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Notes on the Development of Heliocidaris Crassispina with Special Reference to the Structure of the Larval Body

By

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With Plates V-VII and 26 Text-figures

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Contents

Introduction

In the summer of 1927, at the suggestion of Prof. Taku KOMAI, I undertook the investigation of the larval forms of sea-urchins found in the neighborhood of the Seto Marine Biological Laboratory, including *Heliocidaris crassispina* (*A. Ag.*) which is one of the commonest sea-urchins in the waters around Japan. Early in May of the next year, I went to the Tomioka Marine Biological Laboratory of the

Kyushu Imperial University to renew my study with the same species as material. There I succeeded in rearing the larvae through the metamorphosis, the time taken from insemination to the formation of the young sea-urchin being altogether 37 days.

This species of sea-urchin is one of the favorite materials for the laboratory practice of embryology in Japan. However, so far as I know, no one has ever reared the pluteus to metamorphosis, and MORTENSEN's paper in 1921 in which he records the change up to the formation of the first pedicellaria, is the only systematic description of the development of this sea-urchin. Under such circumstances, it may not be improper to publish my findings at some length, although they are in large measure little more than a verification of the accounts given by previous authors on other species of sea-urchins, especially that given in GORDON's excellent paper on *Echinus miliaris* and *Echinocardium cordatum*.

Before proceeding any further, I wish to express my hearty thanks to Prof. Taku KOMAI under whose suggestion and guidance my work was done and to Prof. Hiroshi OSHIMA, to whom I owe not only the privilege of working in the Laboratory under his charge, but also the facilities of access to important literature and many valuable suggestions. My acknowledgments are also due to Mr. Yoshiyuki HIRAIWA of the Tomioka Laboratory and Asst. Prof. Kozo AKATSUKA and Mr. Jiro IKARI of the Seto Laboratory, who gave me every help I needed during my stay.

Methods

All the instruments used for rearing the larvae were sterilized with boiling water. The vessels used for culture were of the capacity of 2 litres. Before being cut open, each material was immersed in running fresh water to destroy any sperm which might be adhering to the outside of the test. To obtain eggs, pieces of ovary were filtered through gauze, or eggs were isolated by means of a pipette. The water containing the fertilized eggs was changed from time to time until the blastulae began to swim. This was apparently indispensable for keeping the culture in good condition. The blastulae were transferred to other vessels to avoid overcrowding.

About one third of the water in the vessel was changed every day. To avoid a sudden change of temperature, the water to be used for the culture was kept at the room temperature for one day beforehand. The temperature of the water in the vessel varied from 20°

to 25° C., which was usually ca. 3° C. higher than the outside sea-water. The water was also stirred from time to time with a glass rod.

Each stage was carefully compared with the corresponding stage found in plankton in order to ensure a normal course of development even under cultural conditions.

In older larvae it is rather difficult to observe the internal structure on account of the opacity of the body. To make fixed material transparent, it was transferred into a dilute solution of NaOH, and then to distilled water, and sealed in glycerin. Care must be taken not to leave the material too long in NaOH, because the calcareous elements are very liable to be disintegrated in the medium.

The time required for the developmental changes after insemination was approximately as follows:

The beginning of gastrulation	16	hours.
The appearance of triradiate spicule	20	,,
Four-armed larva (full size)	8	days.
Six-armed larve (fairly advanced stage)	11	,,
Eight-armed larva (mature stage)	15	,,
The appearance of the rudiment of first pedicellaria	17	,,
The appearance of first larval spine	29	,,
The appearance of primary tube feet	33	,,
Metamorphosis completed	37	,,
The appearance of five paired tube feet	39	,,

PART I. DEVELOPMENT OF PLUTEUS

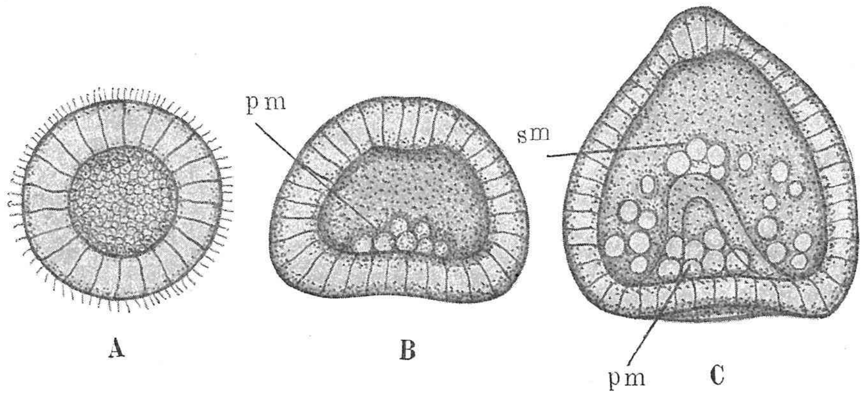
Section 1. Stages from Fertilization to Gastrula

The eggs are small, ca. 0.09mm. in diameter, quite opaque owing to the presence of greenish yolk granules which hinder the observation; from about the time of the beginning of the formation of primary mesenchyme cells, however, the blastula gradually becomes transparent.

The first cleavage takes place about one hour after insemination. The cleavage is total and regular.

The blastula is at first spherical, and with a small blastocoel, surrounded by a rather thick wall made up of small polygonal cells, and the whole surface is covered uniformly by a coat of cilia (Text-fig. 1, A).

In about 8 hours from insemination, the blastula starts to swim. The direction of locomotion is perpendicular to the antero-posterior axis

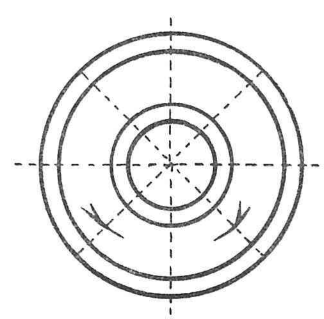


Text-fig. 1.

A. Blastula.
B. Gastrula, showing the primary mesenchyme cells.
C. Gastrula, showing the arrangement of the primary and secondary mesenchyme cells.
pm.—primary mesenchyme cells.
sm.—secondary mesenchyme cells. (300/1).

of the body, often taking a spiral course. Gradually, the posterior portion of the wall becomes thickened, and then flattened, whence some primary mesenchyme cells begin to be budded off into the blastocoel (B, *pm*).

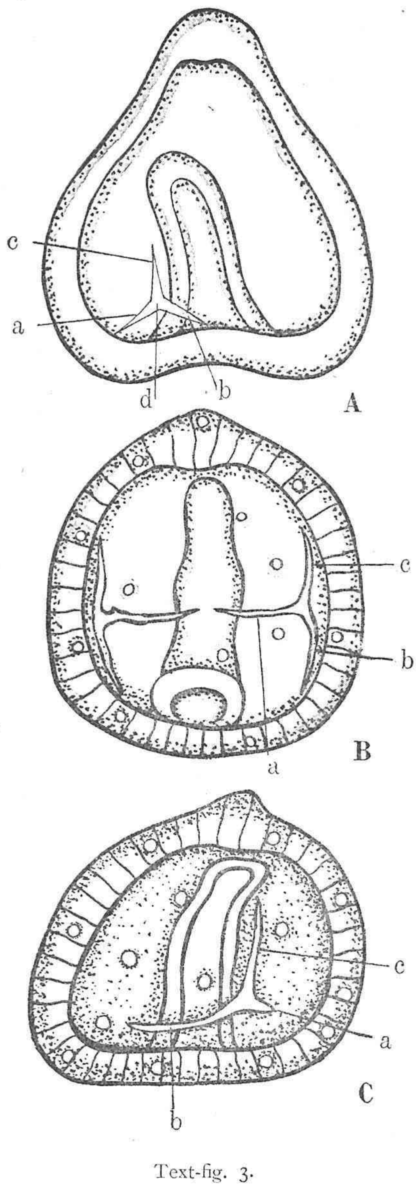
Gastrulation begins in about 16 hours after insemination. The archenteric invagination gradually bends towards one side, and marks out the ventral side of the gastrula. The primary mesenchyme cells are divided into two groups, located on each side of the base of the archenteron, which has become more elongated in the meantime (C). The secondary mesenchyme cells are budded off from the tip of the archenteron shortly afterwards (*sm*). The gastrula gradually changes its external form and assumes a conical shape, with a slightly thickened animal pole (C).



Text-fig. 2.

Diagrammatical gastrula, viewed from animal pole, showing the position of triradiate spicule. 265/1.

In each group of primary mesenchyme cells a small characteristic triradiate spicule makes its appearance in about 20 hours after insemination.



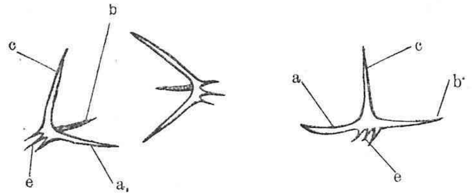
Text-fig. 3.

A. Left-side view of gastrula, showing the position of triradiate spicule.
B. Gastrula, ventral view, circles show pigment cells.
C. Gastrula, right-side view.
a. —rudiment of the ventral transverse rod.
b. —rudiment of the body rod.
c. – rudiment of the dorso-ventral connecting rod. 300/1.

These spicules are situated nearly on the interradial plane on the ventral side, as shown in Text-fig. 2. Thus the embryo becomes distinctly bilaterally symmetrical in form. For convenience, I shall call these rays of the triradiate spicule *a*, *b* and *c* respectively (Text-fig. 3, A, *a*, *b* and *c*). Ray *a* is directed to the ventral side, and ray *b* to the dorsal side, the line connecting the tips of these rays being nearly parallel to the posterior plane of the gastrula, while ray *c* runs upwards to the anterior pole. All the three rays are somewhat inclined towards the inner side, so that they meet one another at an angle of 60 degrees. With the growth of the rays, rays *a* and *b* become almost parallel to the posterior plane, so that both the angles *a*, *d*, *c* and *b*, *d*, *c* are gradually converted into right angles. Ray *a* becomes parallel also to the ventral side with development of the larva, and finally meets its fellow on the median plane of the embryo (B and C). This ray *a* gives rise to the ventral transverse rod, and ray *b* to the body rod, while *c* becomes the dorso-ventral connecting rod.

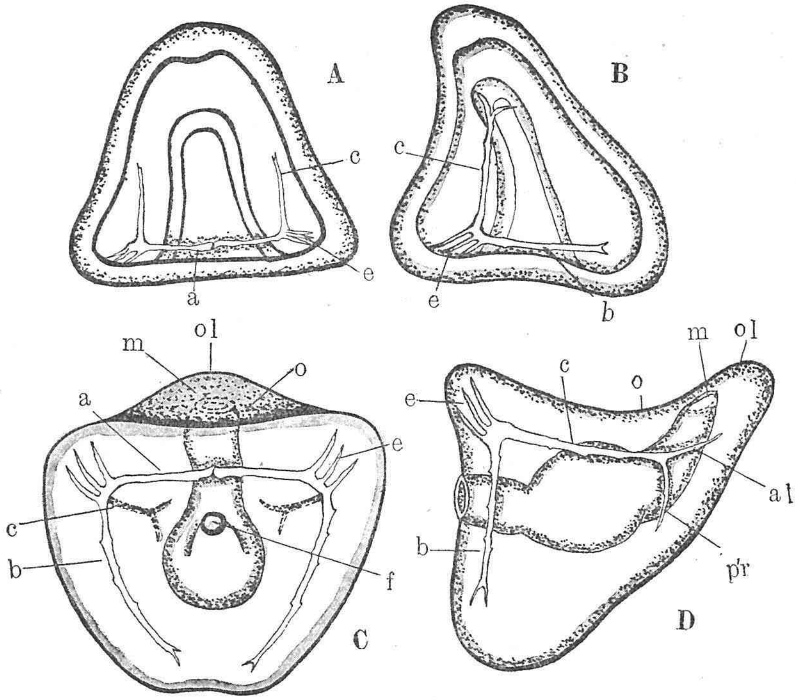
In about 25 hours after insemination, pigment cells make their appearance in the ectoderm.

In a more advanced stage, three processes directed laterally in parallel rows, are given out from the point of junction of the three rays (Text-fig. 4, *e*). These processes as they develope incline gradually towards the ventral side and also slightly to the posterior side. In the fully-advanced pluteus stage these processes become longer than all the



Text-fig. 4.

Triradiate spicules with the rudiment of the postoral rod.
e.—rudiment of the postoral rod. 135/1.



Text-fig. 5.

A. Gastrula, ventral view, showing the junction of the ventral transverse rods.
B. Gastrula, left-side view.
C. Prism-like larva, ventral view.
D. Prism-like larva, left-side view.
al. — antero-lateral rod.
f. — anus.
m. — mouth.
o. — oral field.
ol. — oral lobe.
p'r.— primary recurrent rod.
Further as in preceding figs. 300/1

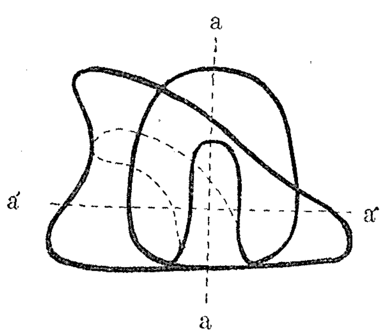
rays, owing to the very rapid growth, and develop into the fenestrated (latticed) rods. This is the so-called postoral rod.

The change in form of the larval body in the mean time may now be described. As time progresses, owing to the rapid development of the skeletal elements, especially to the elongation of the postoral rods, the posterior region of the larva becomes much broader and the dorsal side more rounded than before, so that, in general appearance, the larva of this stage looks helmet-shaped in the ventral view and D-shaped in the left-side view (Text-fig. 5, A and B).

The archenteron continues to grow, until its apical end comes in contact with the antero-ventral end of the gastrula, whence develops the stomodaeal invagination.

Section 2. The First (Young) Stage of Pluteus.

In about two days after insemination, the mouth is formed on the ventral side near the anterior end and the digestive tract is completed. The tract is sectioned into three parts by two slight constrictions. On the surface an adoral ciliated band becomes conspicuous. The dorso-ventral axis shortens, and there appears on the ventral side of the body a concavity, the oral field (C and D, *o*). At the same time, the postero-dorsal region stretches out strongly backwards and becomes the posterior end of the pluteus. As is clear from the above description, the posterior side of the gastrula becomes the ventral side of the pluteus and the ventral side of the former forms the oral field of the latter, while the protruded anterior portion of the oral field becomes the oral lobe (C and D, *ol*).

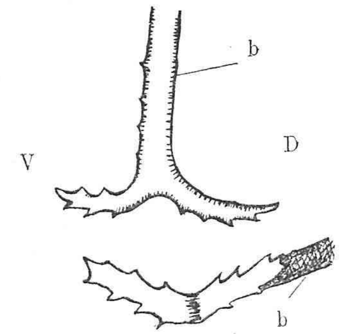


Text-fig. 6.

Diagram, showing the relation of longest (principal) axes of gastrula and pluteus.
a—a. longest axis of gastrula.
a'—a'. longest axis of pluteus.

Thus, the main axis of the gastrula makes an angle of 90° with the longest axis of the pluteus, although falling in the same bilateral median plane of the larval body (Text-fig. 6, a–a and a'–a').

By the close of the second day, the prism-like larva as presented in Text-fig. 5, C and D is formed. In the mean time, the oral field has been encircled with the ciliated band. The postoral rod (C, *c*)

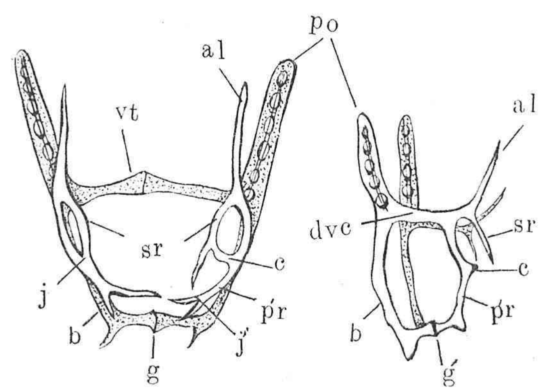


Text-fig. 7.

Two serrated branches at the end of the body rod.
b. — body rod. V.—ventral side.
D.— dorsal side. 600/1.

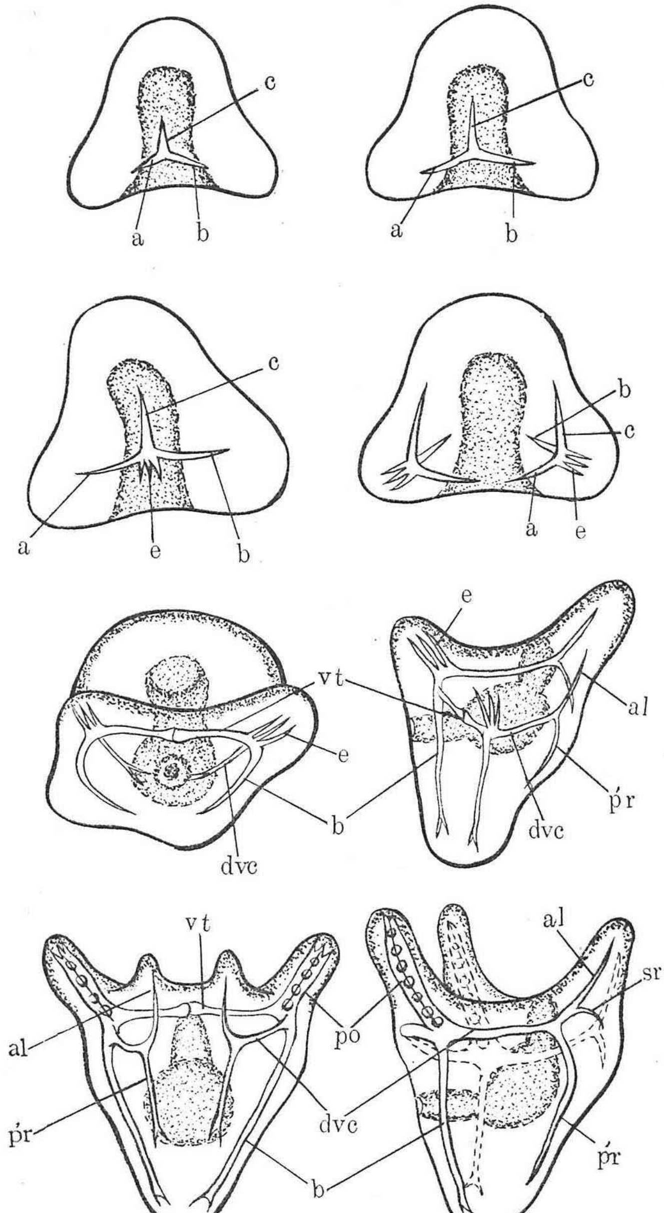
has grown anteriorly, the body rod (*b*) posteriorly and the dorso-ventral connecting rod (*c*) dorsally. A little later a two-armed pluteus larva is formed (Pl. 1, fig. 1). The general outline of the body described above is now almost gone. Already, the postoral rod presents the latticed form ; the rod is armed with short thorns, more on the outer side than on the inner. The body rod which is short and coarsely spinous terminates in two serrated branches (Text-fig. 7), of which one branch is connected with its fellow on the other side at the ventral side of the body (Text-fig. 8, g), while the other joins with a branch from the primary recurrent rod on the lateral side (g′).

The dorso-ventral connecting rod (*dvc*) which arises at the junction of the postoral and the body rod soon gives off a branch ; this branch stretches posteriorly along the dorsal wall to form the primary recurrent rod (*p′r*). A little later, the dorso-ventral connecting rod again furcates into two branches, of which one stretches anteriorly to form the antero-lateral rod (*al*), while the other is directed posteriorly along the primary recurrent rod and forms the secondary recurrent



Text-fig. 8.

Skeleton of pluteus in early stage.
vt. — ventral transverse rod.
dvc. — dorso-ventral connecting rod.
po. — postoral rod.
al. — antero-lateral rod.
sr. — secondary recurrent rod.
p′r. — primary recurrent rod.
c. — process from the primary recurrent rod.
g. — point of junction of the branch from the end of body rod with its fellow.
g′. — point of junction of the branch from the end of body rod with primary rec. rod.
j. j′. — points of junction of the secondary rec. rods with the primary rec. rods. 200/1.



Text fig. 9.

Successive stages of development of the skeleton from gastrula to pluteus. Legends as in preceding figs. 170/1.

rod (*sr*). The secondary recurrent rod joins with the primary recurrent rod at the point of curvature of the latter, but sometimes at a point proximal to a point of curvature (*j* and *j'*). Further connection between the primary and the secondary recurrent rod is realized by a process arising from the former usually on one side or sometimes on both sides of the body (*c*).

The stomach is yellowish in color; pigment cells are aggregated at the tip of each arm; the region surrounding the anal aperture is protruded (Pl. I, fig. 3). The posterior portion of the body rod is serrated densely. The preoral lobe has a distinct ectodermal thickening on the margin, and the postoral ciliated band is sectioned into three slightly concave portions.

The pluteus in the first stage reaches its maximum size which is 0.7 mm. including the arms, and the postoral rod becomes about five times as long as the body rod.

Section 3. The Second Stage of Pluteus.

By the 8th day after insemination, the pluteus reaches the size of 0.8 mm. including the arms, and there appear a pair of new centers of calcification on the posterior side of the base of the postoral rod. In each of these centers arise three rays, directed laterally, anteriorly and posteriorly respectively. Meanwhile, three processes are added to the side of the laterally-directed process of the above three, and the remaining two rays are turned towards the median line of the pluteus diagonally, so that the whole structure assumes a comb-like appearance as shown in Pl. I, fig. 6 (*pd'*).

By the 11th day after insemination the additional three processes develop into a latticed rod just as is the case with the postoral rod, and forms the postero-dorsal rod (fig. 8, *pd*). Meanwhile the rod which is directed diagonally posteriorly, gives off a branch which intersects with the corresponding branch from the opposite side and terminates freely. This branch is the dorsal transverse rod (*dt*). The other two rays, one anteriorly directed and the other laterally directed, on the other hand, remain short; the latter ray is inclined somewhat posteriorly.

In the meantime another calcareous center has appeared at the base of the stomodaeum on the dorsal side of the pluteus. This center develops an unpaired triradiate spicule which is the rudiment of the dorsal arch (fig. 6, *da*). At about this time the tip of the ventral transverse rod, which has been closely fitted to the tip of its fellow something like a pair of clasping hands on the median line of the ventral side, shows signs of intersection with its fellow (*vt*).

The end of the body rod begins to be absorbed; this is the first indication of the destruction of the larval skeleton (fig. 8).

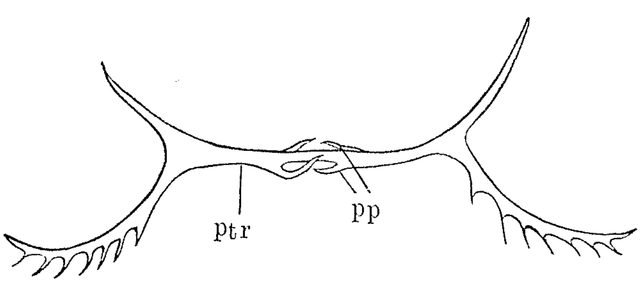
Section 4. The Third Stage of Pluteus.

This stage is characterized by the formation of the preoral arms (Pl. 2, fig. 2, *pr*). The preoral arm rods are given rise to by the elongation of the anteriorly-directed two rays of the dorsal arch along each side of the oral area, while the posterior arm rods remains relatively short.

At the age of 15 days, dating from insemination, the mature pluteus possesses eight arms, and meassures ca. 0.9 mm. including the arms.

The body skeleton begins to be absorbed from the tips of the arms, and component parts of it become separated gradually (fig. 4). The posterior transverse rod, which divides into two branches at either end now appears. One of the branches, the lower branch, which curves laterally, is provided with a series of 6-7 thorns along its lower margin and looks not unlike a comb. The other branch, the upper, which also curves slightly laterally, remains short and simpler than the lower branch (Text-fig. 10). The lower branch mentioned above, supports a pair of small postero-lateral processes (Pl. 2, fig. 4, *plp*) which appear on both sides of the posterior margin of the body.

There are in addition two pairs of small processes arising from points close to the median point of the posterior transverse rod, one on the anterior side, and the other on the posterior; these are the posterior processes (Text-fig. 10, *pp*). The two corresponding processes on the same side join with each other and end in a single process as



Text-fig. 10.

Posterior transverse rod (*ptr*) with posterior processes (pp). 200/1.

development goes on (Pl. I, fig. 7, *pp*). There arise on these processes small thorns, indicating a tendency of the process to ramify.

On the dorsal arch, at some distance from its center, there are small processes (lateral processes) directed laterally and posteriorly (Pl. 2, fig. 2, *lp*). The unpaired posterior prolongation of the dorsal arch runs towards the posterior end, and small thorns which suggest the ramification of the more advanced stage are already seen near its center (fig. 2).

The larva at this time is provided with two pairs of well-developed vibratile lobes, one on the dorsal, and the other on the ventral side, of which the ventral pair develop from the two external sections of the deep trisection of the posterior ciliated band (fig. 3, *vl*), while the dorsal lobes arise from the outgrowth of the ciliated band in the corresponding regions on the dorsal side (*dvl*). On each vibratile lobe there are strong cilia which serve as the principal locomotory organ; the preoral and the posterior ciliated bands are also prominent, each having a sinuation in the middle. The above-stated postero-lateral process is fringed by the ciliated band which arises as the posterior extension of the ciliated portion situated between the postoral and the postero-dorsal arm (fig. 4, *plp*).

In the median region of the posterior part of the larva, there is a deep groove between the postero-lateral processes. Thus, although the set of these ciliated bands of this stage is rather complicated, we can trace them back to the simple band surrounding the oral field of the youngest pluteus.

The pigment cells are arranged so as to form a line bordering the vibratile lobes as well as the postero-lateral processes.

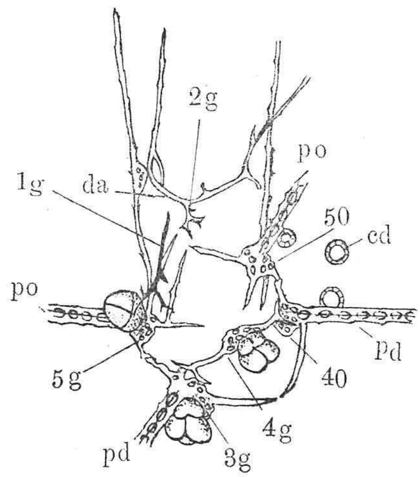
PART II. DEVELOPMENT OF SEA-URCHIN.

Section 1. The Larva in the Beginning of Metamorphosis.

Towards the end of the 17th day after insemination, the larva attains its full size with eight well-developed arms. The meshworks existing in the basal portions of the four main arm-rods as well as in the dorsal arch are gradually brought into existence (Text-fig. 11, 50, 40, *3g* and *5g*).

A pair of small processes which are the rudiment of the lateral process from the dorsal arch make their appearance in this stage from the dorsal arch (Pl. 2, fig. 4, *slp*). The first pedicellaria has appeared in the middle of the posterior end, at the bottom of the median groove (figs. 3 and 4 *1p*). The vibratile lobe has grown in size, and along its dorsal border a new vibratile band appears in the form of a pair of lobes, which are apparently supported by the processes from the dorsal arch (fig. 3, *vb*). At the same time the postero-lateral processes become apparent.

There are green pigment cells along the postoral and postero-dorsal rod and very conspicuous clusters of red pigment cells beneath the postoral ciliated band as well as on the margin of the vibratile lobes on the dorsal side. All the arms of this stage are linear in shape with a slightly broadened basal portion.



Text-fig. 11.

Skeleton viewed from ventral side. Legends as in preceding figs., further: 40 and 50.—ocular plate 4 and 5. 1g, 2g, 3g, 4g and 5g.—genital plate 1, 2, 3, 4 and 5.
cd. — calcareous disc. da.—dorsal arch. pd. — postero-dorsal rod. 50/1.

On the 23rd day after insemination the second pedicellaria makes its appearance; it is situated dorsally at the base of the postero-dorsal arm on the right side of the larva, opposite the echinus rudiment (fig. 5, *2p*). Shortly afterwards, the third pedicellaria appears on the ventral side in a position precisely corresponding with that of the second pedicellaria and at the base of the right postoral arm (*3p*).

Thus, the larva is provided with three pedicellariae, two of which lie on the right side, one dorsally and the other ventrally, the third one being found at the posterior end of the larva.

The posterior ciliated band becomes more prominent and a pair of vibratile lobes on both the dorsal and ventral sides grow laterally so as to encircle half of the base of the four main arms. However, there is no indication of the epaulet as yet.

In the 29th day old larva, the arms begin to be absorbed. This absorption takes place first at the tip of each arm and advances centripetally, the process going on most rapidly in the postero-dorsal arm on the left side (fig. 6). In the course of the absorption the plasm condenses into small spherules at the tip of the arm.

The vibratile lobes on the dorsal and ventral sides extend towards the median line of the body and fuse with each other into the transverse ciliated band; thus the larval body, except the right and left sides, is encircled at about the middle by the ciliated bands. Also, the ciliated band on the postero-lateral process is elongated towards the median line of the body, and fuses with its fellow; thus the posterior portion of the body becomes encircled completely by the ciliated band. The ciliated band has the appearance of a half ring in the side view, and is elliptical in the aboral view (Pl. 3, fig. 4).

Meanwhile, tetraradiate spines (larval spines) make their appearance almost simultaneously at the dorsal and ventral sides of the first pedicellaria; they are carried by genital plate 4 as will be seen later. These spines may be named spine A and B, for convenience in identification (Pl. 2, fig. 6, *sA* and *sB*). A similar spine begins to appear on genital plate 3 on the dorsal side of the second pedicellaria. This spine will be called C (*sC*). Soon on the ventral side of the third pedicellaria is formed a similar spine resting on genital plate 5. This is spine D (*sD*). Some time later, spine E appears; it is immediately posterior to, and slightly to the right side of, the center of the dorsal arch in the dorsal view (sE and fig. 7, *sE*). This spine is carried by genital plate 2. There is little doubt that the genital plates appear in the same order as the spines which rest on the plates.

By the 33rd day the roof of the amniotic cavity on the left side of the body is ruptured and five primary tube-feet make their appearance. These primary tube-feet are arranged in a distinct pentagon, of which one is situated on the median line towards the anterior end of the left side of the body (fig. 7 and Pl. 3, fig. 1, *tf*). The tube-feet are all very thick, and capable of much extension; in its internal cavity are found small corpuscles going to and fro giving an appearance of the circulation of blood-corpuscles in a blood-vessel.

The echinus rudiment has grown so much in size that it occupies not only the entire left side of the larva, but extends farther to the dorsal side and also to the ventral side. Each of the primary tube-feet terminates bluntly at the tip where it is provided with a well-developed sucker.

A tetraradiate spine carried on genital plate 1, appears on the right side of spine E; this will be called spine F (Pl. 2, fig. 7, *sF*).

A little later, close examination reveals similar spines under the epidermis on the internal side of the bases of the right postero-dorsal and postoral arms. These spines may be named G and H (Pl. 3, fig. 1, *sG* and *sH*).

Thus each pedicellaria is provided with a spine on both the ventral and dorsal sides.

Around the periphery of the echinus rudiment, the ordinary (ventral or typical) spines belonging to the corona in the adult are already being formed, something like a fence encircling an arena (fig. 2, *ts*). These spines are imbedded in the amniotic coat which they support.

The larva in this stage is of the shape of a sphere resting on a disc, the disc being formed by the junction of the two postero-lateral processes (fig. 3).

The larva becomes more and more opaque and gradually takes to the bottom of the aquarium, because of the body being made heavier by the calcareous matter, and at the same time, because of the arms becoming proportionally shorter as the larva develops.

Section 2. Metamorphosis.

This is perhaps the most striking stage throughout the whole course of development, and is reached in 35 days after insemination.

The transformation takes place within a very short time. During the change a number of ordinary spines become exposed through the absorption of the membranous amnion, and the larval arm rods which have become naked through the absorption of the surrounding plasm are broken off, leaving, usually, only the basal parts; the greater part of the protruded mouth area is absorbed, leaving only a small basal part.

It is important to notice that the left side of the larva becomes the ventral side of the imago, and the right side of the former becomes the dorsal side of the latter. The imago directly after metamorphosis is approximately pentagonal in form.

Fig. 4 (Pl. 3) represents the right-side view of the larva in the course of metamorphosis, and shows the precise situation of the pedicellariae and the appendant spines. In figs. 4 and 5 all the bare arm-rods are about to fall off. In fig. 6 we see the ventral view of a young sea-urchin just after metamorphosis (37 days old). The ectodermal skin of the larva still covers the whole surface of the sea-urchin, the red pigment cells are scattered over the greenish ground and give the larva a beautiful appearance. As is shown in this figure the antero-median region on the right side of the larva, which is indicated by the remnant of the basal part of the mouth area and by the dorsal arch, becomes one of the ambulacral zones, while the posterior region forms one of the inter-ambulacral zones. Each of the ambulacral and inter-ambulacral zones bears, usually, two tetraradiate spines and four ordinary spines respectively. All the spines have round their bases a thick collar, which becomes very prominent some time after metamorphosis. The five terminal (primary) tube-feet are the principal organs of locomotion in this stage.

The dorsal view of the same specimen is illustrated in fig. 7. The pedicellaria, tetraradiate spine and the dorsal arch serve for the orientation of the body. As may be seen from a comparision of fig. 4 and fig. 7, the first pedicellaria bearing spines A and B is situated at the dorso-median end of the adult body, the second one carrying spines C and G, at the middle of the left side (the dorsal side of the larva), and the third one with spines D and H, at the middle of the right side (the ventral side of the larva). Spine E is located directly anterior to the second pedicellaria a little nearer the median line, and spine F on the opposite side at the corresponding point.

Inspection of the dorsal aspect of the imago reveals clearly that the dorsal area of the imago is derived from two parts of the larval body, viz. the main part of it is formed by the right side of the larva and the remainning part of it, which is occupied by the first pedicellaria, by the posterior side of the larva. This fact indicates that the posterior surface of the larva has turned 90 degrees towards the right side of the larva to assume the present situation, as in the case of Ubisch's *Arbacia pustulosa*.

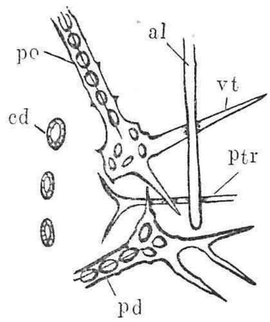
Figs. 8 and 9 show the ventral and the dorsal side respectively of a more advanced sea-urchin, in which a pair of ambulacral tube-feet (ordinary tube-feet) are present at the base of each primary tube-foot. These tube-feet are very small and do not have a blunt termination as in the primary tube-feet.

The adult mouth, anus and sphaeridia are not found as yet.

PART 3. THE RELATION BETWEEN THE LARVAL SKELETON AND THE TEST OF THE SEA-URCHIN.

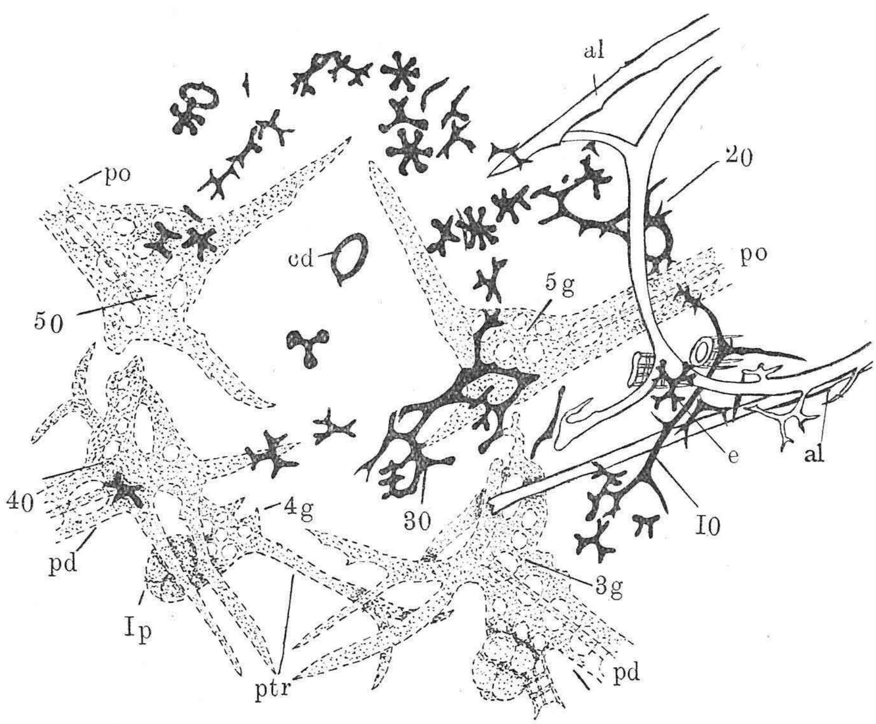
Special attention was paid to the kind of rôle the larval skeleton plays in the formation of the sea-urchin test. In this connection, I may state at the outset that the test of the sea-urchin is derived from two sources, namely, the larval skeleton and the newly-formed elements laid down in the echinus rudiment.

The first trace of calcification appears in the echinus rudiment of the fairly advanced larva. This change is initiated by the appearance of the calcareous disc in the terminal tube-foot (*Text-fig. 12, cd*). In the meantime the rudiments of the ocular plates (1, 2, 3) and their associated spines have appeared (Text-fig. 19). Somewhat later the rudiments of the inter-ambulacral plates and their associated spines seem to appear and also the rudiments of the ambulacral plates are laid down. By this time, the proliferations from the bases of the four main rods and from the center of the dorsal arch, which are destined to become genital (Text-fig. 11, *2g*, *3g*, *4g*, and *5g*) and ocular plates (*40* and *50*), are growing in size.



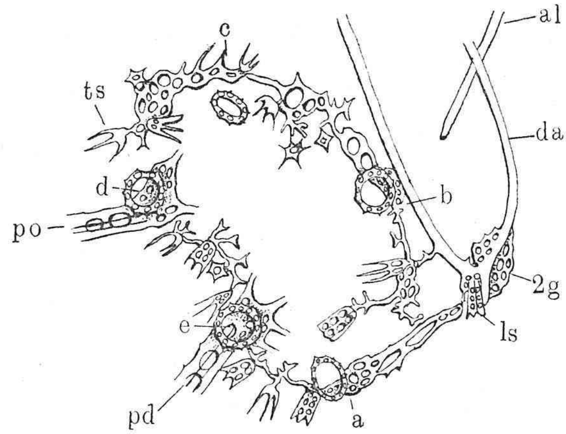
Text fig. 12.

Showing the calcareous disc of terminal tube-foot.
Legends as in preceding figs.
120/1.



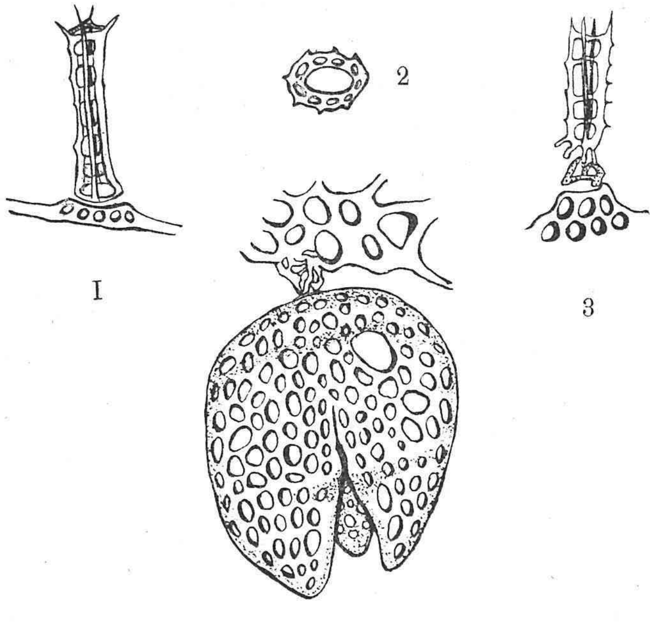
Text fig. 13.

Various calcareous elements laid down in the echinus rudiment. Compressed from dorsal side. Legends as in preceding figs., further:
Ip.—first pedicellaria. e.—rudiment of spine E. 200/1.



Text fig. 14.

Calcifications of the ocular and inter-ambulacral plates in the echinus rudiment. Legends as in preceding figs., further: ts.—typical spine. ls.—larval spine. 120/1.



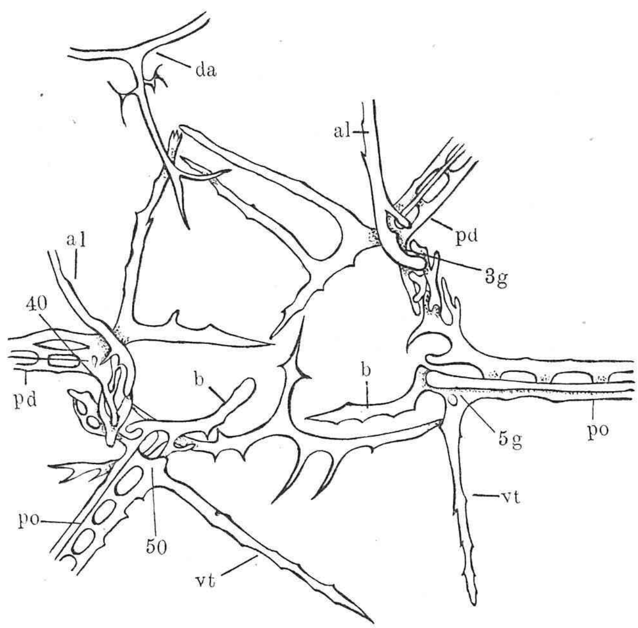
Text fig. 15.

Various calcareous elements.
1.—tetraradiate spine.
2.—disc of a primary tube-foot.
3.—typical spine.
4.—pedicellaria.

It is worth noting that all the oculars do not arise in the echinus rudiment; of the five oculars only three arise in the rudiment and the remaining two, oculars 4 and 5, have their origin in connection with the larval skeleton.

In Text-fig. 14 there are found the rudiments of the tetraradiate and typical spines on the ocular and inter-ambulacral plates, the difference in size among them indicating the order of their appearance. No indication of the dentary apparatus is found at this time.

In Text-fig. 15 are shown various calcareous elements found in this stage. Spicules of various types, six-rayed, four or five-rayed and triradiate, are depicted in fig. 13. I could not, however, trace the successive developmental stages of these spicules. According to



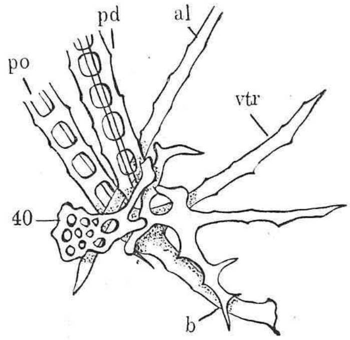
Text fig. 16.

Part of skeleton, showing the meshworks in 20-day old larva; compressed from dorsal side. Legends as in preceding figs. 120/1.

GORDON's report on these elements in *Echinus miliaris*, the typical spine is derived from a six-rayed star, the tetraradiate spine from a triradiate spicule and sphaeridia from a four- or five-rayed star as a rule.

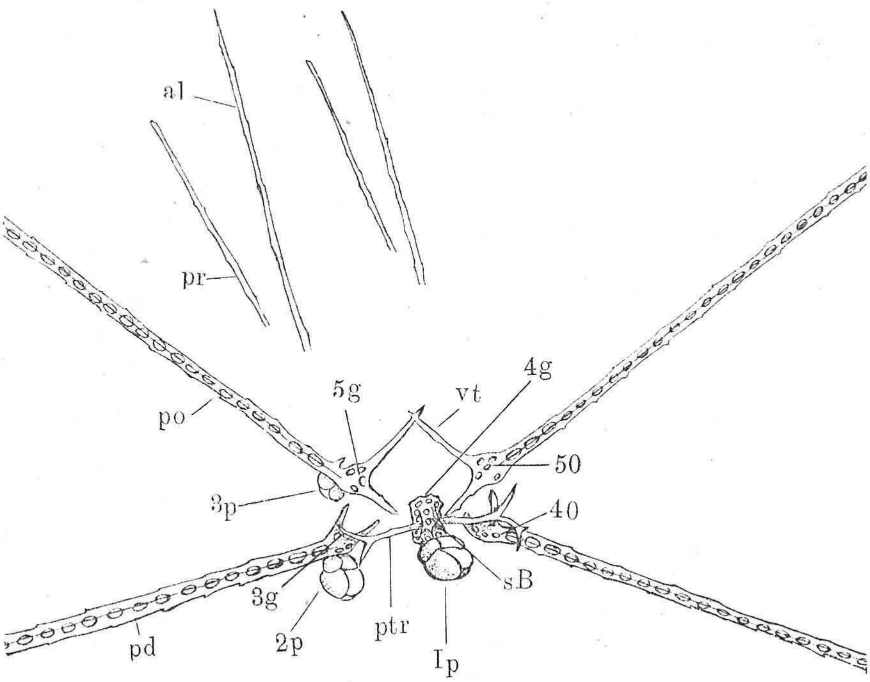
Ocular plates 4 and 5 arise from the proliferations in the basal portions of the left postero-dorsal and postoral rod respectively. These changes are fairly advanced in the 19-day old larva, as is shown in Text-figs. 11, 16 and 17, 40, 50. As to the mode of formation of the ocular plates, my finding conforms well to the observations of UBISCH on *Arbacia pustulosa* and of GORDON on *Echinus miliaris*. The relative situation of the ocular plates is tolerably clear in Text-figs. 13 and 14.

Of the genital plates, four (2, 3, 4 and 5) arise in connection with the larval skeleton; only genital plate 1 appears to be laid down from a new center on the right side of the larva;



Text fig. 17.

Part of skeleton, showing the meshwork. Left side of the 23-day old larva, viewed from the dorsal side. Legends as in preceding figs. 120/1.



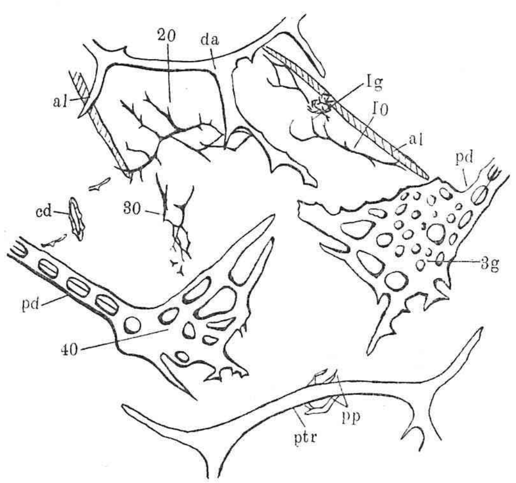
Text fig. 18.

Part of skeleton, showing the meshwork of genital plate 4. Legends as in preceding figs., further:
pr.—preoral rod. 2p.—second pedicellaria. 3p.—third pedicellaria.
sB.—spine B. 100/1.

this fact is in sharp contrast to what has been confirmed with regard to the ocular plate (Text-fig. 11, *1g*).

When one compares the mode of appearance of the genital plates in this species with that in *Strongylocentrotus lividus* (UBISCH), *Echinus microtuberculatus* (UBISCH) and *Echinus miliaris* (GORDON), a rather great difference may be noticed; viz. whereas in the present species genital 4 originates as a proliferation of the larval skeleton, in those species the plate appears to be laid down in the posterior portion of the larva without any relation to the larval skeleton. *Arbacia pustulosa* (UBISCH) agrees with the present species in this respect.

As has been described previously, the tetraradiate spine first to appear is that which rests on genital plate 4. As shown in Text-fig. 18, the posterior processes of the posterior transverse rod send out branches which grow out to meet the branches of its fellow on the other side and encircle the posterior transverse rod, and thus a fine meshwork is constructed. This mesh-plate is nothing other than genital 4, and is surmounted by spines A and B (*sB*).

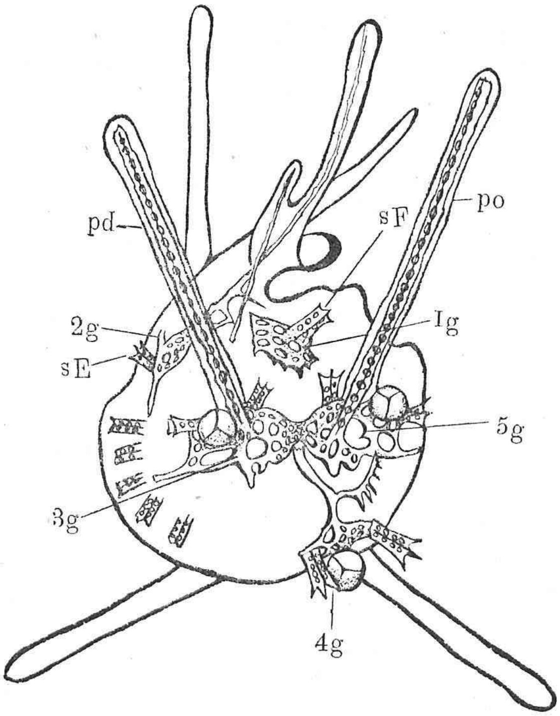


Text fig. 19.

Part of skeleton, showing the meshworks of genital 3 and ocular 4.
Legends as in preceding figs., further:
Ig.—genital 1. (dorsal view). 120/1.

No definite tubercles are formed on the genital plates. After metamorphosis, genital plate 4 is located in the eccentric position, which is due to the fact that it originates from the posterior end of the larva. Genital plate 3 is formed as a proliferation from the base of the right postero-dorsal rod (Text-figs. 11 and 19, *3g*), while genital 5 is formed similarly from the right postoral rod (*5g*). These plates appear nearly at the same time as ocular plates 4 and 5 (Text-fig. 18, *3g*, *5g*, *40* and *50*).

As regards genital 2, it originates as a proliferation of the dorsal arch as has been described by UBISCH (1913). From the center of the dorsal arch a few small branches are sent out, which undergo branchings and anastomoses and bring about a mesh-work and give rise to genital plate 2 (Text-figs. *20* and *21*, *2g*). In the meantime another calcareous center appears in the genital plate 2, which is the rudiment of the tetraradiate spine E (*sE*) (Text-fig. 13, *e*).



Text fig. 20.

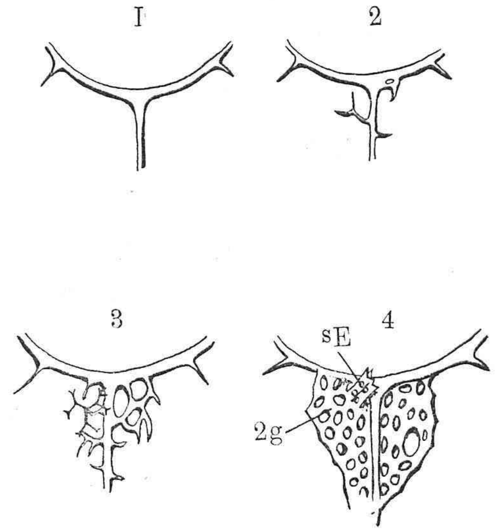
Pluteus, showing the relative position of genitals. Legends as in preceding figs., further :
sE.—spine E. sF.—spine F. (dorsal view). 100/1.

About this time, two other calcareous centers are laid down on the right side of the larval body and these develop into genital 1 and its associated spine F (Text-fig. 20, *1g* and *sF*).

The relative position of the genital plates is shown in Text-fig. 20.

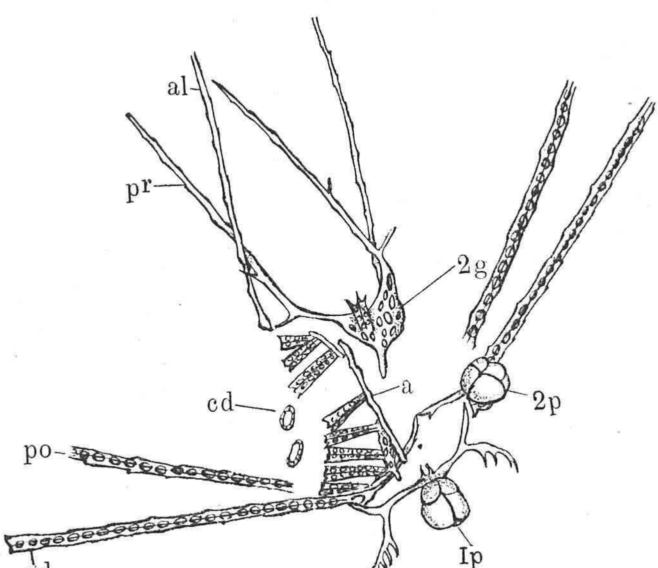
As may be clear from the above description a greater part of the larval skeleton is utilized for the formation of the test of the imago, and it is only the larval appendage that the young urchin loses during metamorphosis.

Text-fig. 14 represents the calcification in the echinus rudiment of the slightly older larva. In this figure the arrangement of the ocular and inter-ambulacral plates is clearly shown. Of the plates found in that figure ocular plate a (*a*) develops from ocular plate 3 (*30*) shown in Text-fig. 13 and in a similar way ocular plate b (*b*) develops from ocular plate 1 (10), ocular plate c (*c*) from ocular plate 2 (*20*) of the latter figure, while ocular plate d (*d*) and e (*e*) are the plates which have been originated from the left postoral rod and the left postero-dorsal rod respectively (*50* and *40*). At each inter-ambulacral zone a



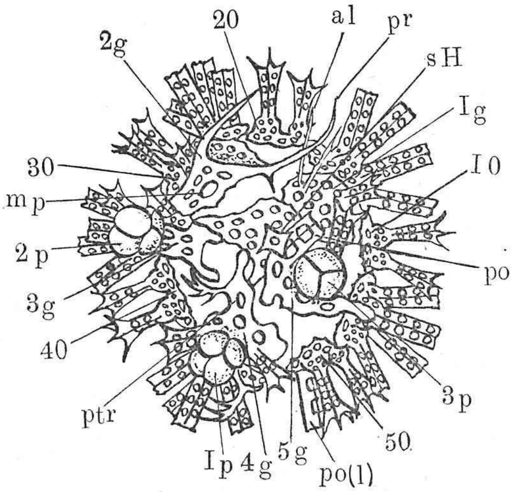
Text fig. 21.

Part of skeleton, showing the process of proliferation in the center of dorsal arch (genital 2). Legends as in preceding figs. 100/1.



Text fig. 22.

Skeleton of pluteus, showing the spines in the echinus rudiment. Legends as in preceding figs. (dorsal view). 50/1.



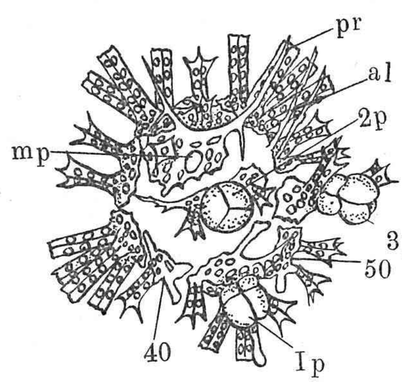
Text fig. 23.

Dorsal view of the young sea-urchin test, slightly leaned towards the left side.
Legends as in preceding figs., further:
mp.—madreporic pore.
po (1).—left postoral rod. 70/1.

plate showing a fairly complete meshwork, and its associated spines, are formed as development proceeds.

In Text-fig. 22 we have a side view of the echinus rudiment in a more advanced stage, showing that the spines stand in a row on the ocular and inter-ambulacral plates. Plate a in the figure (*a*) probably becomes ocular plate 3.

Text-fig. 23 represents the dorsal view of the urchin just metamorphosed slightly leaning towards the dorsal side of the larval stage. The arrangement of the various elements conforms exactly with the description given above. The outline of the imago in this view is pentagonal. Genital 4g (*4g*) is eccentric in situation; however, it moves towards the center as the development proceeds.

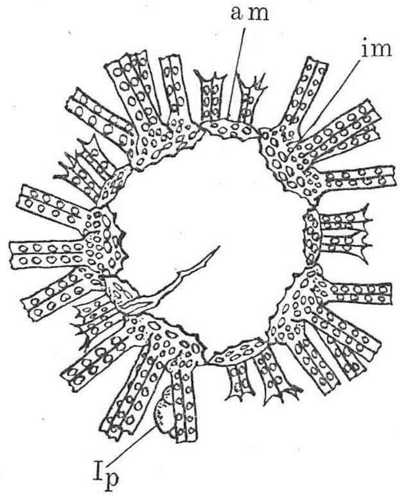


Text fig. 24.

Dorsal view of the young sea-urchin test, slightly leaned towards the right side.
Legends as in preceding figs. 70/1.

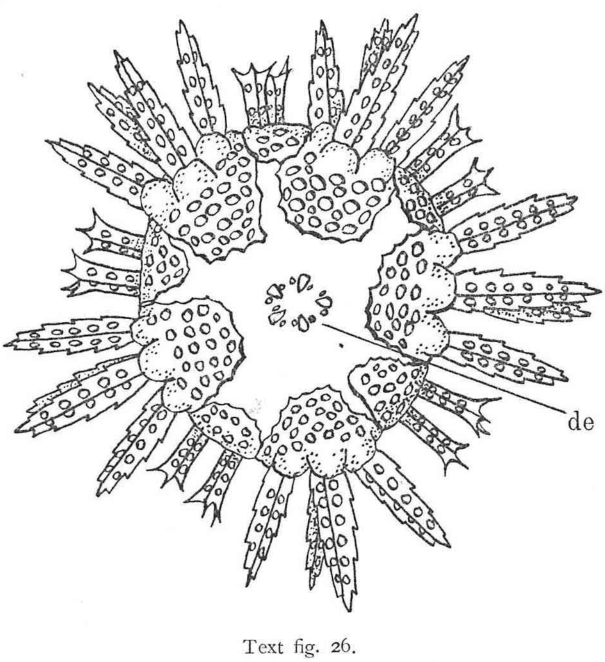
It should be noticed that the arrangement of the genital plates, pedicellariae and spines in the apical system of the imago, shows a clear bilateral symmetry. The plane of symmetry passes through ocular plate 2 (*2o*) and genital plate 4 (*4g*). The plane coincides with the longest axis of the larva, but it intersects with the bilateral symmetrical plane of the pluteus larva at an angle of 90°. Thus, by locating this symmetrical plane it is easy to determine the orientation of the larva even in the advanced stage.

In Text-fig. 23 the remnant of the right postoral rod (*po*) is visible between the third pedicellaria (*3p*) and spine H (*sH*), the remnants of the right antero-lateral rod (*al*) as well as of the preoral rod (*pr*) and the left postoral rod [*po* (*1*)] are also quite evident. The dorsal arch (*da*), the posterior transverse rod (*ptr*) and its appendices are the elements most prominent in the figure. The position of the posterior transverse rod in this figure is especially noteworthy as it shows that the dextral rotation of the larval body has proceeded as much as 90 degrees, with the longest axis of the larva as the rotational axis



Text-fig. 25.

Ventral view of the young sea-urchin test. Legends as in preceding figs., further: am.—ambulacral plate. im.—inter-ambulacral plate. 70/1.



Text fig. 26.

Ventral view of more advanced sea-urchin test than in fig. 25, showing the dental apparatus and corona.
Legends as in preceding figs., further: de.—dental apparatus. 100/1.

(the direction being determined from the view from the ventral side).

Genital plate 2 is pierced by a single, rather large pore which is apparently the water pore (madreporic pore) (Text-figs. 23 and 24, *mp*).

The space surrounded by genital plates 1, 2, 3 and 5 is the adult periproct, but no calcareous plates are formed as yet in this region. On the ocular plate and inter-ambulacral plate there are found, usually, two tetraradiate and four ordinary spines respectively. Of the genitals, 1 and 2 bear only one tetraradiate spine each, while 3, 4 and 5 each bear two tetraradiate spines and also one pedicellaria (ophiocephalus).

Text-fig. 24 represents the dorsal view of a specimen of the same age as that in Text-fig. 23 slightly leaning towards the ventral side of the larval stage. Ocular plates 4 (*4o*) and 5 (*5o*) are seen on both sides of the first pedicellaria; they are distinguished from the other three by the peculiarity of their form.

Thus, the apical system which forms the dorsal area of the young urchin consists of five genital and five ocular plates, all of which are still delicate reticulate structures and not in contact with each other to form the sutures.

The ventral view of the young urchin test is represented in Text-fig. 25 in which the peristome area is still large. In a more advanced stage, illustrated in Text-fig. 26, the peristome is smaller and the corona part larger because of the growth of the ambulacral and inter-ambulacral plates, moreover the dental apparatus has already come into existence (*de*).

REMARKS.

The difficulty in raising the echinoid larva beyond the pluteus stage is due to various facts. Above all, it seems to be absolutely necessary for this purpose to secure gonads in the prime of maturity. As regards food, my experience shows that the larvae are able to continue their development if given fresh sea-water every day, without being fed with diatoms, etc.

The results described above for *Heliocidaris crassispina* are essentially the same as those given by UBISCH (1913) in *Arbacia pustulosa*, so far as the constitution of the genital system is concerned; genital plates 2, 3, 4 and 5 arise in connection with the larval skeleton and only genital plate 1 is formed from an independent calcareous primordium. In *Strongylocentrotus lividus* (UBISCH), *Echinus microtuberculatus* (UBISCH) and *Echinus miliaris* (GORDON), both genitals

1 and 4 arise from independent calcareous primordia (centers), while in *Echinocardium cordatum* (GORDON) genital 4 arises from a part of the right anterior extension of the aboral rod, and in *Arbacia punctulata* (GORDON) genital 1 arises in connection with the right antero-lateral rod. In *Echinarachnius parma* (GORDON) genital 1 is missing, and although genital 4 is derived from a part of the larval spicule, it arises from the point of connection of the two branches of the two postero-dorsal rods (see GORDON 1929, fig. 15).

With regard to the mode of development of the ocular plates, the present species agrees with *Arbacia pustulosa* (UBISCH), *Echinus miliaris* (GORDON), *Arbacia punctulata* (GORDON) and *Echinarachnius parma* (GORDON).

As in the case of *Arbacia* (GORDON), the buccal plates do not appear until after metamorphosis, but this, according to Gordon, is due to the fact that the buccal plates always lag behind the first ambulacral plates.

The calcareous element formed first in the metamorphosing larva in the present species is the discs found in the terminal tube-feet, while in *Echinus miliaris* (GORDON) and *Arbacia punctulata* (GORDON) oculars 1, 2 and 3 appear first.

In *Strongylocentrotus lividus* (UBISCH), *Echinus microtuberculatus* (UBISCH) and *Echinus miliaris* (GORDON) the dorsal side of the imago corresponds with the right side of the larval body, while in *Arbacia pustulosa* (UBISCH), *Echinocardium cordatum* (GORDON), *Arbacia punctulata* (GORDON), *Echinarachnius parma* (GORDON), as well as in the present species, the posterior portion of the larva takes part in the formation of the dorsal portion of the imago together with the right portion.

The difference existing between the two types just stated is apparently related to the difference in the origin of genital plate 4. In the type in which genital plate 4 is formed independently, as in *Strongylocentrotus lividus*, the dorsal side of the imago corresponds with the right side of the larva, while in the other type where genital plate 4 is originated from the larval skeleton, as in *Arbacia pustulosa*, it is made up of the right and posterior portions of the larva, and it is very likely that this distinction occurs widely in the whole group of echinoids.

It is indeed of some interest to note that *Heliocidaris crassispina* shows in the above respect a closer relationship to *Arbacia pustulosa*, which is a primitive form of regular sea-urchin, than to other higher members of the same species, contrary to what is commonly known as regards the systematic position of the former species.

Evidently the larva of the present species belongs to MORTENSEN's *Echinometridae* type, which has the following characteristics; in the first stage a short, obliquely truncated body, supported by a complicated basket structure, the recurrent rod being double; in the second stage there is a posterior transverse rod; postero-lateral and vibratile lobes are found, but no epaulets; the rods of the main arms are fenestrated.

SUMMARY.

(1) The blastula is at first perfectly spherical in form, but later assumes a conical shape with the vegetative pole more flattend and thickened than the animal pole.

(2) The first stage of the pluteus (four-armed larva) is reached in about 18 hours after insemination, the skeleton has a basket structure with double recurrent rod and fenestrated postoral rods.

(3) The second stage of the pluteus (six-armed larva) is reached about the 11th day after insemination. This stage is characterized by the presence of more fenestrated postero-dorsal rods.

(4) The absorption of the larval skeleton begins first at the tips of the body rods of the 11-day old larva.

(5) The third stage of the pluteus (eight-armed larva) which is marked by the presence of the preoral arms is reached in about 15 days after insemination. The total length of the pluteus at this stage including the antero-lateral arms is approximately 0.9 mm., the four main arms measuring about 1.5 times as long as the body proper.

(6) The posterior transverse rod has developed fairly well at the end of 15 days, having postero-lateral processes at its extremity, the proliferations at the basal parts of the four main arm rods and the centers of the posterior transverse rod and of the dorsal arch, growing in size from about the time when the larva enters its third stage.

(7) By the 17th day, well-developed vibratile lobes develop on both the ventral and dorsal sides, but there is no indication of epaulets.

(8) The first rudiment of pedicellaria has appeared on the 15th day, and on the 23rd day after insemination the larva is provided with three pedicellariae.

(9) On the 29th day after insemination, all the arms begin to be absorbed and this absorption begins in the left postero-dorsal arm.

(10) On the 33rd day after insemination, the first terminal (primary) tube-feet emerge on the surface of the body, and soon afterwards,

the eight larval spines and the ventral spines surrounding the echinus rudiment come into existence.

(11) Metamorphosis is reached on the 37th day after insemination.

(12) The dorsal area of the imago is made up of two parts of the larval body; the main part of the imago arises from the right side of the larva and the remaining part, occupied by the first pedicellaria, is formed by the posterior side of the larva. The diameter of the imago including the spines is 0.8 mm.

(13) On the 33rd day after insemination a pair of ordinary tube-feet are present at the base of each unpaired primary tube-foot. The adult mouth as well as the sphaeridium are not formed by the 40th day after insemination.

(14) The test of the sea-urchin is derived from two sources, namely, the larval skeleton and the newly formed elements laid down in the echinus rudiment.

(15) The new element to appear first is the discs of the terminal tube-feet.

(16) Of the genitals, genitals 2, 3, 4 and 5 are formed as proliferations of the larval spicules; genital 2 appears at the center of the dorsal arch, genitals 3, 5 and 4, on the right postero-dorsal, the postoral and the posterior transverse rod respectively, while genital 1 is formed in a new center.

(17) Of the oculars, oculars 4 and 5 are formed from proliferations of the left postero-dorsal and postoral rods of the larva, while oculars 1, 2 and 3 are formed by the elements newly laid down.

(18) The buccal apparatus comes into view on the 37th day after insemination.

(19) The various elements in the apical system are arranged symmetrically about Ubisch's "Primordialebene des Seeigels" and this symmetrical plane is at right angles to the plane of the bilateral symmetry of the larva (Ubisch's larvale-symmetrieebene).

(20) With regard to the formation of the apical system, the present species resembles *Arbacia pustulosa*, which is a primitive form of the regular sea-urchin, more than the higher members of the group.

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EXPLANATION OF PLATES 1—3.

All the figures are drawn from life by the aid of ABBE's apparatus and magnified 100 times the natural size, unless otherwise stated.

List of Abbreviations

al	antero-lateral rod.	slp	secondary lateral process
ad	adoral ciliated band.	sA	spine A.
b	body rod.	sB	spine B.
dt	dorsal transverse rod.	sC	spine C.
dvl	dorsal vibratile lobe.	sD	spine D.
da	dorsal arch.	sE	spine E.
D	dorsal side.	sF	spine F.
ec	enterocoelic pouch.	sG	spine G.
e	anal opening.	sH	spine H
lp.	lateral process.	sr	secondary recurrent rod.
oe	oesophagus.	st	stomach.
pr.	preoral arm.	trs	tetraradiate spine.
plp	posterior lateral process.	ts	typical spine.
pb	posterior ciliated band.	tf	tube-foot.
ptf	paired tube-foot.	vl	vibratile lobe.
po	postoral rod.	vt	ventral transverse rod.
pd	postero-dorsal rod.	vb	vibratile band.
pd'	rudiment of postero-dorsal rod.	V	ventral side.
p'r	primary recurrent rod.	Ip	first pedicellaria.
pob	preoral ciliated band.	2p	second pedicellaria.
ptr	posterior transverse rod.	3p	third pedicellaria.

PLATE I

Fig. 1. Young larva, ventral view; 72 hours old.

Fig. 2. The same specimen as in fig. 1, dorsal view.

Fig. 3. The same specimen as in fig. 2, left side view. Triparted gut distinct and anal area protruded.

Fig. 4. Young larva, five days old; ventral view. Drawn from preserved specimen.

Fig. 5. Dorsal view of the same specimen as in fig. 4. Drawn from fixed material.

Fig. 6. Pluteus, dorsal view; 8 days old, showing the rudiments of the dorsal arch and postero-dorsal rod.

Fig. 7. Aboral view, 11 days old larva.

Fig. 8. Pluteus, dorsal view; 11 days old, showing destruction of the body rod.

Fig. 9. Larva 8 days old, side view. Drawn from fixed material. Notice shrinking when compared with fig. 10.

Fig. 10. Larva 8 days old, dorsal view.

Fig. 11. Pluteus 11 days old, dorsal view; drawn from fixed material. Postero-dorsal arms are fairly developed and also dorsal arch has already appeared. Notice that the posterior ends of the body rods have been absorbed.

PLATE II

Fig. 1. Pluteus 11 days old, seen from anterior side, but in such a position that the body is leaning somewhat to the dorsal side.

Fig. 2. Pluteus 15 days old, ventral view. The preoral arms and the posterior transverse rod have been developed.

Fig. 3. Full developed pluteus, 17 days old; ventral view. First pedicellaria (rudiment) has began to form in the posterior end of the body. The vibratile lobes and the dorsal ciliated bands have been developed.

Fig. 4. Pluteus of the same age as in fig. 3, dorsal view. Drawn from preserved specimen.

Fig. 5. Pluteus 23 days old, ventral view. First pedicellaria well developed. Second and third pedicellariae already seen on the right side of the body. The bases of the four main arms are encircled by the well developed vibratile lobes.

Fig. 6. Pluteus 29 days old, ventral view. Notice the commencement of absorption of the arms. Vibratile lobe connects with its fellow and encircles the body in the middle as well as at its end. Five tetraradiate spines are seen, but one of them (sE) does not come into view in an actual state, as it is situated on the dorsal side.

Fig. 7. Pluteus 33 days old, left side view. Notice tetraradiate spines sE and sF.

PLATE III

Fig. 1. Pluteus in the beginning of metamorphosis, 33 days old; ventral view. Five terminal tube-feet are seen in the projecting condition, tetraradiate spines sG and sH also visible.

Fig. 2. Pluteus of the same age as in fig. 1, left side view, showing the arrangement of the ventral spines and terminal tube-feet which are seen through the ectoderm.

Fig. 3. Aboral view of the pluteus, 33 days old, showing the relative position of the pedicellariae and the tetraradiate spines.

Fig. 4. Pluteus in the act of metamorphosing, right side view. Some ventral spines already projecting. Notice the bared arm rods and the relative position of the pedicellariae and the tetraradiate spines.

Fig. 5. Pluteus in the act of metamorphosing, seen from the anterior end of the larva.

Fig. 6. Young sea-urchin just after metamorphosis, ventral view; 37 days old. The upper part of this figure is the anterior end and to the left is the ventral side in the larval stage. There is a terminal tube-foot in each ambulacral zone which bears one or two tetraradiate spines. The protuberance in the anterior end is the relic of the mouth area.

Fig. 7. The dorsal aspect of the same specimen as in fig. 6. The upper part of this figure is the anterior end, and to the left is the dorsal side in the larval stage. Compare this figure with fig. 4 and the orientation of the larva will be seen clearly.

Fig. 8. Ventral view of sea-urchin, 39 days old. Five paired ordinary tube-feet have appeared.

Fig. 9. The dorsal view of the same specimen as in fig. 8.

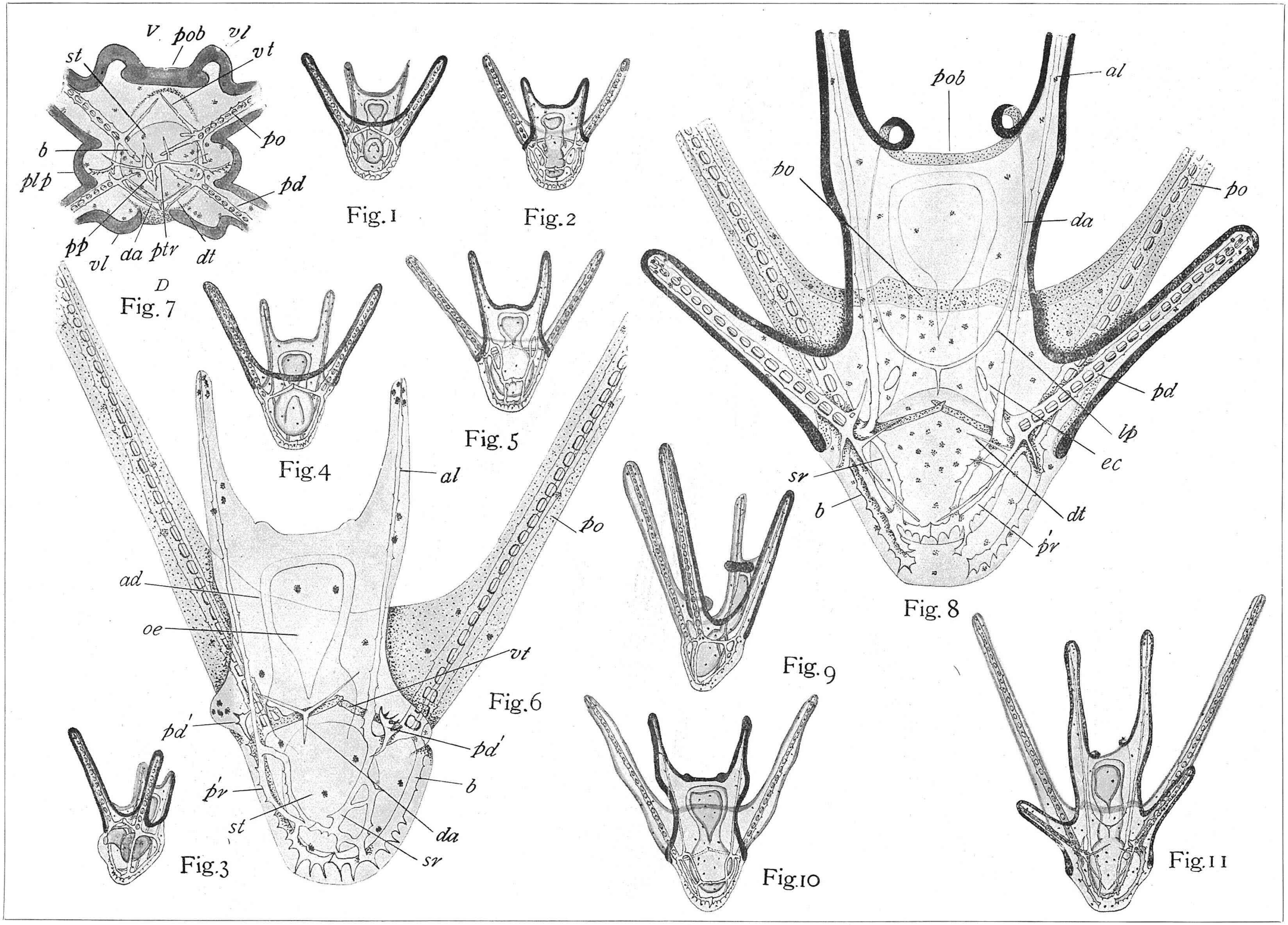
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Fig. 7
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Fig. 11

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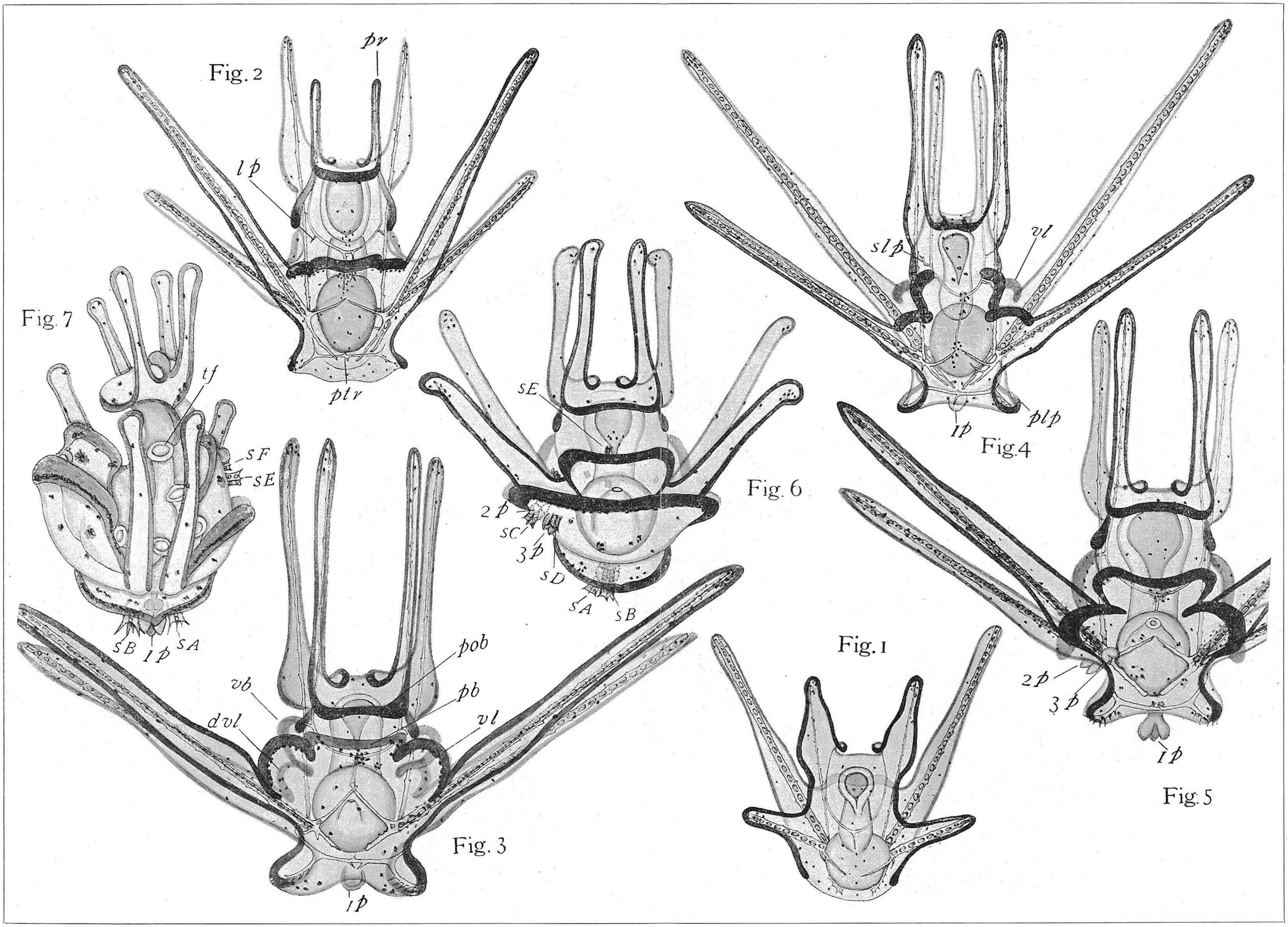
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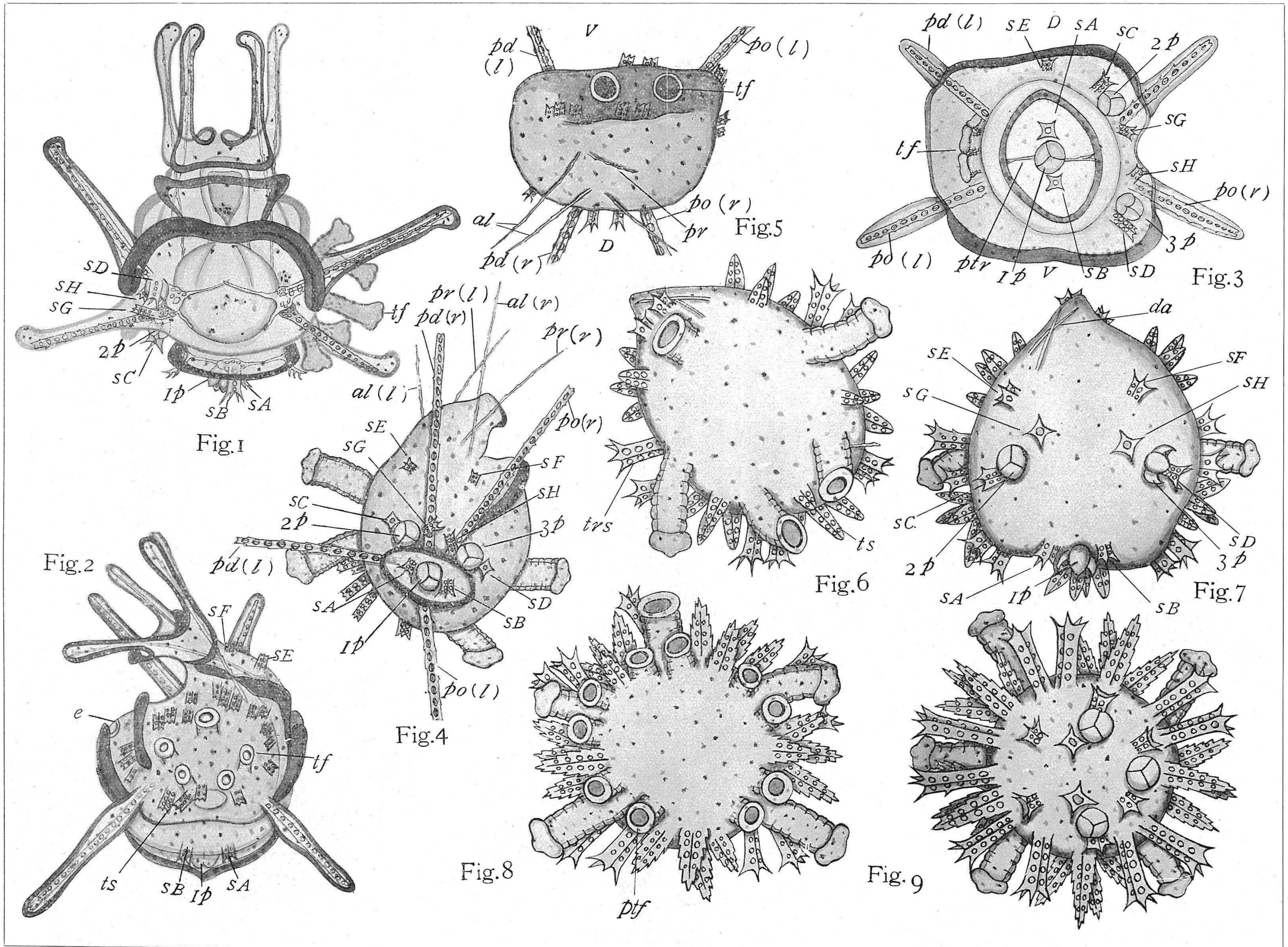
Fig. 1
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Fig. 2
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Fig. 5
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Fig. 3
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pd (r)
al (r)
pr (r)
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Fig. 4
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Fig. 6
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sH
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sD
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sA
1p
sB
Fig. 7
Fig. 8
ptf
Fig. 9

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