, calcareous rods, and plates.

The spines are relatively larger and more prominent than in the adult. In many of these structures the superficial layer of the spine closely hugs its calcareous centre forming the shank, while in others, mostly younger, the thickness of the outer transparent layer is perceptible in lateral profile. The sea-urchin, when seen from the abactinal area, is found to be oblong, a diameter passing through the anus being a third longer than that at right angles to it. The anus is slightly excentric, and has the form of a crescentic slit, which is formed by a circular plate, "centrale," *ap*, almost closing the circular opening, leaving a crescentic orifice with concavity towards the apex. The ambulacral areas are distinguishable from the interambulacral at the apex of the body, while near the periphery of the test (seen from the aboral region) their discovery and separation is more difficult. The spines, *sp*, around the rim of the body are large and long. The ambulacral feet, *am*, are widely extended.

* *Loc. cit.*

Seen from the actinal side, Pl. VII. fig. 16, it will be noticed that the oral opening is very large, and that the rim, *ed*, of the test surrounding this opening is notched. The diameter of the opening is about one half that of the test of the sea-urchin. The larger part of this opening is closed by a muscular wall, in which are imbedded the teeth and dental apparatus of the Aristotle's lantern, *lan*. The five teeth of the last-mentioned structure are all well developed. The young of Echinocyamus, figured by Müller, is probably in about the same stage as the young Echinarachnius just described. As far as the form of the spines and their position on the test goes there is little question that the young Echinoid ascribed by Müller to Echinocyamus has a somewhat different form after the absorption of the pluteus from that of Echinarachnius, although the differences are slight. In both Echinarachnius and Echinocyamus we seem to have at first the spines arranged in a single row about the margin of the test, an approach to the arrangement of spines in Arbacia and some other genera. The young Echinarachnius is less spherical than that of Echinocyamus. The forms of the ambulacral feet are alike. The spines are movable, but their motion is small. The motion of the spines in the young Echinarachnius is observed long before the absorption of the pluteus. The external changes subsequent to the stage last described, passing from the young Echinarachnius into that described by A. Agassiz, consist in a diminution in size of the vertical axis and a migration of the anal opening, *ap*, more towards the margin of the disk.

The existence of large spines in the young Echinarachnius and their subsequent diminution in size in the adult may show a likeness of the young Echinarachnius to certain embryonic types where the spines attain a relatively large size. The primary position of the centrale and subsequent migration of the anal opening from its normal position seem to indicate a likeness in the young flat sea-urchin to round forms like those which have an apically placed anus.

The following summary may be made of the preceding observations :

1. The egg of Echinarachnius can be artificially fertilized, and resembles that of other Echinoderms in its mode of segmentation. It has no polar globules, while the egg is free in the water.

2. A gastrula is formed by invagination, as in some other Echinoderms.

3. The pluteus referred to Echinarachnius by A. Agassiz is an immature pluteus of Echinarachnius.

4. The development of the young Echinarachnius on the water-tube of the pluteus resembles that of other sea-urchins. The rosette form of

the water-tubes described in other Echinoderms is likewise found in Echinarachnius.

5. The first-formed calcareous deposits of the test in the young Echinarachnius are trifid in shape, varying in number in different specimens. The extremity of each trifid division later in its growth bifurcates, and the calcareous body thus formed appears to be enclosed in a transparent wall, which has a spherical outline.

6. Spines are very early formed, and are proportionally very large as compared with those of the adult, as in other Echinoderms.

CAMBRIDGE, March, 1886.

EXPLANATION OF THE PLATES.

a. Space between the vitellus and the egg capsule.
ach. Archenteron.
a cl. Amœboid cells, mesoblastic cells.
air. Antero-internal calcareous rod.
al. Anterior lobe.
alr. Antero-lateral rod.
am. Ambulacral tube, or member of primitive rosette.
ap. Anal plate, "centrale."
ar. Anterior rod.
c. Transparent body, in which is contained a branched calcareous rod.
cap. Capsule.
cav. Cavity.
cl. Cell.
cl pl. Cleavage plane.
1 *cl pl.* First cleavage plane.
2 *cl pl.* Second cleavage plane.
c pl. Central plate, "centrale"?
cr. Calcareous rod.
d. Polar globule. In Pl. I. fig. 2, polar globule?
e. Body of young Echinarachnius.
ed. Edge of the test and junction of the muscular oral structure.
f. Muscular filament connecting the wall of the stomach with the epiblast.
g. Abnormal segmentation sphere.
ga. Stomach.
gm. Mouth of gastrula, blastopore.
i. Intestine.
lan. Aristotle's lantern.
l. Lateral arm.
lr. Lateral rod.
ma. Madreporic opening; dorsal pore.
mm. Large segmentation spheres, macromeres.
o. Thickened floor of a retort-shaped structure. (This is the same as that which Metschnikoff calls the invagination of the wall of the pluteus.)
oe. Oesophagus.
ol. Oral lobe.
or. Mouth.
p. Primitive furrow. In Pl. I. fig. 1, cortical hyaline layer.
pig. Pigment.

pl. Calcareous plate.
pr. Posterior rod and posterior arm.
r. Smaller segmentation spheres, micromeres.
rd. Anal rod.
s. Stellate calcareous body.
sp. Spine. In Pls. I. and IV. the trifid calcareous rod of the pluteus.
spi. Spicules of the two posterior rods after absorption of the body of the pluteus by the growing urchin.
t. Transparent layer.
v. Anus.
vp. Vaso-peritoneal vesicle, water-tube.
vt. Vitellus.
wt. Water-tube.

PLATE I.

Figures drawn with camera lucida, Obj. D. D. eye-piece 2, Zeiss. Reduced one third in photography.

Fig. 1. Ovum of *Ophiopholis aculeata*, found free in water after ? fertilization. *p*, Superficial cortical layer of the yolk.

" 2. Same, showing a single polar globule (?) at *d*.

" 3. Egg in the 2-cell stage with polar globules, *d* and first plane of cleavage, 1 *cl pl*.

" 4. The same without polar globules.

" 5. Egg in the 2-cell stage with the first cleavage plane, so turned as to show the thickness of the transparent plasmic region following the first plane of cleavage.

" 6. Egg in 4-cell stage.

" 7. The same.

" 8. Egg in 8-cell stage.

" 9. The same showing the segmentation cavity, *cav*. Twelve hours old.

" 10. Blastosphere. The cluster of cells in the middle of the figure are not cells in the blastocœlic cavity, but cells of the blastoderm with granulations. All the blastodermic cells have the same appearance. One day old.

" 11. Blastosphere, showing the beginning of an invagination of the archenteron, *ach*, forming the blastopore.

" 12. The same seen from the side. Amœboid, mesoblastic cells at *a cl*.

" 13. A little older larval stage, from the ventral side. A small cluster of cells represented with granulations near the right-hand lower corner.

" 14. Older gastrula from ventral side.

" 15. The same from a lateral view.

" 16. An older gastrula from ventral side. Some of the epiblastic cells in the lower left-hand side are granulated. This character is not confined to the cells of this region.

" 17. An older gastrula in which the lateral prominences, which later form the lateral arms, are shown. The prominence in the medial line above is the oral lobe, *ol*, of later figures.

Fig. 18. An older larva with circumoral ciliated band, and prominent anterior lobe. The prominences on the sides are lateral arms.

" 19. An older larva, in which the calcareous rods, *sp*, are found in the body. Thirty-six hours old.

" 20. Still older larva, one side outlined, in which the length of the calcareous rods has greatly increased. Three days old.

" 21. An older pluteus from the dorsal side.

" 22. The same from ventral side.

" 23. Dorsal view of the oldest pluteus of Ophiopholis which was studied. Mouth, *or*, seen through the oral lobe.

PLATE II.

All figures drawn to a scale with camera lucida. Obj. B. B. Eye-piece 2, Zeiss. Reduced one half in photography.

Fig. 1. Ovum of *Echinarachnius parma* in its capsule.

" 2. Same ovum with the primitive constriction, *p*, which forms the first plane of cleavage. The external pigmented layer of the capsule is not represented.

" 3. Ovum in 2-cell stage.

" 4. Ovum in which the second plane of cleavage has begun to divide each of the two cells of the 2-cell stage.

" 5. A stage somewhat older.

" 6. Outline of the four cells of the 4-cell stage, showing the two planes of cleavage at right angles to each other.

" 7. Ovum in 4-cell stage.

" 8. Ovum in which constrictions have begun to form new planes of cleavage, which later divide the four cells of the 4-cell stage to form the 8-cell stage.

" 9. Ovum in the 8-cell stage.

" 10. The same seen in a plane at right angles to the last.

" 11. Ovum in 8-cell stage showing the segmentation cavity, *cav.*

" 12. Segmented ovum of a stage with more cells than the 32-cell stage. Two of the cells are represented with nuclei.

" 13. The same, older, with segment spheres more angular and segmentation cavity shown.

" 14. Blastosphere, "planula stage." The cells have taken the form of a hollow sphere. The larva has left the egg-capsule.

" 15. The same showing the flattening at one pole preparatory to an invagination. (Optical section, in which the cells are not shown in the hemisphere turned to the observer.)

" 16. Young gastrula with partially infolded blastoderm, forming the stomach, *ga.*

" 17. Older gastrula. Outlines of blastodermic cells not represented.

PLATE III.

Fig. 1. Abnormal ? egg of *E. parma*, in which the groove-like constriction which generally encompasses the ovum and forms the first cleavage plane, is limited to a furrow at one pole, *p*.

" 2. Ovum in which this furrow, *p*, has deepened, forming a slit.

" 3. Same egg in an older stage.

" 4. Ovum in 2-cell stage, the original connection between the two cells in the undivided part of the original ovum turned from the observer. This egg is seen at right angles to the plane in which Figs. 1–3 are drawn.

" 5. An ovum in 4-cell stage, in which we have two large segment spheres and two small. This condition is thought to be uncommon.

" 6. Ovum in 4-cell stage, in which the beginning of the furrow destined to divide each of the four cells is found on one side, inner side, of all cells, and does not take the form of a groove-like constriction reaching wholly about the blastomere.

" 7. Ovum in 4-cell stage in which each of the four blastomeres is divided later into two of unequal size.

" 8–12. Formation of the 3-cell stage. They first represent the formation of a 4-cell from a 2-cell stage, and then the subsequent breaking away of a part of one segmentation or cleavage plane, so that two of the blastomeres are reduced to one. This is thought to be abnormal, pathological, or at all events unusual.

PLATE IV.

Fig. 1 Gastrula of Echinarachnius, in which the archenteron, *ach*, has made its way to the ventral side of the body. Lateral view.

" 2. The same, ventral view.

" 3. An older stage in which the limestone rods, *sp*, have begun to form. Lateral view.

" 4. View of the last from anterior pole.

" 5. An older larva, showing the differentiation of the anterior lobe and the posterior rods or arms. Ventral view.

" 6. A slightly younger larva seen from one side.

" 7. An older larva seen from one side.

" 8. A larva older than the last, seen from the side.

PLATE V.

Fig. 1. Gastrula of *E. parma*. Lateral view. (The cilia on the body are too faintly photographed.)

" 2. Older gastrula showing the " water-tube " at *vp*. Lateral view. As the larva is under slight pressure, the spicule or calcareous rod, *spi*, is slightly thrown out of position.

Fig. 3. Youngest pluteus with well developed posterior arms. Lateral view.
" 4. The same, older. Lateral view.
" 5. An older pluteus seen from the ventral side. The posterior arms are well developed; the anterior lobe is not divided.
" 6. Ventral view of a pluteus, of about the same age as the last.
" 7. View of a somewhat older pluteus in which the two anterior rods, *ar*, are formed from the anterior or oral lobe. Ventral view.
" 8. Anal lobe of a pluteus about as old as fig. 5, seen from the ventral side. The appendages are not figured. *cl*, cell nuclei ? Filaments, *f*, connecting the epiblast and hypoblast.
" 9 Lateral view of a pluteus, a little younger than the last.
" 10. View of a pluteus from opposite (lateral) side. This stage is a little younger than the last.
" 11. Side view of a pluteus of approximately the same age as the last.
" 12. Pluteus in which the antero-lateral arms have begun to form. Ventral view.

PLATE VI.

Fig. 1. Lateral view of a pluteus just before the formation of the antero-lateral rods.
" 2. A figure showing the relation of the antero-lateral rod, *alr*, when first formed, to the posterior, *pr*.
" 3. An older pluteus seen from the dorsal side and laterally.
" 4. A still more mature pluteus, seen from the ventral side.

PLATE VII.

Fig. 1. Adult pluteus of *E. parma*, showing the young echinus forming at *e*. Dorsal view. Camera lucida, obj. B. B., eye-piece 2, Zeiss. Reduced one third in photography. Calcareous rods on the right-hand appendages not represented in the arms.
" 2. Adult pluteus. Ventral view. Camera B. B., eye-piece 2, Zeiss. Reduced about one third in photography. Right-hand rods not represented.
" 3–17. Stages in the development of the young sea-urchin. All drawn with Camera D. D., eye-piece 2, Zeiss. Reduced one third. All from dorsal side of the pluteus, except Fig. 16.
" 3. Young sea-urchin with five ambulacral tubes, *wt*, and a single external opening, *ma*. Formed on the left-hand water-tube. *e*, in Fig. 1.
" 4. The same, older.
" 5. Still older stage, slightly elevated from the wall of the stomach of the pluteus, which it closely hugs in Figs. 3, 4. Pigment spots of dendritic shape have appeared. A spherical calcareous body is seen at *pl*.
" 6. The same, older.
" 7. Older stage with five ambulacral tubes, seen in profile, an external opening, *ma*, and two trifid limestone formations, *pl*.
" 8. The same, older.

Fig. 9. The same, still older.

" 10. An older stage, with numerous limestone bodies of dendritic shapes.

" 11. An older condition of the sea-urchin, in which the ambulacral tubes have developed very considerably, and the dendritic calcareous body is enclosed in a transparent "cell," *pl*, resembling "Hohlkehlen," described by Johannes Müller.

" 12, 13. Still older stages, similar to Fig. 11.

" 14. A young stage, in which the pluteus is so twisted that a central body, *c pl*, "centrale," and five peripheral bodies, *pl*, are shown. At *am* are the ambulacral tubes. The view is at right angles to that of Figs. 3-13. *ma* is turned out of sight. The bodies, *pl*, may be five radial water-tubes.

" 15. A sea-urchin of about the same age as Fig. 13, showing its relation to the anal rods, *rd*, of the pluteus.

" 16. View of a young sea-urchin from the ventral? side, submitted to slight pressure. At *c pl* there is a central plate without calcareous deposits. Around this plate is a ring of five polyhedral plates, in which, *pl*, there is a deeper stellate calcareous system, "stellate cells," and superficial, "lace-work cells or rods." If this is a ventral view, and we are looking at the plates from below, the stellate rods would be superficially placed on the test, and the "lace-work" rods more profound. The lace-work of rods would then be the beginning of the plates of the test of the sea-urchin.

" 17. A sea-urchin older than the last, with attachment to its pluteus.

" 18. The mouth and adjacent region of the anterior rods of a pluteus of about the same age as Fig. 2, showing the muscular fibres at the end of the dotted line without letters. The antero-internal rods are moved in part by these muscles. Free-hand drawing.

PLATE VIII.

Fig. 1. View of the surface of the pluteus between the posterior rods, *pr*, and the anterior rod, showing a structure similar to the so-called loop, *t*, described by A. Agassiz in Strongylocentrotus.

" 2. The same "loop' with the external opening partially closed, and the whole structure more retort-shaped.

" 3. Relation of the "loop"-like structure to the posterior rod, *pr*.

" 4. The relation of the same structure, "loop," to the water-tube, *wt*.

" 5. A view of the same very much reduced in size, with the orifice almost closed.

" 6. The arrangement of the calcareous rods in the body of the pluteus. Soft parts removed. Lateral view. Anal pole at left of the sketch.

" 7. The relation of the infolded part of the external surface to the water-tube.

" 8. A well formed sea-urchin with spines. Dorsal view of pluteus. Arms of pluteus removed.

" 9. An older sea-urchin. Ventral view. Arms of pluteus removed.

Fig. 10. Lateral view of a sea-urchin of about the same age, showing alternating ambulacral tubes and spines.

" 11. A young sea-urchin, under slight pressure, showing a central and five peripheral plates in its apical region.

" 12. A young sea-urchin before the absorption of the pluteus, showing two ambulacral zones. Oral view.

" 13. Three contiguous plates from a very young sea-urchin, showing characteristic double calcification.

" 14. A young Echinarachnius raised from a pluteus. The sea-urchin has absorbed all the soft parts of the pluteus. Three limestone rods, *spi*, still remain unabsorbed. Lateral view. Oral region below.

" 15. Aboral view of a young Echinarachnius obtained by dredging.

" 16. Oral view of an older stage also obtained by dredging. The animal from which Figs. 15 and 16 were drawn do not differ much in size.

Plate I.

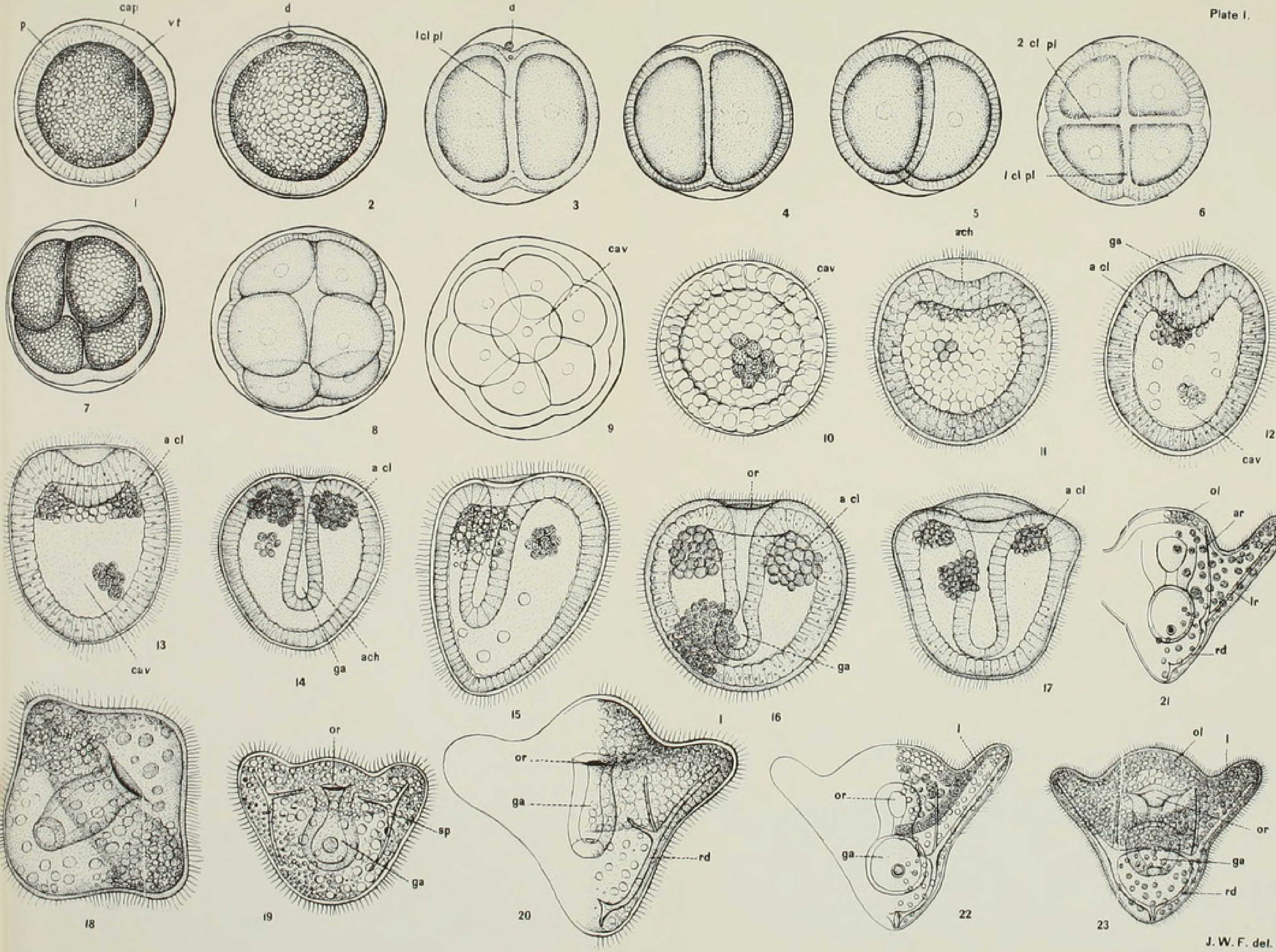

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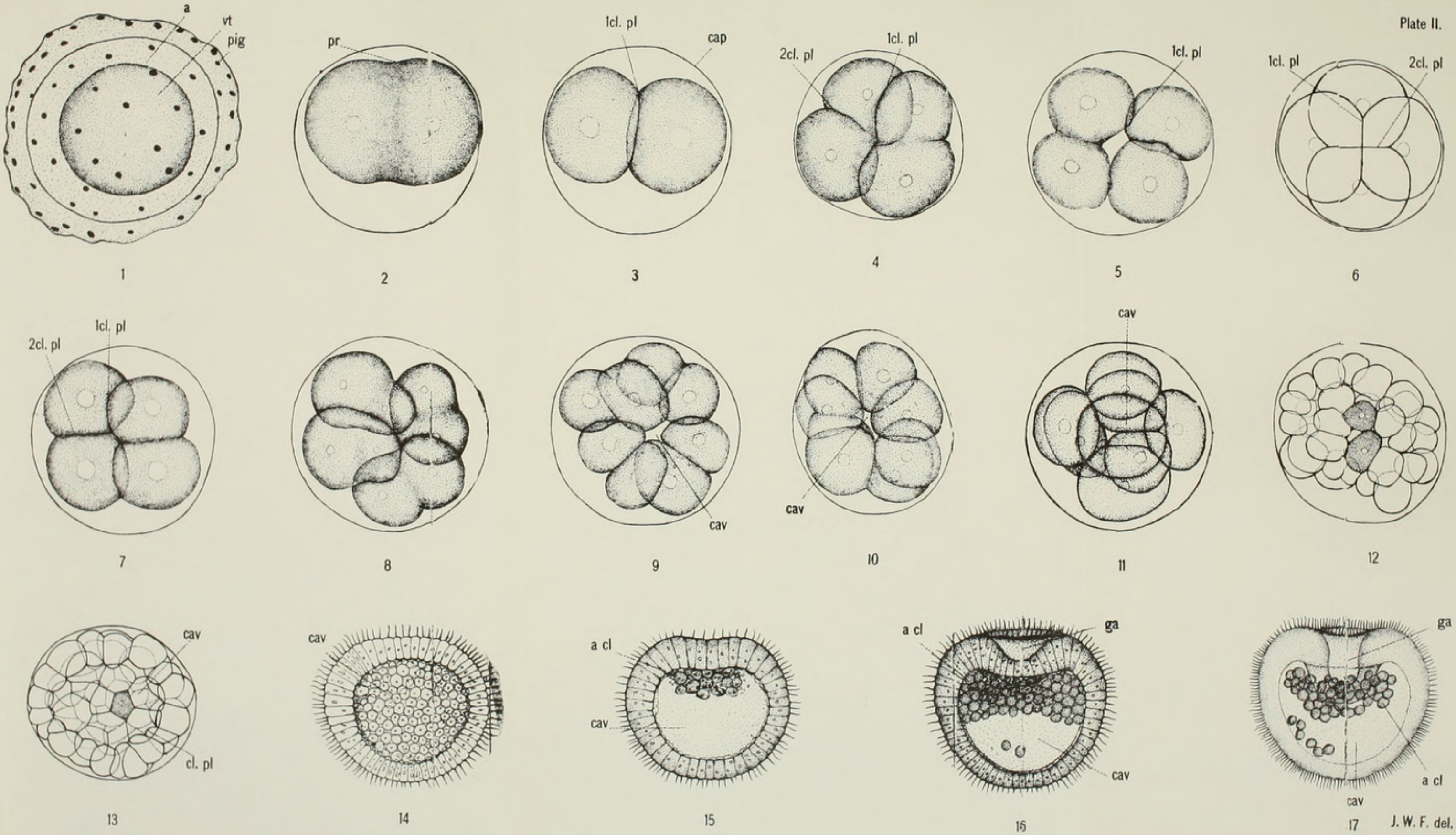
Plate II.



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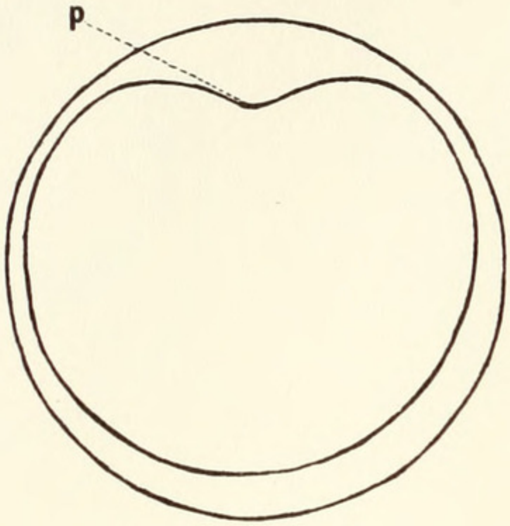

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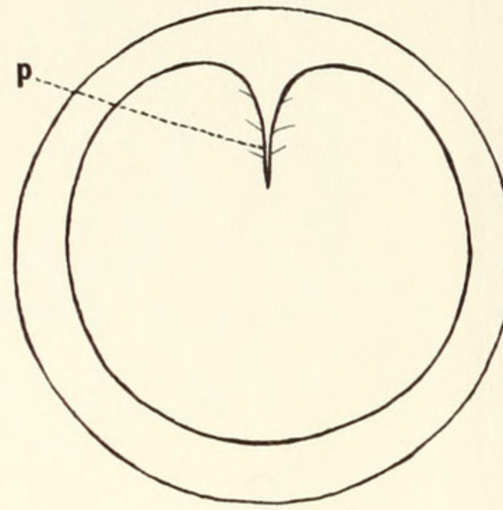

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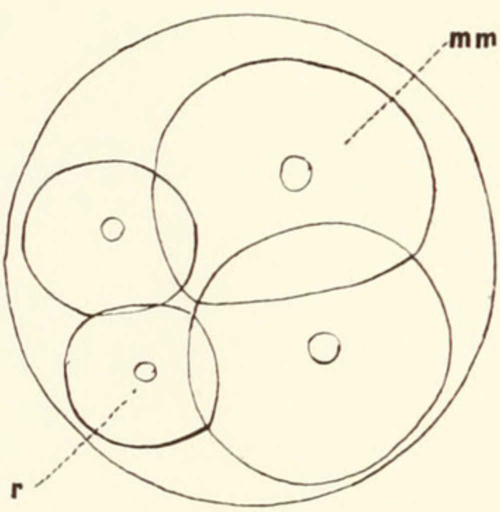

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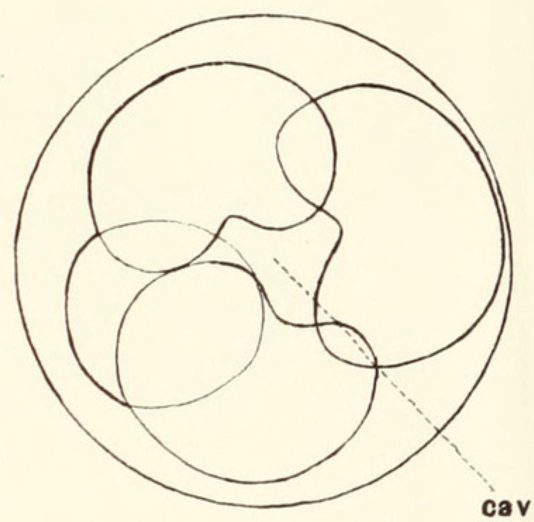

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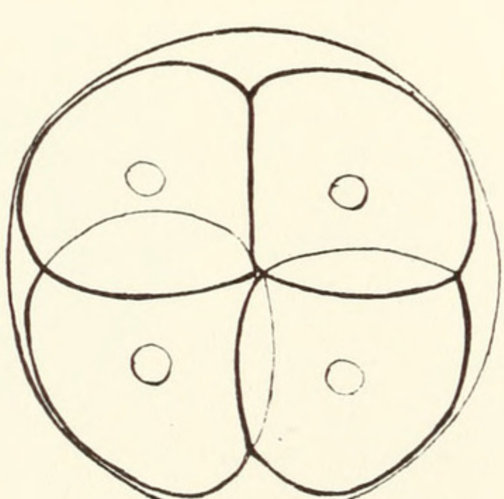

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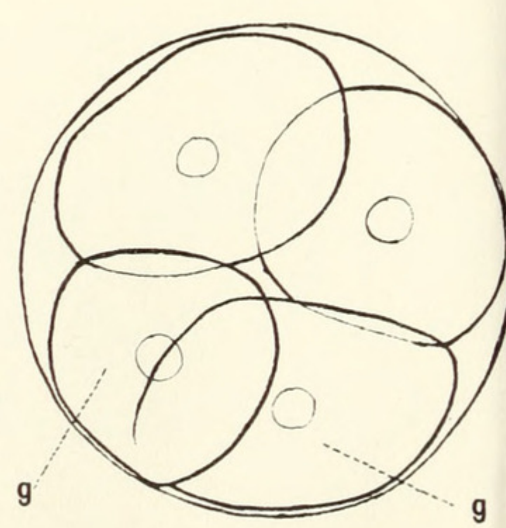

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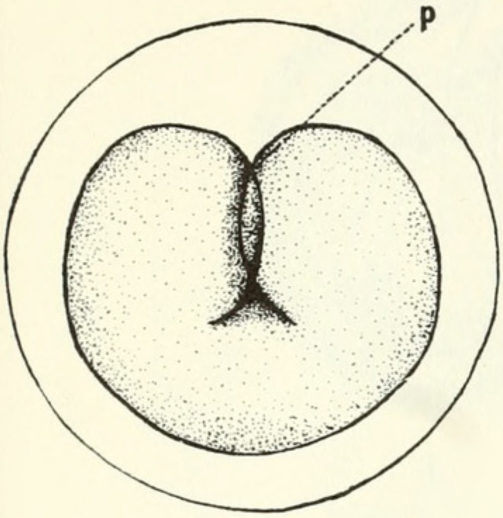

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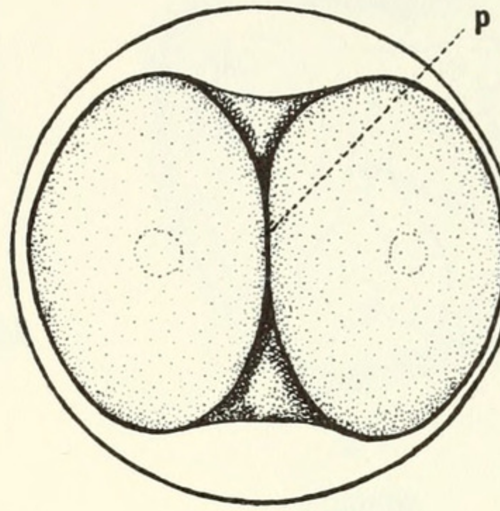

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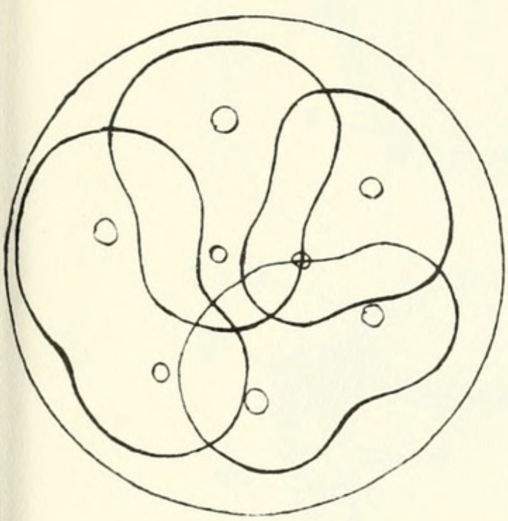

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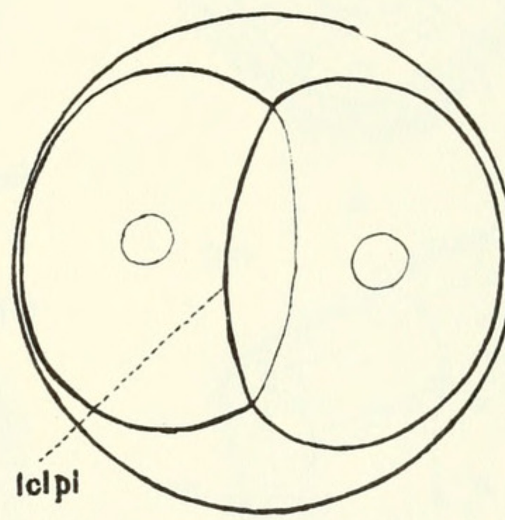

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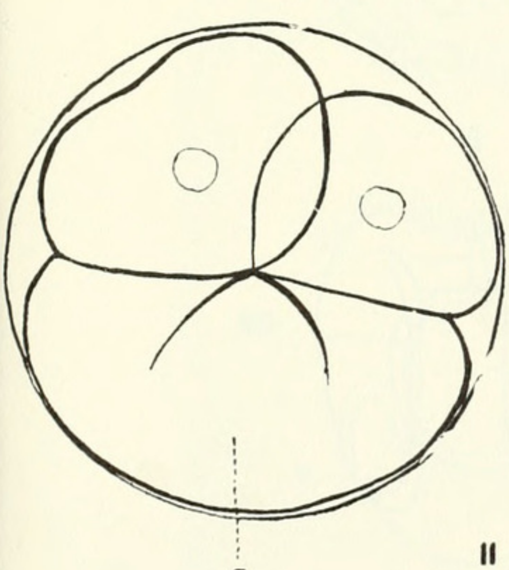

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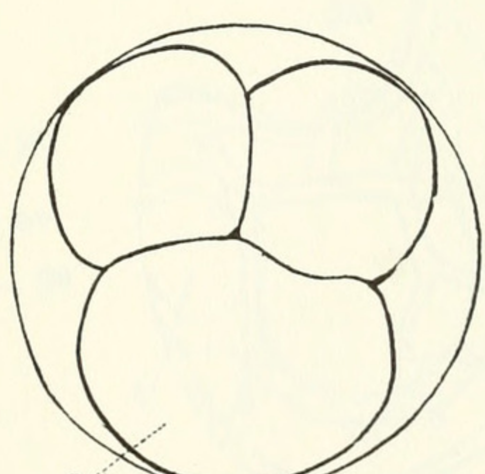

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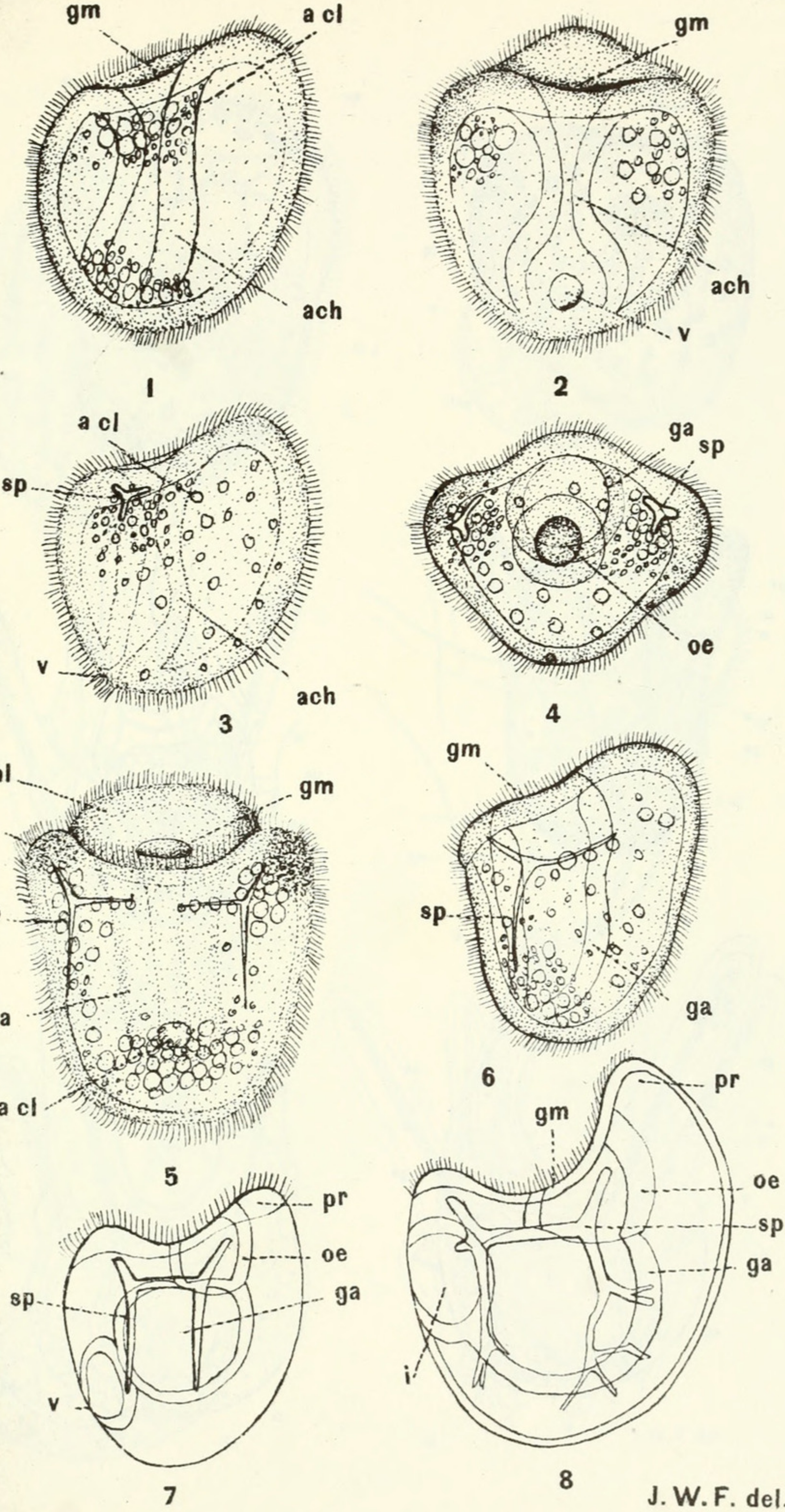
Plate IV.



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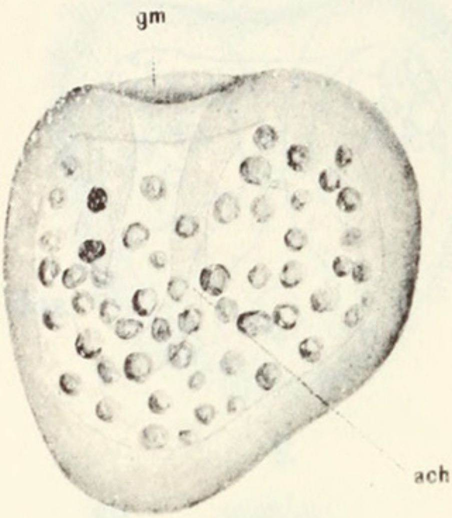
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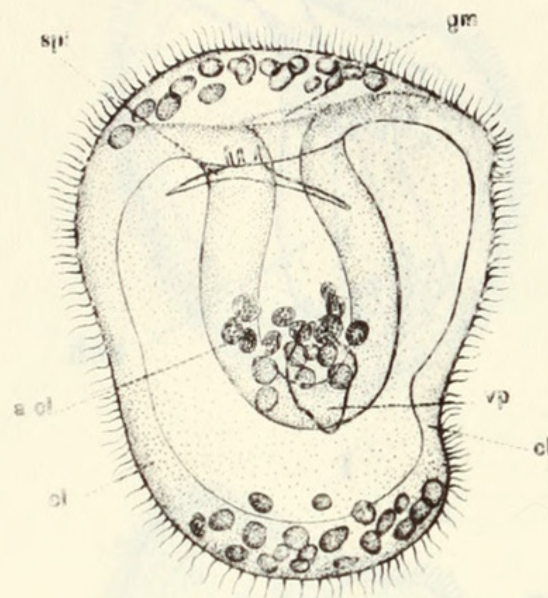
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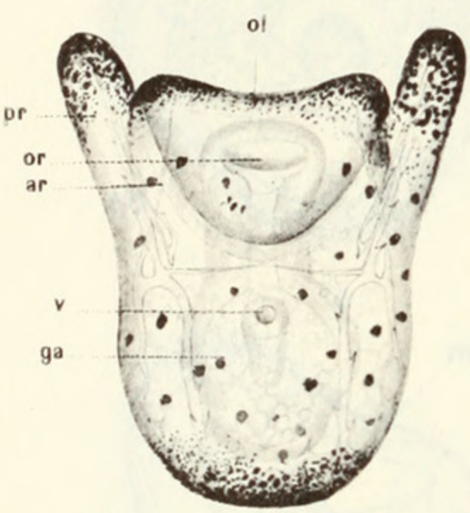
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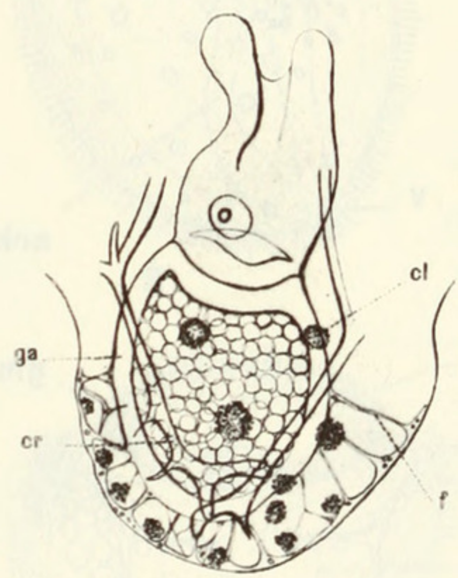
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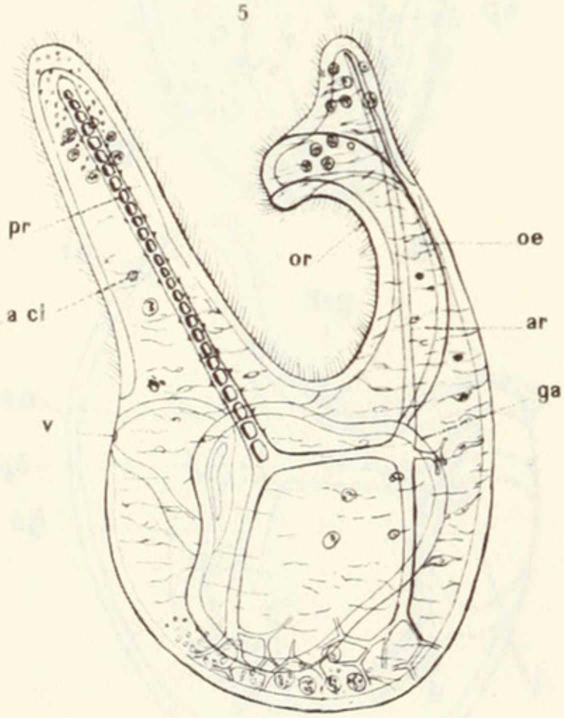
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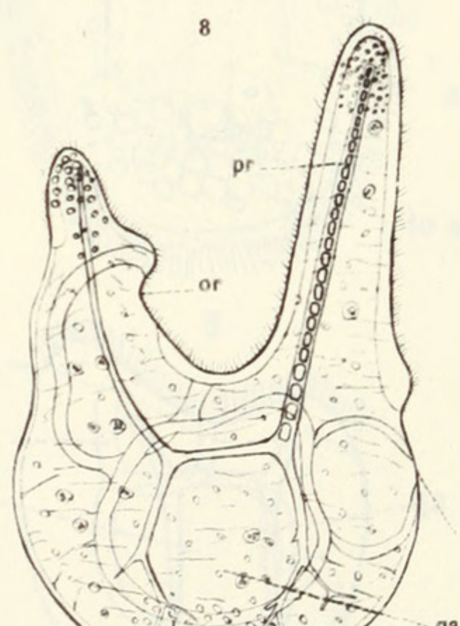
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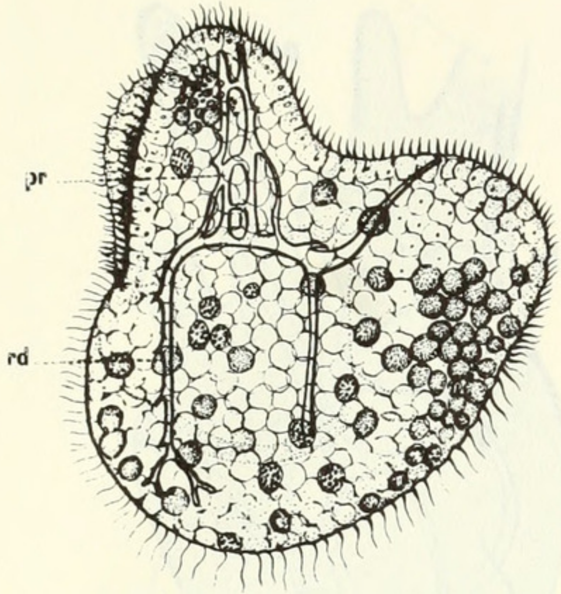

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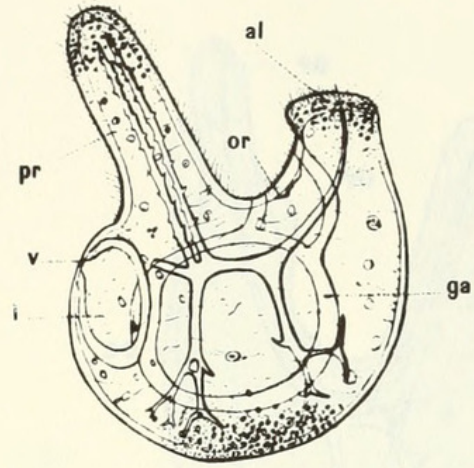

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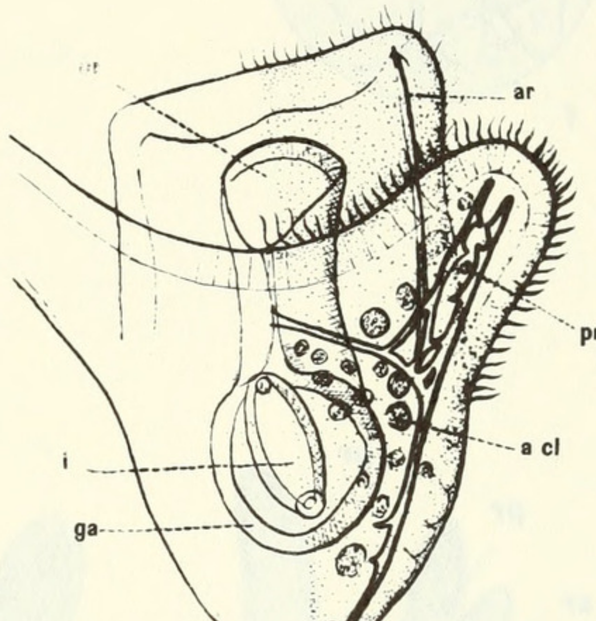

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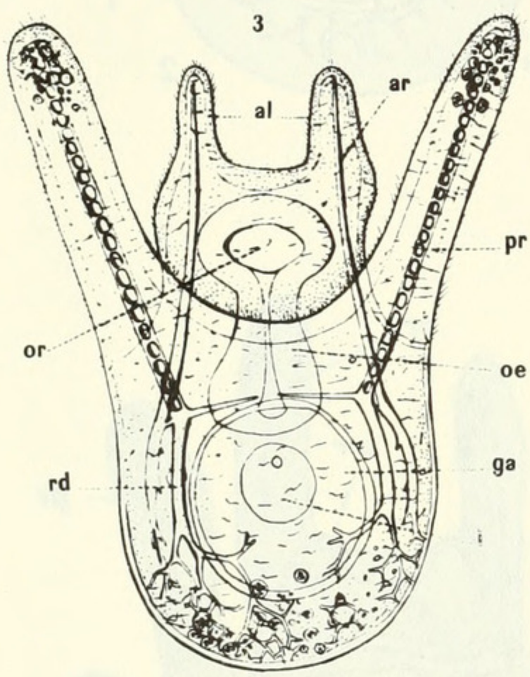

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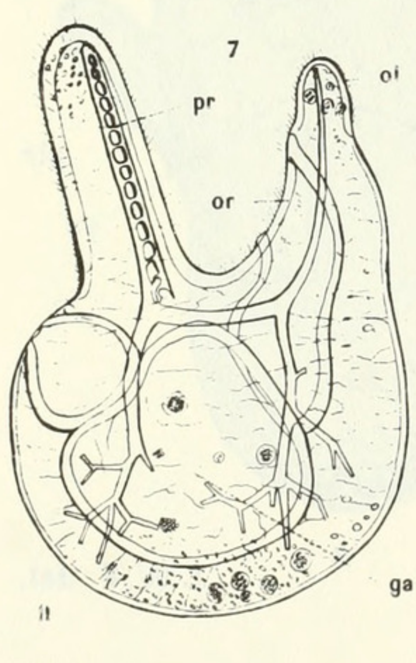

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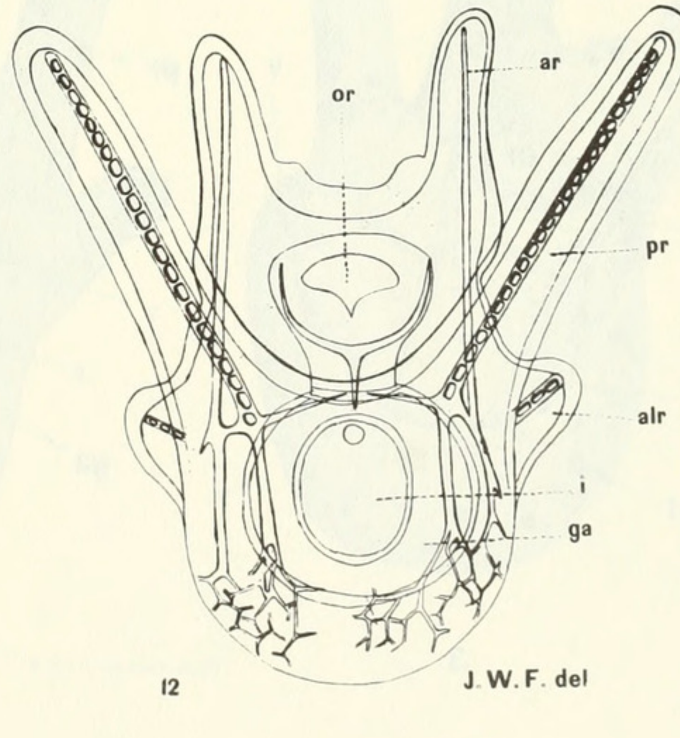

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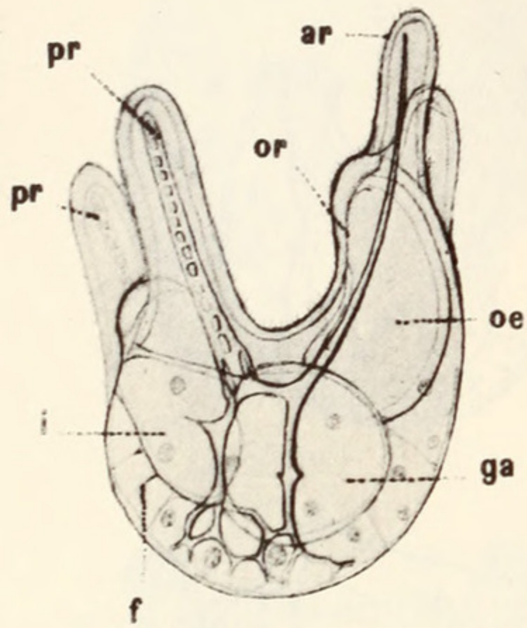
Plate VI.



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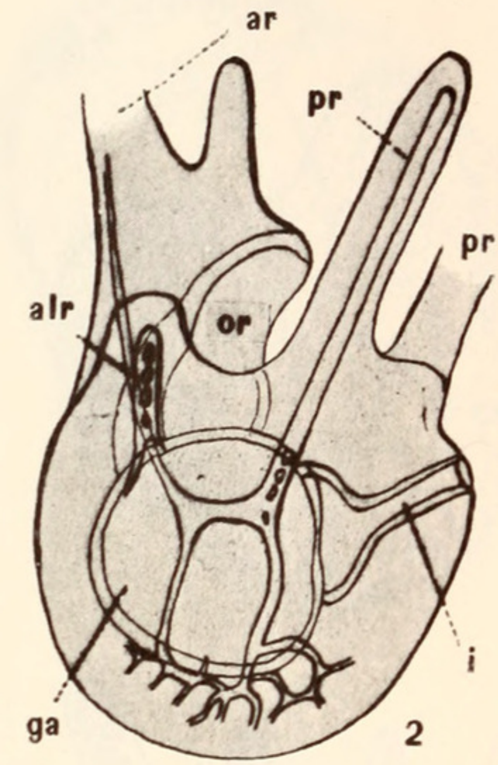

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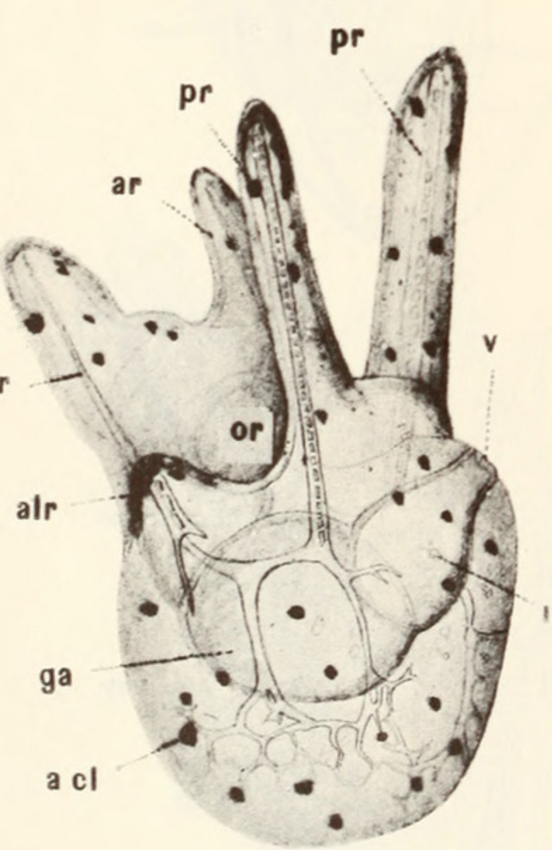

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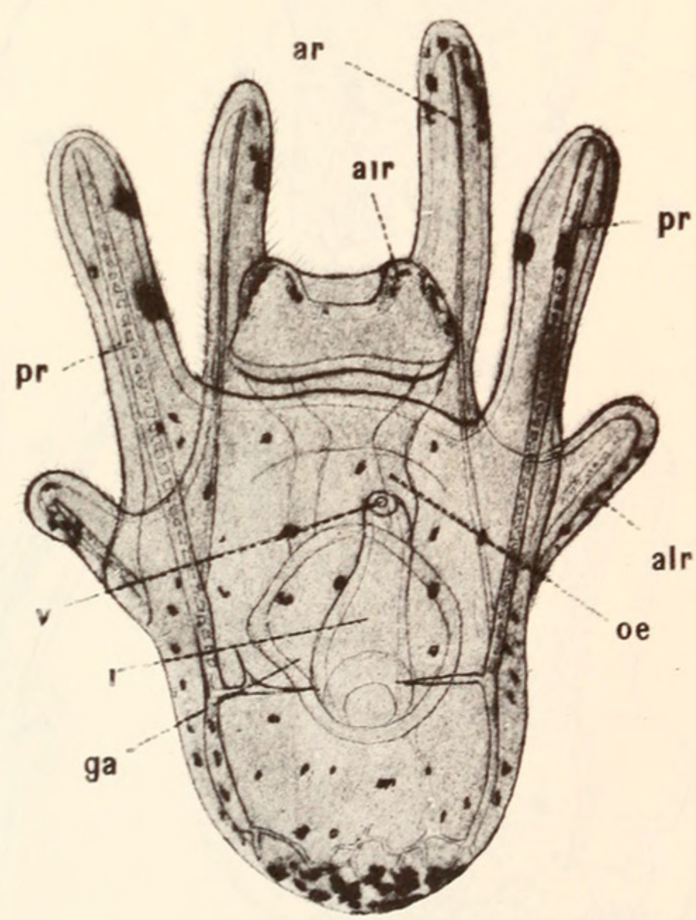

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Plate VII.

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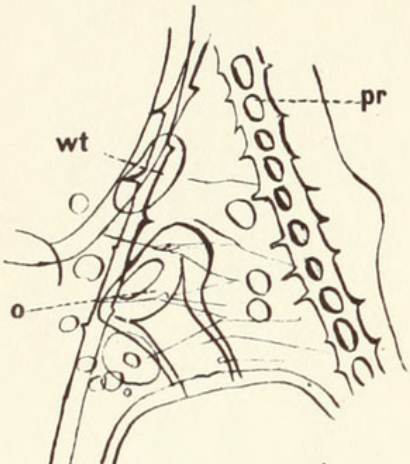

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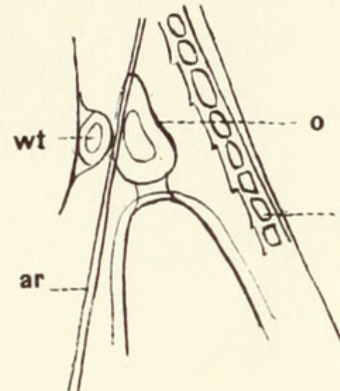

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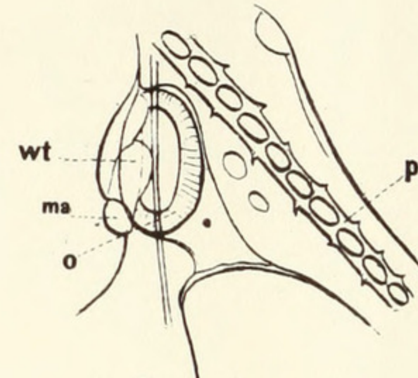

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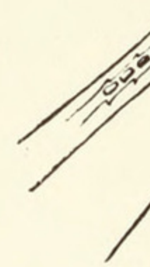

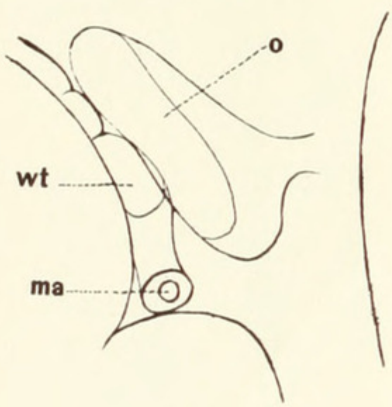

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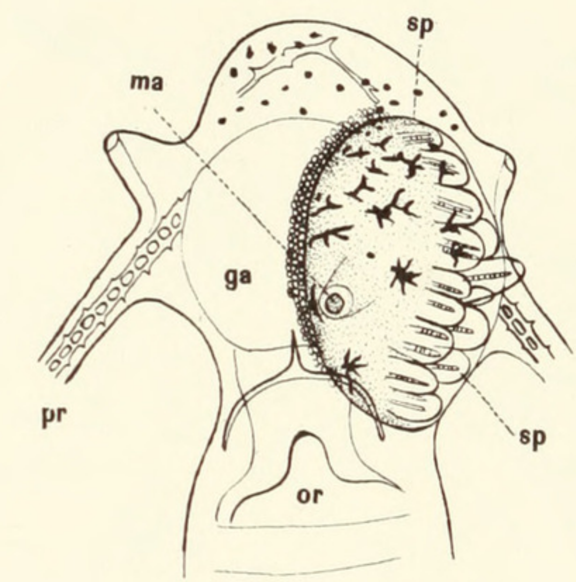

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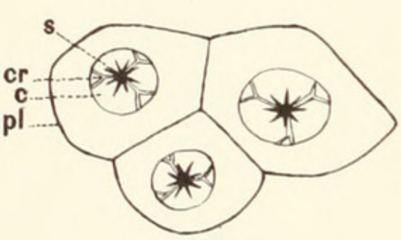

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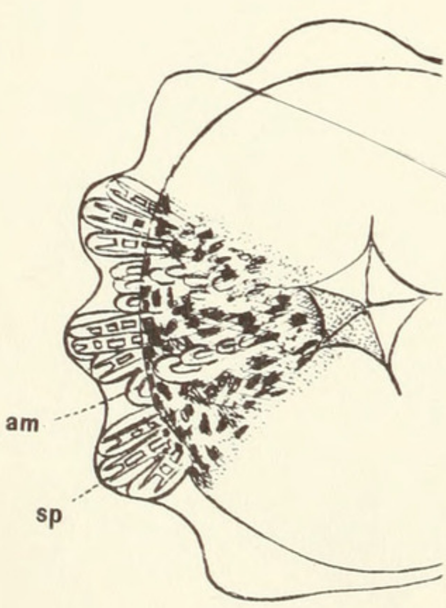

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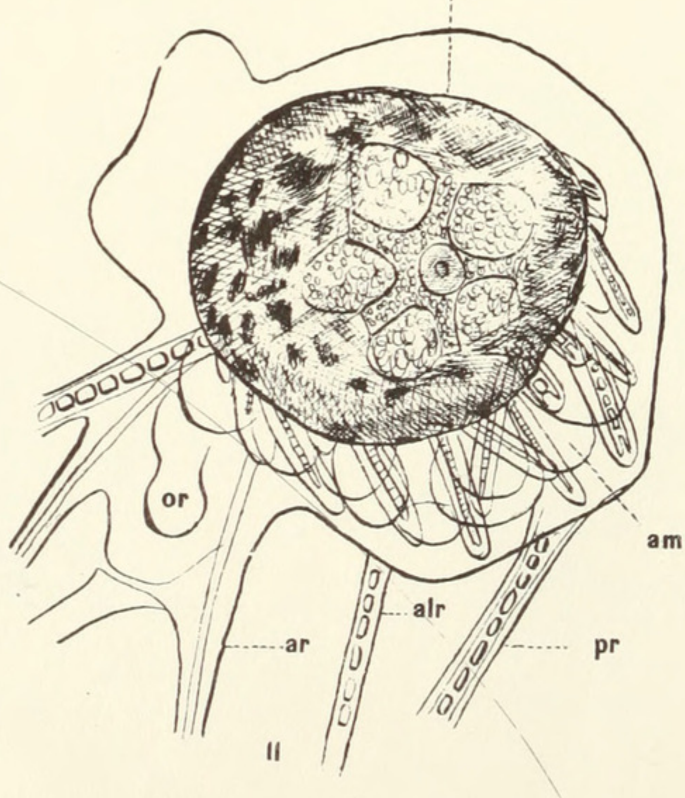

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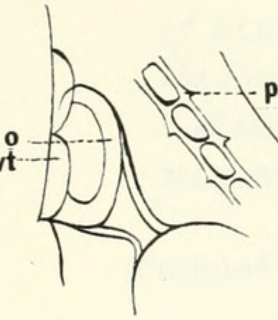

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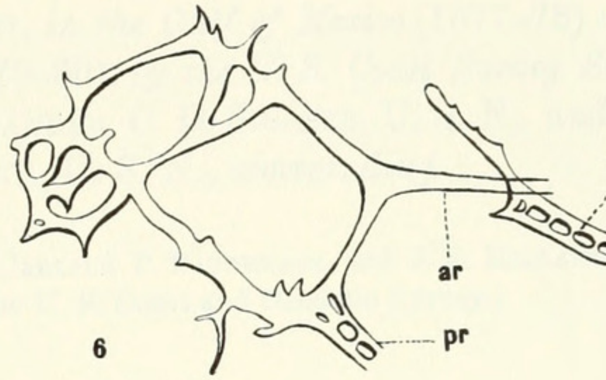

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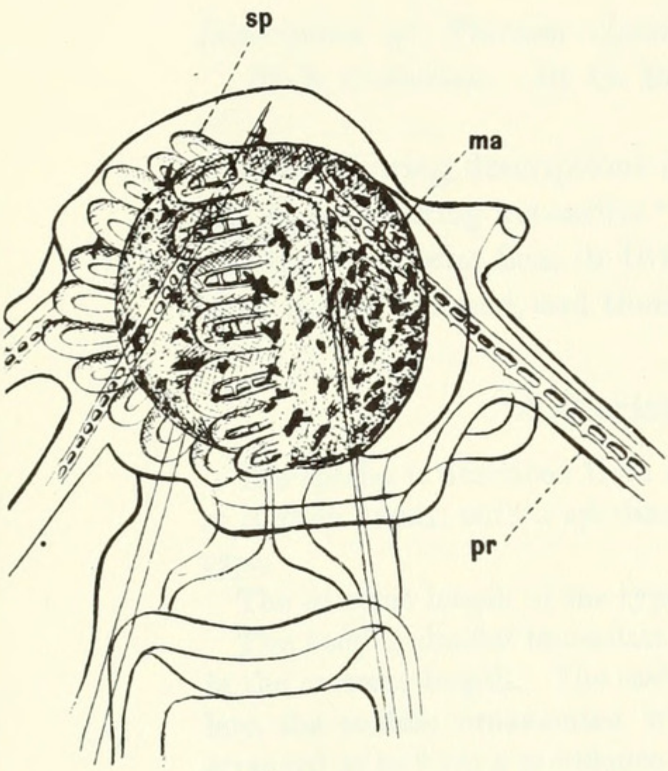

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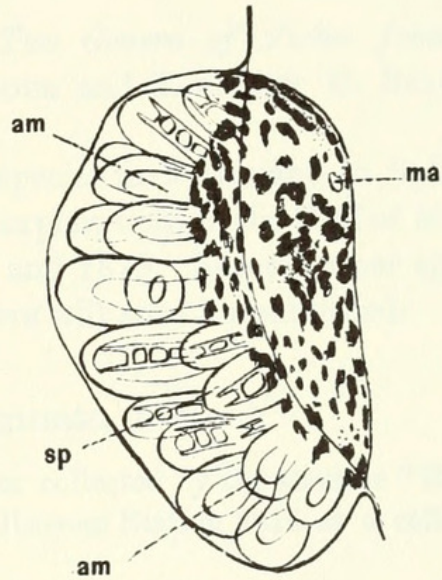

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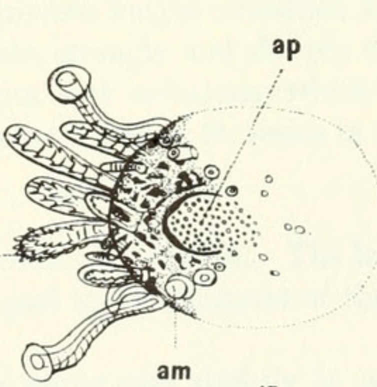

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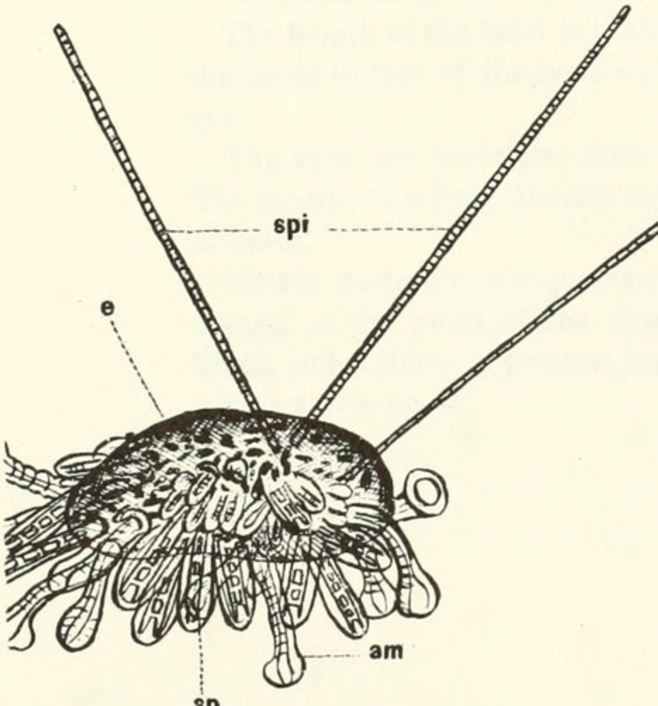

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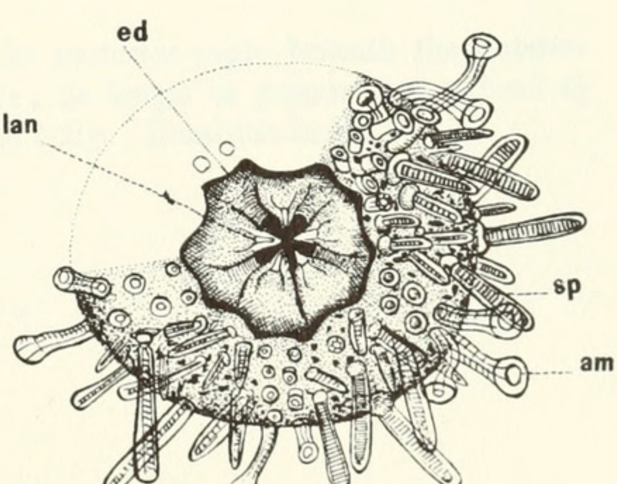

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