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THE FATES OF ISOLATED BLASTODERM CELLS OF SEA URCHIN BLASTULAE AND GASTRULAE INSERTED INTO THE BLASTOCOEL[1]

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Recently, some attempts have been made to approach the problem of morphogenetic movement in the sea urchin embryo by tracing the movements of the embryonic cells (Dan and Okazaki 1956; Gustafson and Kinnander 1956a, b, 1960; Gustafson and Wolpert 1961a, b, c; Kinnander and Gustafson 1960; Okazaki 1960; Okazaki, Fukushi and Dan in press) and by histochemistry (Motomura 1960).

In this paper the developmental potencies of blastoderm cells, such as primary mesenchyme cells, endodermal cells, ectodermal cells and ectodermal cells from Li-embryo, of sea urchin blastulae and gastrulae in the blastocoel of animal half larvae are described. On the secondary mesenchyme cells the potency is tested by removing the primary mesenchyme cells from the whole larvae. As the results a remarkable regulation in the secondary mesenchyme cells, the cells of the archenteron and in the lithium-treated ectodermal cells was observed. The relation between the implant and the host tissue is also discussed.

Thanks are due to Professor Isao Motomura for his guidance and to Professor Sven Hörstadius and Professor Eturo Hirai for his valuable criticism.

MATERIAL AND METHOD

Two species of sea urchins, *Strongylocentrotus nudus* and *Glyptocidaris crenularis*, collected at Asamushi were used as the materials.

The operations were carried out in Ca-free sea water with glass needles under the binocular microscope.

In the case of primary mesenchyme cells, the larvae were centrifuged at 4000G for 15 minutes at the stage when the mesenchyme cells were shed into the

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blastocoel. The vegetal half of the larvae in which the primary mesenchyme cells were pushed to the animal pole side were removed by cutting at the equatorial region. The animal half thus treated and containing the primary mesenchyme cells was cultured in normal sea water.

The experiments of implantation of endoderm, pressumptive apical plate as well as the presumptive ectoderm of the lithium-treated larvae were carried out in the following way. The animal half of the early mesenchyme blastula was cut in Ca-free sea water and used as the host. The middle part of the well developed archenteron of the gastrula, the presumptive apical plate of the early mesenchyme blastula taken out from normal or the lithium-treated larvae was inserted into the animal half through the cut opening. As the insertion was carried out in normal sea water, the opening closed soon after the operation. The lithium-treated larvae used as the donner were cultured in sea water containing six percent of 0.5 M LiCl from ten minutes after fertilization till the time of operation. In the experiment of the secondary mesenchyme cells, the primary mesenchyme cells were first removed by shaking moderately in Ca-free sea water at the early mesenchyme blastula stage. The primary mesenchyme cells attaching to the vegetal wall run out through the opening of the vegetal wall, which is broken by the above mentioned treatment. The larvae without primary mesenchyme cells were cultured further in normal sea water, in which the opening of the vegetal wall closed soon. In the other series of the experiments, the primary mesenchyme cells were sucked out with capillary through the wall of the animal pole of the primary mesenchyme blastula packed in a narrow glass tubing in a direction parallel to the animal-vegetal axis.

RESULTS

I. Implantation of primary mesenchyme cells into the animal half.

The differentiation of the animal halves with or without the primary mesenchyme cells was observed (Table 1). After the closure of the cut opening of the animal halves the implanted primary mesenchyme cells migrated toward the vegetal side. The thickness of the ectodermal wall was reduced, as the size of the larvae increased. The spicules were usually formed at the site of the migrated primary mesenchyme cells. The shape of the spicules was normal or deformed uniting with two lateral spicules at the vegetal side (Fig. 22B). The spicules were formed in the animal half with implanted primary mesenchyme cells in all cases, while it was not the case in the majority of the animal half only. In the latter case the size of the spicule was small, probably due to the presence of a few mesenchyme cells of unknown origin. The archenteron was rarely formed both in the animal half only or in the animal half containing the implanted primary mesenchyme cells (Table 1).

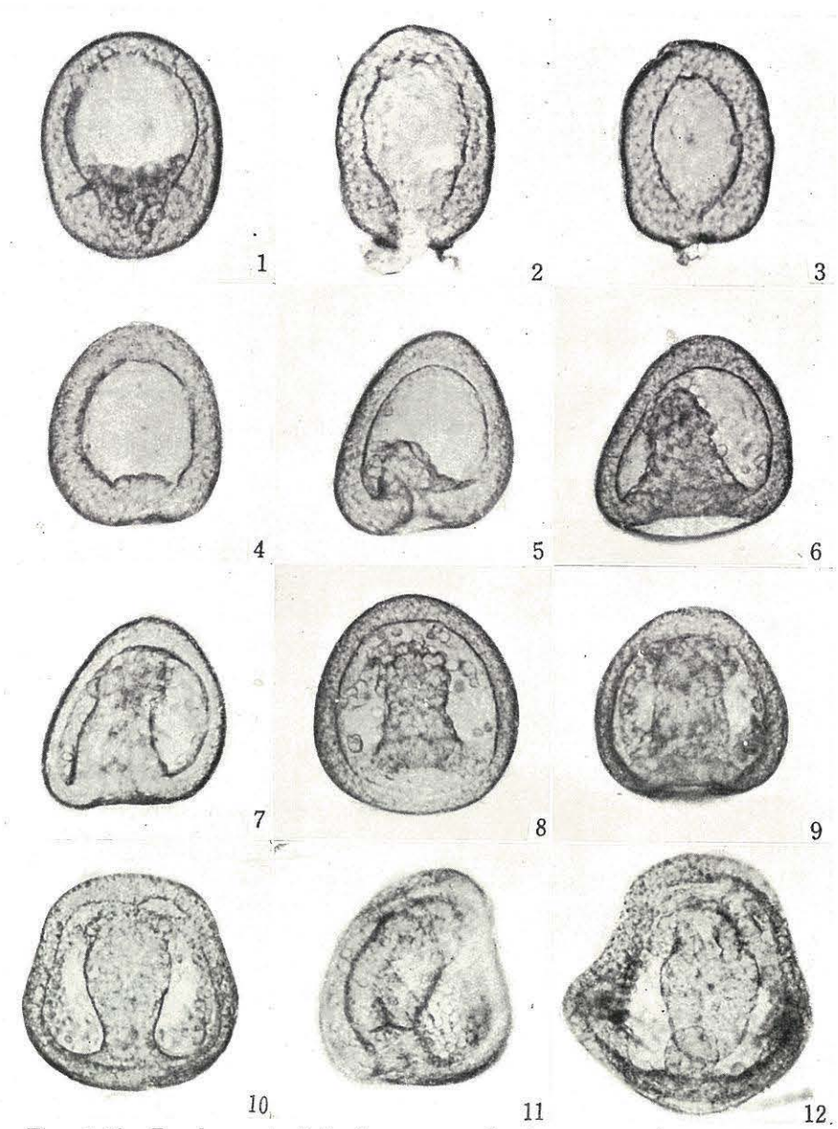
Table 1

Development of animal halves with or without primary mesenchyme cells in the eggs of *Strongylocentrotus nudus*.

Experiments	Hours after operation	Larvae without spicule and gut	Lar. with spicule only	Lar. with gut only	Lar. with spicule and gut	Total
(A) Animal half	24	9	1	1	0	11
	48	8	1	1	1	11
(B) Animal half with primary mesenchyme cells	24	0	5	0	1	6
	48	0	5	0	1	6

II. The role of the secondary mesenchyme cells in the larvae without the primary mesenchyme cells.

With the above mentioned method the primary mesenchyme cells were removed from the early mesenchyme blastula soon after the shedding of primary mesenchyme cells into the blastocoel. The procedure was easily carried out at the mentioned stage in *Glyptocidaris*, in comparison with the later stages. The larvae without primary mesenchyme cells were picked up, and cultured in normal sea water. In the embryos destitute of primary mesenchyme cells the invagination proceeded normally. The shedding of the secondary mesenchyme cells was also observed at the tip of the invaginated archenteron, where the secondary mesenchyme cells emitted the pseudopodia in diverging directions (Fig. 4). Accordingly, up to the present stage, the movement of archenteron as well as the secondary mesenchyme cells was normal. Later, the secondary mesenchyme cells showed a remarkable regulative movement. Namely, some of the secondary mesenchyme cells dispersed in the blastocoel, and migrated across the blastocoel toward the vegetal side stretching long pseudopodium in each cells (Figs. 6–9). The majority of the dispersed secondary mesenchyme cells settled on the lateral wall of the vegetal side, where the primary mesenchyme cells aggregated in the normal embryo. The ring of the mesenchyme cells thus formed was poor in comparison with the ring, which was formed by the primary mesenchyme cells in the normal development. The secondary mesenchyme cells retracted their pseudopodia after settling at the lateral wall. Even at this stage, a few secondary mesenchyme cells remaines at the dorsal as well as the ventral wall protruding long pseudopodia into the blastocoel. Small spicules appeared at the centre of the secondary mesenchyme cell mass on both sides of the lateral wall (Fig. 11). Further development of those embryos was nearly normal forming normal pluteus with arms and spicules (Fig. 12), although the development was remarkably retarded in comparison with the



Figs. 1–12. Development of the larva removed primary mesenchyme cells. Figs. 4–12 were followed in the same material. Fig. 1. Early mesenchyme blastula. Before operation. Fig. 2. Immediately after operation. Aperture of the vegetal wall opening. Fig. 3. Opening closed. Fig. 4. Initiation of invagination. Primary mesenchyme cells completely absent. Fig. 5. Secondary mesenchyme cells appear. Fig. 6. Secondary mesenenchyme cells coming down toward vegetal side. Fig. 7. Archenteron tip reaches to the animal pole. Side view. Fig. 8. The same as Fig. 7. Dorsal view. Fig. 9. Mesenchyme cells migrating and taking an arrangement at the lateral side. Fig. 10. More advanced stage than Fig. 9. Fig. 11. Showing the triradiate spicule formed by secondary mesenchyme cells. Fig. 12. 45 hours after operation. Coelomic vesicles formed.

normal one. The above mentioned results show that the secondary mesenchyme cells have a remarkable regulative potency in the embryo, in which the primary mesenchyme cells were removed at the early stage of gastrulation.

III. The fate of endodermal cells inserted into the blastocoel of the animal half.

The isolated cells from the archenteron were adhesive each other forming a lump convenient for insertion procedure. Observations were carried out in nine cases. Twenty four hours after operation, the inserted cells were situated at the animal side forming a lump, and some of the cells had stretching pseudopodia. In many cases a cavity was observed at the centre of the lump of the inserted cells (Fig. 13). Control larvae, fertilized simultaneously were at the stage of early pluteus. The majority of the implanted archenteron cells tended to move to the lateral or vegetal side protruding pseudopodia, and formed the spicules at that region in all cases 48 hours after the operation, as in the case of the secondary mesenchyme cells (Figs. 14–15). Well developed archenteron was observed in two cases. In two cases the archenteron was small.

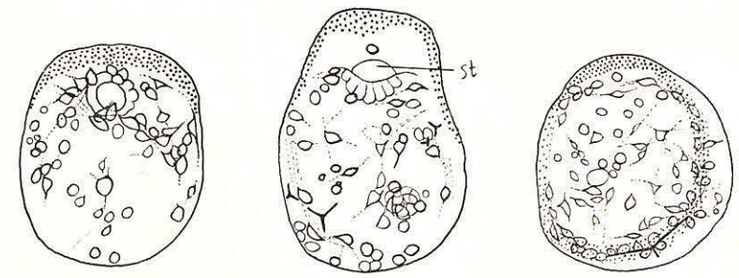


Fig. 13. Fig. 14. Fig. 15.

Figs. 13–15. Animal halves with isolated endodermal cells in the blastocoel. Inserted cells have a tendency to lump each other. Cells considerably stretch their pseudopodia and form spicules. Fig 13. 24 hours after operation. Figs. 14–15. 48 hours after operation.

IV. The fate of the presumptive ectodermal cells inserted into the blastocoel of the animal half.

The inserted ectodermal cells dispersed in the blastocoel and increased in number attaching to the ectodermal wall (Figs. 16–18). Nearly one third of the inside wall of the ectoderm was covered by the implanted cells of various sizes at 48 hours after operation. No migration of the cells with pseudopoida was observed. The ectodermal wall of the animal half differentiates the ciliary band and stomodaeum as observed in the animal half without implanted cells. Well developed archenteron was observed in one case, and small ones in 13 cases.

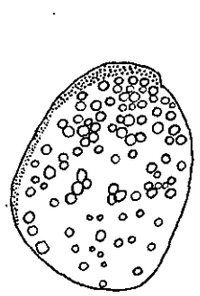
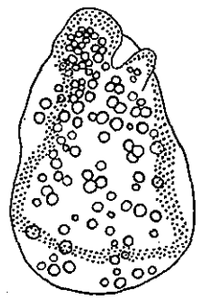


Fig. 16.



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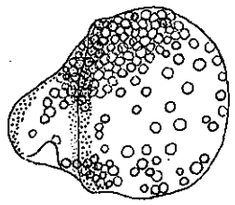

Fig. 18.

Figs. 16–18. Animal halves implanted the ectodermal cells in the blastocoel. Migration of the cells with pseudopodia not observed. The cells increase in number. Fig. 16. 24 hours after operation. Figs. 17–18. 48 hours after operation.

V. The fate of the presumptive ectodermal cells obtained from lithium larvae in the blastocoel of the animal half of normal embryo.

In all cases the inserted cells formed the pseudopoida and the spicules (Figs.

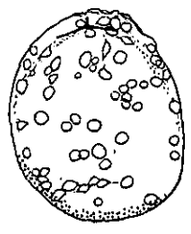

Fig. 19.

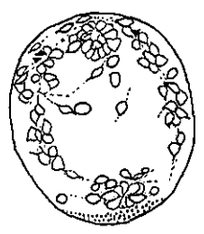

Fig. 20.

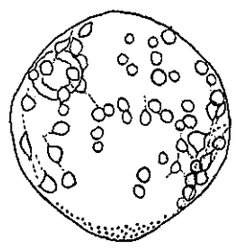

Fig. 21.

Figs. 19–21. Animal halves implanted the ectodermal cells obtained from Li-larvae in the blastocoel. Inserted cells show the characters similar to endodermal cells in the formation of pseudopodia and spicules. Fig 19. 24 hours after operation. Figs. 20–21. 48 hours after operation.

19–21). The process of spicule formation was nearly the same as in the cases of implantation of endodermal cells or of extirpation of the primary mesenchyme cells, described in 2, and 3, but it was slightly retarded in comparison with the two latter cases. The bilaterality was observed in the formation of cell aggregates and spicules at the lateral side. The archenterons were formed in three of seven cases.

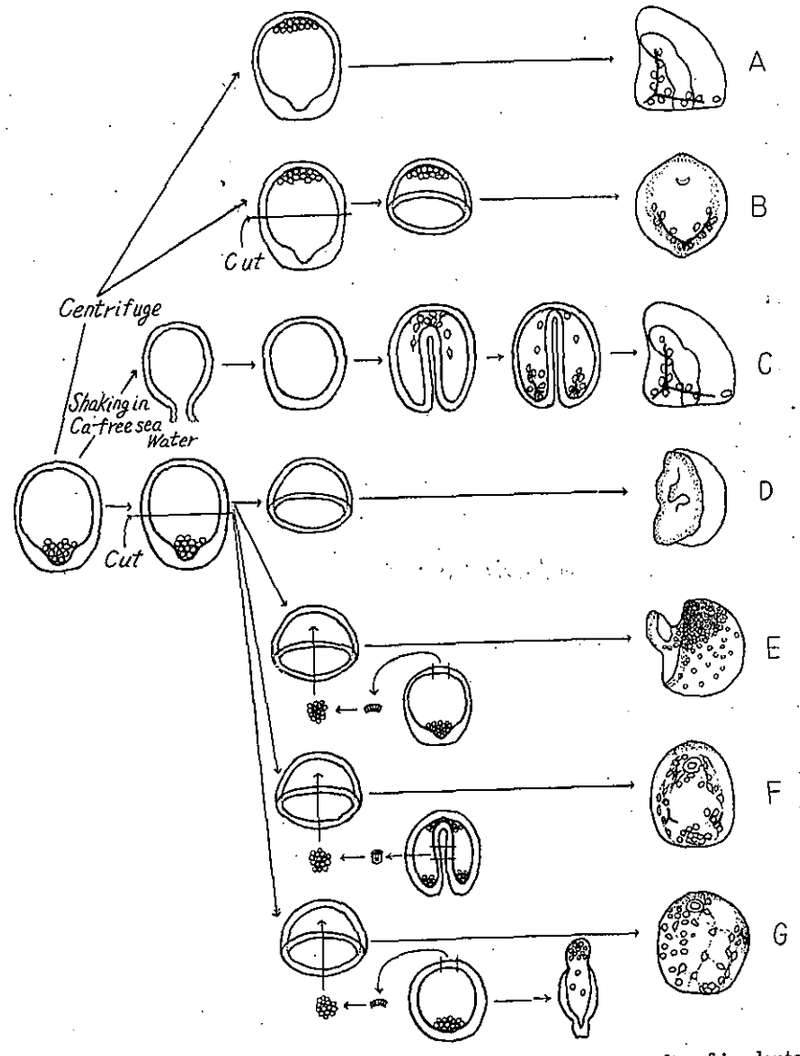

Fig. 22. Schematic representation showing the procedures and results of implantation experiments of blastoderm cells from blastulae and gastrulae in the blastocoel of sea urchin larvae. A. Whole larva with the primary mesenchyme cells amassed at the animal pole by the centrifugal force. B. Animal half cut off from the blastula of experiment A. C. Larva removed primary mesenchyme cells. D. Animal half cut off at the early mesenchyme blastula. E. Animal half implanted the ectodermal cells in the blastocoel. F. Animal half implanted the endodermal cells in the blastocoel. G. Animal half inserted the ectodoermal cells from Li-larvae.

DISCUSSION

Recently, Motomura (1960) has shown histochemically that ectodermal cells of the vegetal region secrete mucosubstance to hold the primary mesenchyme cells. Okazaki, Fukushi and Dan (in press) have observed fan-shaped optical patterns of the ectoderm corresponding to the thickest wall where the primary mesenchyme cells aggregate. These patterns appear soon after hatching and disappear after the completion of invagination. Primary mesenchyme cells once amassed at the animal pole in the early mesenchyme blastula by centrifugal force take ultimately the normal position in the whole larvae (Okazaki, Fukushi and Dan, in press). In the present experiment it was ascertained that the primary mesenchyme cells displaced to the animal side by the centrifugal force migrate again to the vegetal side of the animal half embryo, and that they form spicules at the site of migration. The result agrees with the observations of von Ubisch (1933, 1936) in his implantation experiments of micromeres into the isolated animal half. As mentioned above, the secondary mesenchyme cells migrate to the vegetal side, and form the spicules at that place when the primary mesenchyme cells are removed at the stage of mesenchyme blastula. These facts suggest that a definite part of the ectodermal wall prepares a condition favorable to the aggregation and the arrangement of the mesenchyme cells.

Okazaki (1960) reported from her observation in normal larvae that all the primary mesenchyme cells contribute to the formation of the skeletal matrix, but she could not deny the possibility of the presence of specialized matrix-forming cells from her experiments. The facts reported in this paper show that not only the primary mesenchyme cells but also the secondary mesenchyme cells as well as the cells of the archenteron have the potency to form spicules.

Hörstadius (1936) has shown that the animal half isolated in the 32-cell stage invaginates and grows up to pluteus when the cells of the vegetal side of the animal half treated with lithium chloride are implanted at the vegetal side. In this case the fragments are incorporated in the blastula wall as a part of vegetal wall. Similar results were observed when such fragments were implanted in the blastocoel of the animal half isolated in the blastula.

In the writer's experiments of the implantation of the ectodermal cells, two important facts were observed. The ectodermal cells taken from the normal mesenchyme blastula did not show a remarkable differentiation forming cell debris in the blastoceol. On the other hand, the ectodermal cells taken from the lithium-treated larva showed a tendency of mesodermalization, such as protrusion of pseudopodia and formation of spicules. This shows that the nature of the cells of the animal pole can be changed or approached to the nature of the primary mesenchyme cells, namely to the nature of cells of the vegetal end, by the effect of

lithium ions. Accordingly, the results show a remarkable tendency of vegetalization of animal cells, while the tendency of this extent is not observable in the experiment of lithium treatment in the whole embryo.

SUMMARY

1. The developmental potencies of blastoderm cells, such as primary mesenchyme cells, endodermal cells, ectodermal cells and ectodermal cells from Li-embryo, of sea urchin blastulae and gastrulae in the blastocoel of animal half larvae were described. On the secondary mesenchyme cells the potency was tested by removing the primary mesenchyme cells from the whole larvae.

2. The primary mesenchyme cells, which were amassed at the animal pole by centrifugal force, migrate toward the vegetal side in the animal half larvae and form the spicules at the site of migration.

3. When the primary mesenchyme cells are removed at the stage of early mesenchyme blastula, the secondary mesenchyme cells migrate to the vegetal side and form the spicules at the place, corresponding to the place where the primary mesenchyme cells are arranged in normal development.

4. The majority of implanted archenteron cells move to the lateral or vegetal side protruding pseudopodia and spicules are formed at that site.

5. The inserted ectodermal cells disperse in the blastocoel of the animal half and increase in number attaching to the ectodermal wall. Migration of the cells with pseudopodia was not observed.

6. The inserted ectodermal cells from Li-embryo form the pseudopodia and spicules in the blastocoel of the animal half. The process of spicule formation is nearly the same as in the case of implantation of endodermal cells.

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