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THE FORMATION OF THE ECHINUS RUDIMENT AND THE DEVELOPMENT OF THE LARVAL FORM IN THE SEA URCHIN, *TEMNOPLEURUS HARDWICKII*[1]

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In the sea urchins the main parts of the adult structures are formed only on the left side of the bilaterally symmetrical pluteus. The pluteus become asymmetrical by the differentiation of the coelomic vesicle. Namely, the left anterior coelomic vesicle buds off a small vesicle called the hydrocoele which gives rise to the adult water-vascular system (MacBride 1903, 1914b; von Ubisch 1913). Thereafter, above the hydrocoele an ectodermal invagination occurs, an amniotic invagination, which is cut off from the exterior to form an amniotic cavity before long. Later, this gives rise to the tube feet, spines, central nervous system etc. of the adult in cooperation with the hydrocoele (MacBride 1903, 1914b; von Ubisch 1913). These processes seem to be common in the majority of the sea urchins. The writer's observations in *Glyptocidaris* (1960) also agree with the general principle mentioned above.

In 1959, the writer reported that in the pluteus of the sea urchin, *Temnopleurus hardwickii*, a spherical cell mass is observed at the left side. It is formed by the invagination of the ectodermal cell wall in the early pluteus stage at 25 hours after fertilization. At this stage the coelomic vesicles have been just formed and is earlier from the formation of the pore canal and from the division of the coelomic vesicle into anterior and posterior portions. The cell mass could be found in two speices belonging to the genus *Temnopleurus* among nine species of the Japanese sea urchins examined, namely *Temnopleurus hardwickii*, *Temnopleurus toreumaticus*, *Hemicentrotus pulcherrimus*, *Strongylocentrotus nudus*, *Strongylocentrotus intermedius*, *Glyptocidaris crenularis*, *Echinocardium cordatum*, *Anthocidaris crassispina* and *Astriclypeus manni*.

The present paper deals with the fate of the cell mass with the changes of the larval form of *Temnopleurus hardwickii* reared until the completion of the metamorphosis.

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1) Contributions from the Marine Biological Station of Asamushi, Aomori Ken, No. 267.

course of the present work. Thanks are also due to the staff of Sugashima Marine Biological Station of the Nagoya University for providing the room and materials during the course of observations on the larvae of *Temnopleurus toreumatics*, *Anthocidaris crassispina* and *Astriclypeus manni*.

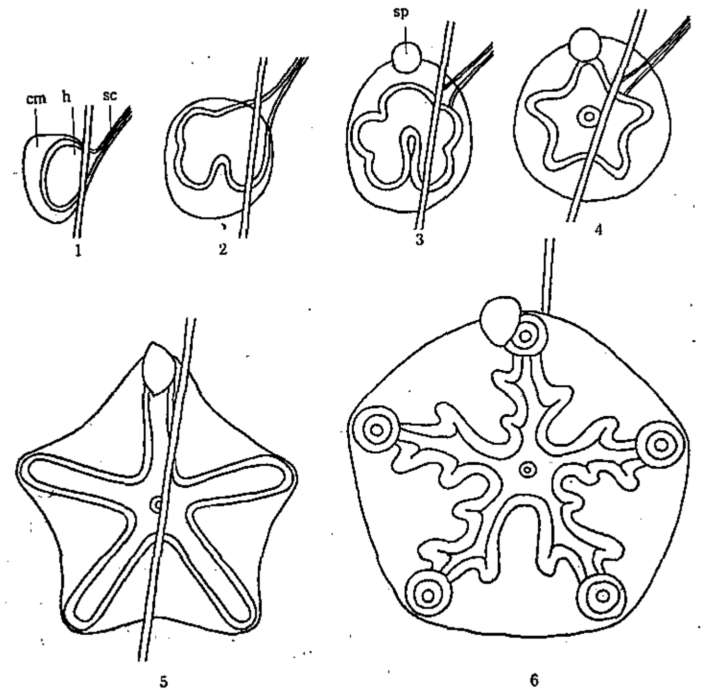
MATERIAL AND METHOD

Material used in the following description was the larvae of *Temnopleurus hardwickii* collected at Asamushi. *Hemicentrotus pulcherrimus*, *Strongylocentrotus nudus*, *Strongylocentrotus intermedius*, *Glyptocidaris crenularis*, *Echinocardium cordatum* collected at Asamushi, and *Temnopleurus toreumaticus*, *Anthocidaris crassispina*, *Astriclypeus manni* collected at Sugashima were used for the comparative study. The eggs and sperms used for the observations of the development were obtained by the KCl method. For rearing the plutei until the completion of metamorphosis a glass vessel of 300 ml and 7 cm in depth was used. Fresh sea water taken from the sea was changed every day. The pluteus feeds mainly on *Peridinium* and *Licmophora* living in fresh sea water. Under these conditions the larvae of less than 20 or 30 were cultured in a vessel. The observations were carried out on the living materials.

OBSERVATION

I. *On the formation and development of the echinus rudiment*

A cell mass appears on the left side of the pluteus 25 hours after fertilization. Ten days after the cell mass gradually increases in size to give rise to a vesicle. The left and right coelomic vesicles, the rudiments of which appeared prior to the cell mass extend to the posterior side along the stomach. The left one divides into the anterior and posterior portions at the connection of the oesophagus and stomach. By the end of the second week the left anterior coelomic vesicle buds off a posterior vesicle, the hydrocoele, contacting with the cell mass. The hydrocoele communicates with the anterior coelomic vesicle by a narrow canal. This is the stone canal. By the middle of the third week, five notches appear in the hydrocoele observable from the left side. The posterior one of them is conspicuous and two ends of it join to form a ring in the centre of the hydrocoele. Five blunt lobes formed by the notches extend out radially and take shape like fingers. Now, the cell mass gives rise to a vesicle covering the hydrocoele and the lobes of the hydrocoele protrude as five processes into the cavity of the vesicle. Several spherical free mesenchyme cells are scattered on these two organs closely touching each other. The anterior end of the vesicle buds off a small process outwards. The fate of this organ was not followed. The vesicle covering the hydrocoele transforms pentagonally in shape as five finger-shaped lobes of the hydrocoele extend. Compound organ of the hydrocoele and the above-mentioned cell mass measures



Figs. 1–6. Development of the compound organ of the hydrocoele and cell mass. Left side view of the larvae. ×243. Figs. 1–4, during the third week after fertilization. Notches appear in the hydrocoele and the posterior one which is conspicuous forms, with a ring of hydrocoele in the centre. Cell mass buds off a small process from the anterior end. Figs. 5–6, during the fourth week after fertilization. Lobes of the hydrocoele extend out like fingers. Distal ends of lobes take a shape like sucker. The stems branching to form the tube feet.

cm, cell mass; h, hydrocoele; sc, stone canal; sp, small process.

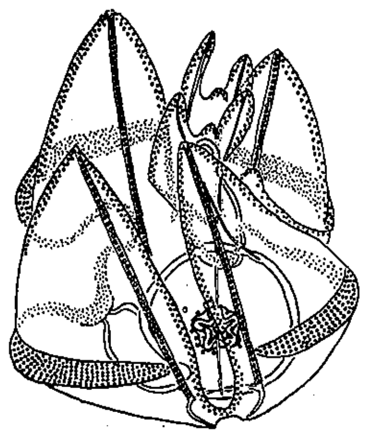


Fig. 7. Larva, 24 days old, viewed from the left side. Showing the position of the compound organ of the hydrocoele and cell mass.

170 microns in maximum diameter. By the end of the fourth week, the finger-shaped lobes have sucker-like structures at their distal parts, and bud off one or two branches on each side of their stems. Later, the ends of the finger-shaped lobes give rise to the primary azygous tube feet and the branches also to the tube feet, respectively (Figs. 1–6).

II. *Changes of the larval form as the development proceeds*

Moore (1933) reported on the outline of the normal development of *Temnopleurus hardwickii* by rearing the plutei for a month, although she had been unable to obtain the metamorphosed imago because of her departure from the Asamushi Marine Biological Station. The following is to supplement her report by rearing the larvae through the whole development up to the completion of metamorphosis and adding some observations taken in more detail.

About eight days after fertilization the pore canal (pc) arises from the left coelomic vesicle communicates with the exterior through the dorsal pore (dp). In the course of further development the pore shifts from its original portion on the left side towards the mid-dorsal line. The posterior branch of the calcareous dorsal arch extends avoiding the pore on the left side. As the result the dorsal pore is situated at the right side of the posterior branch of the dorsal arch in the later stage. By the ninth day two mesenchymal masses appear, situated at the lateral side of the larva in the concavity between the oral lobe and post oral arms. In their centres a triradiate calcareous spicule is formed and grows up to the latticed rod supporting the postero-dorsal arms (pd). One or two days later, the rudiment of the dorsal arch (da) which is to support the pre-oral arm, is formed at the dorsal side of the oesophagus. After the end of the second week the dorsal and ventral ciliated epaulettes (de, ve) begin to grow. The postero-dorsal arms extend as long as two-thirds of the length of the post oral arm, but the tips of the pre-oral rods do not yet reach to the rudiments of the pre-oral arms. By the end of the third week the tips of three pairs of the arms except the pre-oral arms (poa) extend to about the same distance from the body. The ciliated epaulettes grow conspicuously. At the posterior pole, the rudiments of the pedicellariae and spines (rps) appear as a little knob-like outgrowth of the ectoderm, the number of the pedicellariae or spines being variable, and not more than three in sum of both organs. The pedicellariae are formed only at the posterior pole in the larva of *Temnopleurus hardwickii*. By the end of the fourth week the primary tube feet (pt) are recognized at the left side of the stomach. The outgrowth of the anterior end of the cell mass (sp) is observed as a small mass lightly touching to the posterior end of the compound organ of the hydrocoele and cell mass. During the fifth week the larvae reach to the maximum size, measuring 1.1 mm from the posterior pole to the tips of the arms. The square-topped external spines (es) and the patches of calcareous networks supporting them are arising on the surface of the body. In the compound organ of the hydrocoele and cell mass the adult spines become formed around the primary tube feet. The spines and tube feet grow in size so large that the stomach (s) is pushed away to the right side. By the first day of the sixth week, two metamorphosed imagos were obtained for the first time. The metamorphosis

of *Temmopleurus hardwickii* is similar to that of the other species, such as *Echinocardium cordatum* (MacBride 1914a), *Mespilia globulus* (Onoda 1936) and *Glyptocidaris crenularis* (Fukushi 1960). There are a few pedicellariae and several spines on the abactinal surface of the young imago. Thirty spines formed in the compound organ of the hydrocoele and cell mass are regularly arranged at the circumference of the imago. Four spines (isp) in each interradius arranged in the form of a lozenge are pointed, but five pairs of spines (iss) situated at the abactinal side of the secondary tube feet are square-topped. Three secondary tube feet (st) are situated, one of them on one side and the other two on the other side of the primary tube feet at the actinal side. The arrangement of this manner is observed regularly in relation to the larval axis (Fig. 6). There is a spheridium (sph) at the actinal side of each primary tube feet. On the actinal surface five rudiments of the tube feet (rt) are observed and they also take constantly a definite arrangement in regard to the larval axis.

DISCUSSION

It has been observed in the normal development of the sea urchin that the hydrocoele is formed preceding the formation of the amniotic cavity, of the dental sacs from the left posterior coelomic vesicle and of the adult oesophagus from the left side of the stomach. Further, it has been generally believed from the observations of various abnormalities that the former is prerequisite to latters.

MacBride (1911) reported two larvae occasionally possessing an echinus rudiment on the right side as well as on the left. One of them is simple in the echinus rudiment of each side, but the other is preparing a pair of well-developed adult organs. He deduced from these facts that the formation of an amniotic cavity is not due to the pre-existence of an invisible rudiment of the ectoderm; on the contrary, it is the result of a stimulus acting on the ectoderm and emanating from the hydrocoele, and that the whole ectoderm of the larva is so organized that any part of it can form an amniotic cavity if the appropriate stimulus reaches it. MacBride (1918) confirmed his view on the basis of the fact that the amniotic invagination is never formed where no hydrocoele existed.

Ohshima (1922) supported MacBride's view from his works of situs inversus in the larvae of *Psammechinus miliaris*, and proposed an idea that the formative stimuli emanating from the hydrocoele and the further development of the water-vascular system is associated with the communication of the hydrocoele to the exterior. From this view, he explained the production of the double hydrocoele and situs inversus of the sea urchin larvae.

On the other hand, Runnström (1912, 1918b) showed several instances where an ectodermal invagination was formed at a place under which no hydrocoele had been developed. This contradicts to MacBride's view. Runnström (1917–18) further could produce a new amniotic invagination in the larva of *Psammechinus miliaris* from which the echinus rudiment had been removed experimentally. However, Ohshima (1922) doubts whether all of the structures observed by Runnström are to be identified as the normal amniotic invagination with high and cylindrical epithelial cells. But, there is a general agreement that further differentiation of the amniotic invagination cannot proceed without the underlying structure (Runnström 1918a).

In the *Temnopleurus* larvae no amniotic invagination of the ordinary type is observed. On the other hand, the cell mass formed about 25 hours after fertilization gives rise to the echinus rudiment in cooperation with the hydrocoele in *Temnopleurus*. Accordingly, the formation of the cell mass of this species corresponds to the amniotic invagination of other species. The cell mass is formed without the contact of the coelomic vesicle or special aggregation of the free mesenchyme cells namely by self-differentiation. If it is possible to say that the cell mass is homologus with the amniotic invagination in other species of sea urchins, the above-mentioned fact contradicts MacBride's idea.

SUMMARY

1) The cell mass which is formed on the left side of the *Temnopleurus* larva by the invagination of the ectoderm corresponds to the amniotic invagination of other species in its function.

2) The invagination is formed by the self-differentiation in the *Temnopleurus* larvae.

3) The changes of the larval form until the completion of the metamorphosis were described.

REFERENCES

FUKUSHI, T., 1959. On the cell mass observed on the left side of the pluteus of the sea urchin, *Temnopleurus hardwickii*. Bull. Mar. Biol. Stat. Asamushi, **9** : 133–135.

———, 1960. The external features of the development of the sea urchin, *Glyptocidaris crenularis* A.Agassiz. Ibid., **10** : 57–64.

MACBRIDE, E. W., 1903. The development of *Echinus esculentus* together with some points in the development of *E. miliaris* and *E. acutus*. Phil. Trans. Roy. Soc. (London), Ser. B, **195** (*fide* MacBride 1911).

———, 1911. Two abnormal plutei of *Echinus* and the light which they throw on the factors in the normal development of *Echinus*. Quart. Journ. Microsc. Sci., **57** : 235–250.

———, 1914a. The development of *Echinocardium cordatum*. I. The external features of the development. Ibid., **59** : 471–486.

———, 1914b. Text-bood of Embryology. I. Invertebrata. London.

———, 1918. The artificial production of echinoderm larvae with water-vascular systems, and also of larvae devoid of a water-vascular system. Proc. Roy. Soc., Ser. B, **90** (*fide* Ohshima 1922).

MOORE, M.M., 1933. Notes of the development of the sea urchin *Temnopleurus hardwickii*. Sci. Rep. Tôhoku Imp. Univ., Biol., **8** : 263–276.

OHSHIMA, H., 1922. The occurrence of situs inversus among artificial-reared echinoid larvae. Quart. Journ. Microsc. Sci., **66** : 105–150.

ONODA, K., 1936. Notes on the development of some Japanese echinoids with special reference to the structure of the larval body. Jap. J. Zool., **6** : 637–654.

RUNNSTRÖM, J., 1912. Quelques observations sur la variation et la correlation cheq la larve de l'oursin. Bull. Inst. Ocean. Monaco, **247** (*fide* Ohshima 1922).

———, 1918a. Analytische Studien über die Seeigelentwicklung. III. Roux' Arch. Entw.-mech., **43** : 223–328.

———, 1918b. Analytische Studien über die Seeigelentwicklung. IV. Ibid., **43** : 409–431.

———, 1917–1918. Zur Entwicklungsmechanik der Larve von *Paraechinus miliaris*. Bergens Mus. Aarb., 14 (*fide* Ohshima 1922).

VON UBISCH, L., 1913. Die Entwicklung von *Strongylocentrotus lividus* (*Echinus microtuberculatus*, *Arbacia pustulosa*) Zeit. wiss. Zool., **106** : 409–448.

EXPLANATION OF PLATES
LIST OF ABBREVIATIONS

a	anus
ala	antero-lateral arm
cm	cell mass
da	dorsal arch
de	dorsal epaulette
dp	dorsal pore
es	spine formed on the body surface of the larva
h	hydrocoele
isp	pointed spine formed in the amniotic cavity
iss	square-topped spine formed in the amniotic cavity
lacv	left anterior coelomic vesicle
lpcv	left posterior coelomic vesicle
m	mouth
o	oesophagus
p	pedicellaria
pc	pore canal
pda	postero-dorsal arm
poa	post oral arm
pra	pre-oral arm
pt	primary tube foot
rps	rudiment of the pedicellaria or spine
rt	rudiment of the tube foot
s	stomach
sp	small process budded off from the cell mass
sph	spheridium
st	secondary tube foot
ve	ventral epaulette

PLATE VII

Fig. 1. Larva, nine days old, viewed from abanal surface. ×83. Dorsal pore opens. Two mesenchymal masses appear at the both sides.

Fig. 2. Larva, 11 days old, viewed from the abanal surface. ×83. Rudiment of the pre-oral spicule appears. Left coelomic vesicle divides into two parts, anterior and posterior.

Fig. 3. Larva, 14 days old, viewed from the abanal surface. ×83. Hydrocoele arises. Epaulettes develop.

PLATE VIII

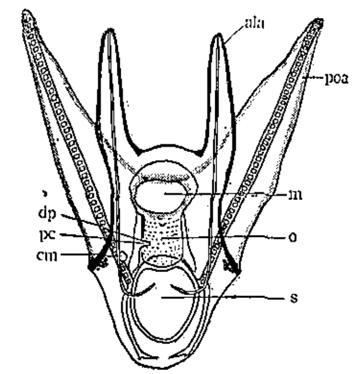
Fig. 1. Larva, 25 days old, viewed from the anal surface. × 60. Vesicle grown from the cell mass covers the hydrocoele. Postero-lateral processes develop. Rudiments of the pedicellaria and spine are not shown as hidden behind the posterior pole.

Fig. 2. Larva, 29 days old, viewed from the anal surface. ×60. Tube feet become grown in size.

PLATE IX

Fig. 1. Larva, 33 days old, viewed from the abanal surface. ×60. Spines and pedicellariae develop.

Fig. 2. Actinal view of a just metamorphosed imago. ×72. The arrow points to the anterior direction of the larva.



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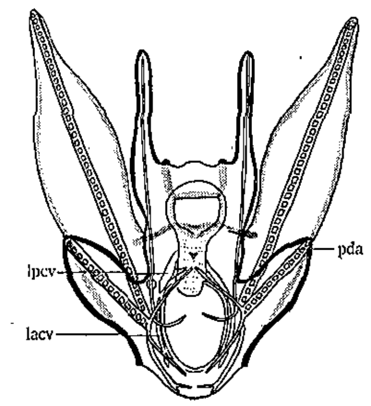

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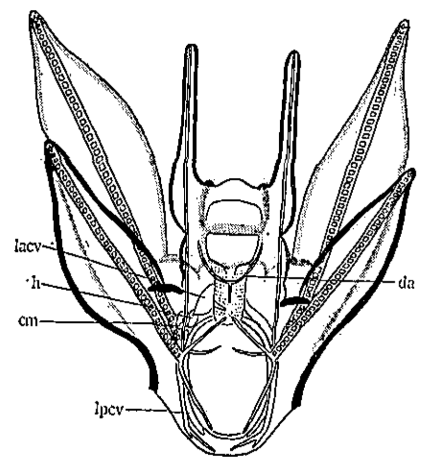

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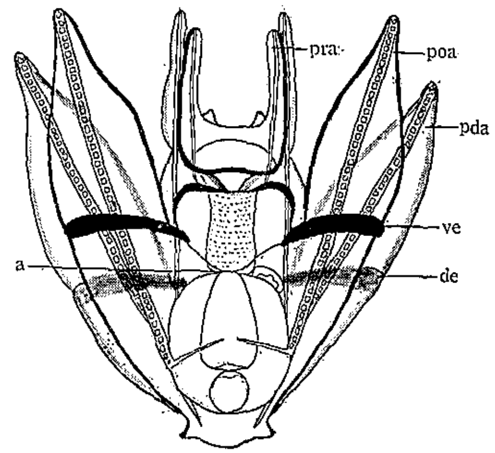

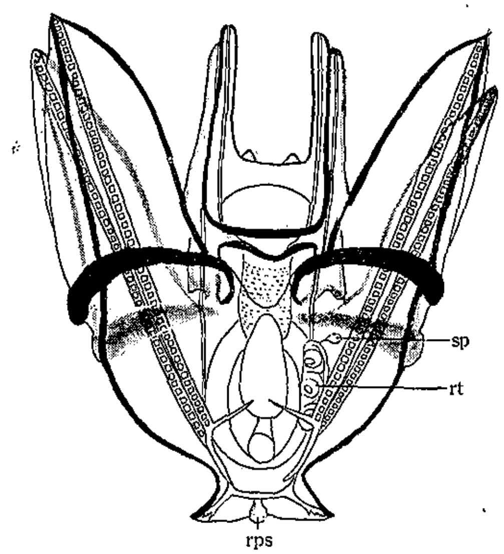

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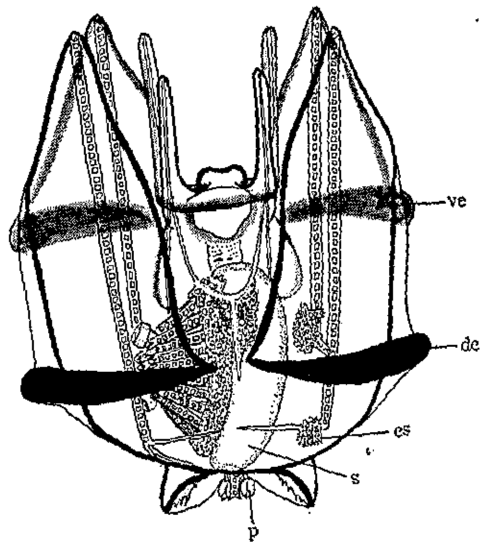

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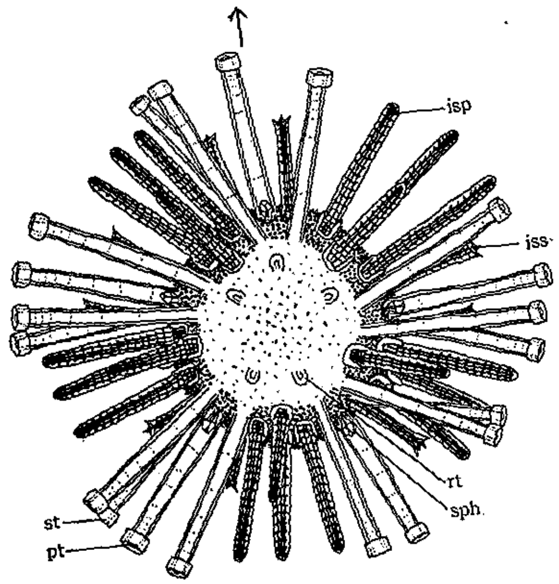

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