FURTHER STUDIES ON CELL DIVISION WITHOUT MITOTIC APPARATUS IN SEA URCHIN EGGS

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ABSTRACT

A large quantity of paraffin oil, sucrose solution, or sea water was injected into the eggs of the heart urchin *Clypeaster japonicus* shortly before the onset of the first cleavage. The injected oil became spherical, pushing the mitotic apparatus aside. The sucrose solution mixed with the protoplasm and caused disintegration of the mitotic apparatus, and the sea water formed a vacuole at the center of the cell. In all these cases, cleavage may take place almost normally in spite of the absence of the mitotic apparatus or its displacement within the cell. In some eggs, furrowing may take place when more than fifty per cent of the endoplasm has been replaced with sea water before onset of cleavage.

In a previous paper (3), it was shown that cleavage may take place in sea urchin eggs, from which the mitotic spindle or both the spindle and the asters have been removed before onset of cleavage (metaphase or anaphase of mitosis), and it was shown further that the position of the furrow is not affected by the removal or by the displacement of the mitotic apparatus within the egg. From these results, it was concluded that, after a definite stage, the furrowing results from mechanical activity of the cortex which is not modified by presence or absence of the mitotic apparatus. Recently, the author succeeded in some experiments further confirming the above conclusion, and these are described in the present paper.[1]

METHODS AND RESULTS

As material, the fertilized eggs of the heart urchin, *Clypeaster japonicus*, were used. The eggs were deprived of the fertilization membranes and the hyaline layers by treatment with 1 M urea solution shortly after insemination, and they were then left in a Syracuse dish filled with sea water. Before experimentation, the eggs were put into a manipulation chamber devised by the author (3). Unless otherwise stated, manipulation was carried out in eggs shortly before onset of cleavage (anaphase). The methods of the microinjection and of the suction of the egg protoplasm have already been mentioned elsewhere (3, 5).

Experiment I: Microinjection of Paraffin Oil

In order to displace the mitotic apparatus within the cell, a large quantity (1 to 4 $\times$ 10^{-7} ml, corresponding to 15 to 60 per cent of the cell volume) of paraffin oil was injected into the central portion of the egg. The oil became spherical, pushing the spindle and the asters aside (Fig. 1 *a*). Cleavage took place in most of the eggs, and the cleavage plane was not modified; *i.e.*, the furrow appeared in the position already determined before the microinjection. When the furrow advanced, the oil drop became oval, as shown in Fig. 1 *b*. It must be noted that the deformation of the drop occurred before the drop came into contact with the cortex, which is believed to be a few microns in thickness (*e.g.* reference 4). This fact may indicate either that viscosity of the endoplasm is so high that the oil drop is deformed by the

[1] A preliminary account of the results of this paper has been given in Japanese (6).

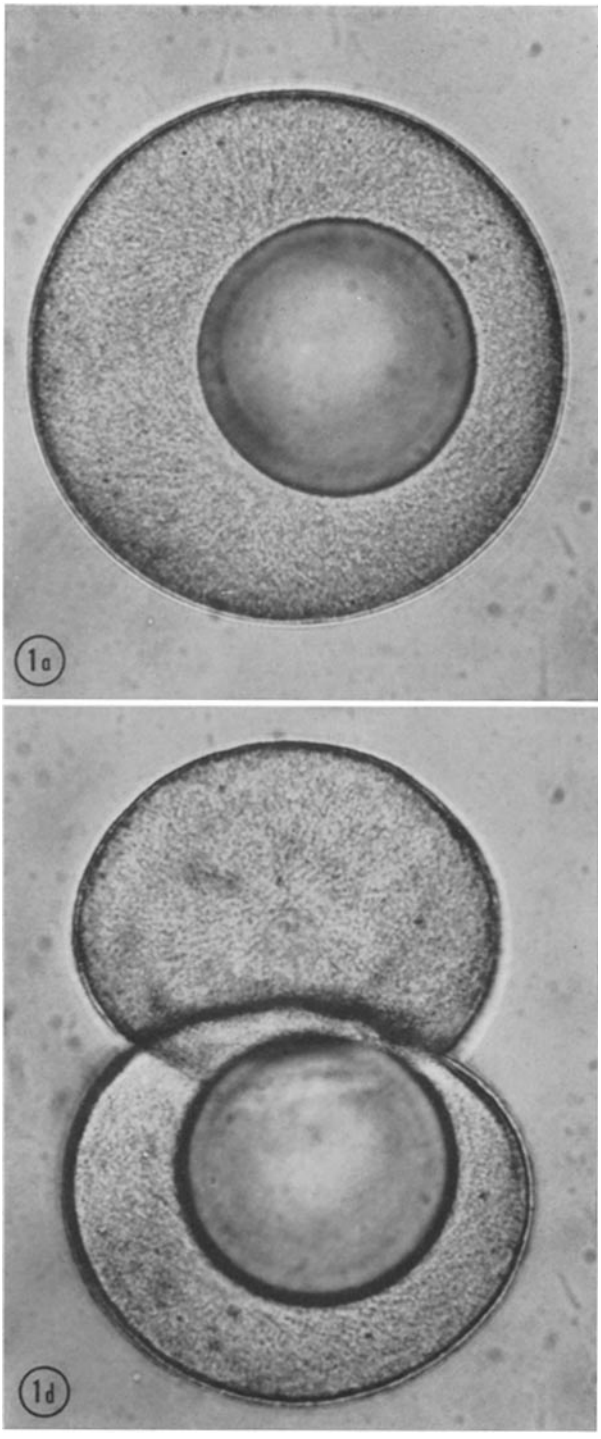
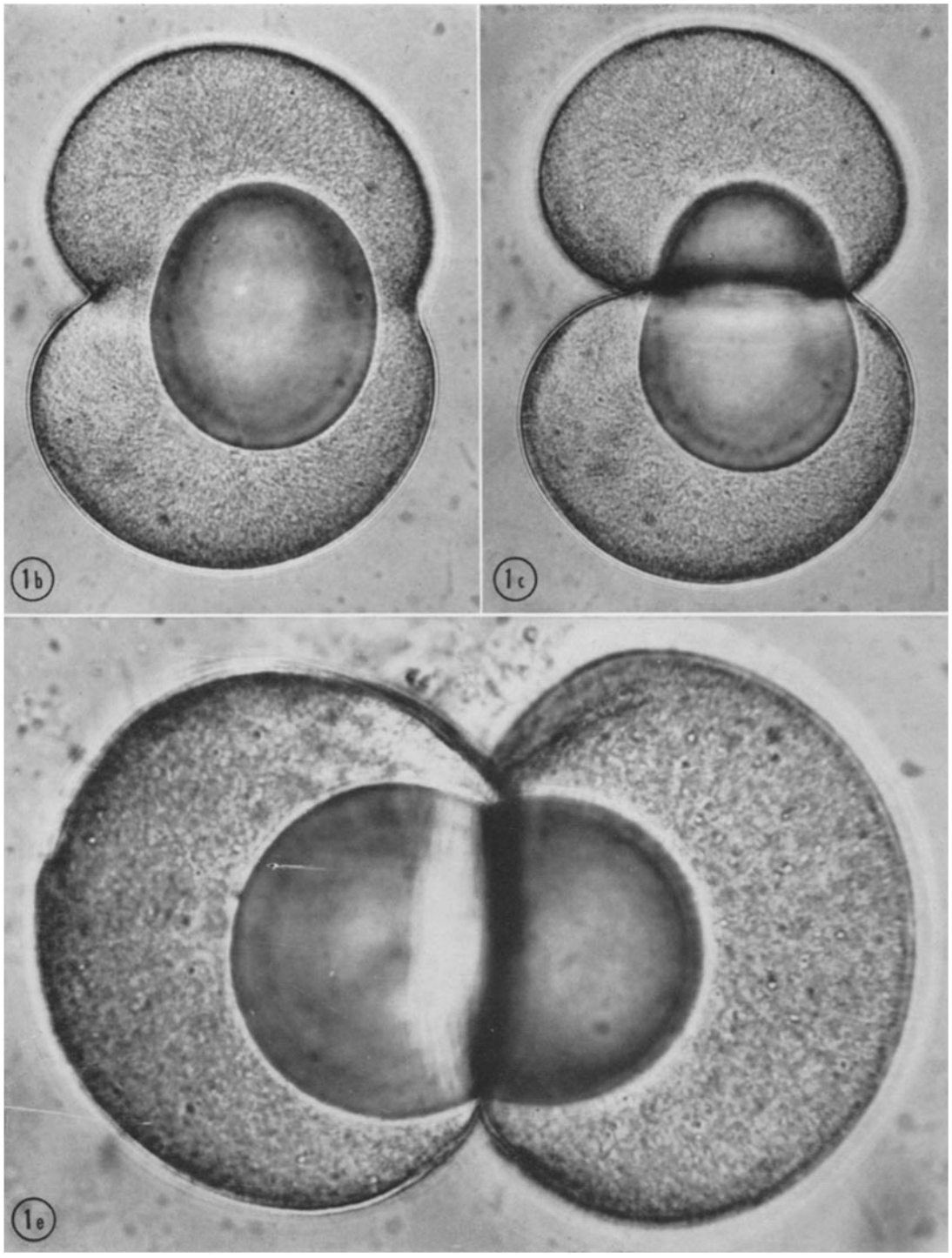


FIGURE 1 Cleavage of *Clypeaster* eggs injected with paraffin oil. 26°C.

a to *d*. Successive stages of the same egg. The oil drop, which had been spherical before onset of cleavage, is deformed and then is pushed out of the cleavage plane as the furrow advances. × 550. *e*. Cleavage of an egg in which the injected oil is constricted by the trough of the furrow. × 820.


1b
1c
1e

movement of the endoplasm during cleavage or that some rigid structures exist within the endoplasm connecting the oil drop and the cortex. As the furrow advanced further, the oil drop was constricted by the trough of the furrow (Fig. 1 *c*, *e*). When the cleavage plane passed through the exact center of the drop, it formed a dumb-bell (Fig. 1 *e*). In this case, although the furrow advanced for a while, it afterwards regressed and the drop resumed a spherical shape. On the other hand, when the cleavage plane did not pass the center of the drop, the larger half of the constricted drop enlarged more and more while the smaller half became smaller with advance of the furrow, and, in consequence, the drop was pushed out of the cleavage plane (Fig. 1 *c*, *d*). In this case, the egg was completely divided into two blastomeres, one of which contained the oil drop (Fig. 1 *d*). It was never observed in the present experiment that the oil drop in the cleavage plane was cut into two by the advancing furrow, as Chambers (1) observed in the case of *Lytechinus* eggs. Irrespective of success or failure of cleavage, daughter nuclei appeared in the egg during cleavage (Fig. 1 *c*, *d*).

Experiment II: Microinjection of Sucrose Solution

In the next experiment, isotonic (0.75 M) sucrose solution[2] was injected into the egg. The solution became mixed with the protoplasm (Fig. 2 *a*) and was fairly rapidly diffused away. When the protoplasm and the sucrose solution came into contact, the mitotic apparatus was rapidly disintegrated, until finally all the radiate structures disappeared. Upon close observation, active Brownian movement of the protoplasmic granules, indicating complete solation of the mitotic apparatus, was recognized in the central portion of the egg. The cleavage furrow appeared in the presumptive position, and the eggs often completely divided (Fig. 2 *b*). The mitotic apparatus, which disappeared at the time of the microinjection, never reappeared, and no resting nucleus was recognized within the egg during, as well as after, cleavage.

[2] Although the volume of the injected solution was not exactly estimated, it was similar to that of the injected oil (1 to 4 $\times$ 10^{-7} ml).

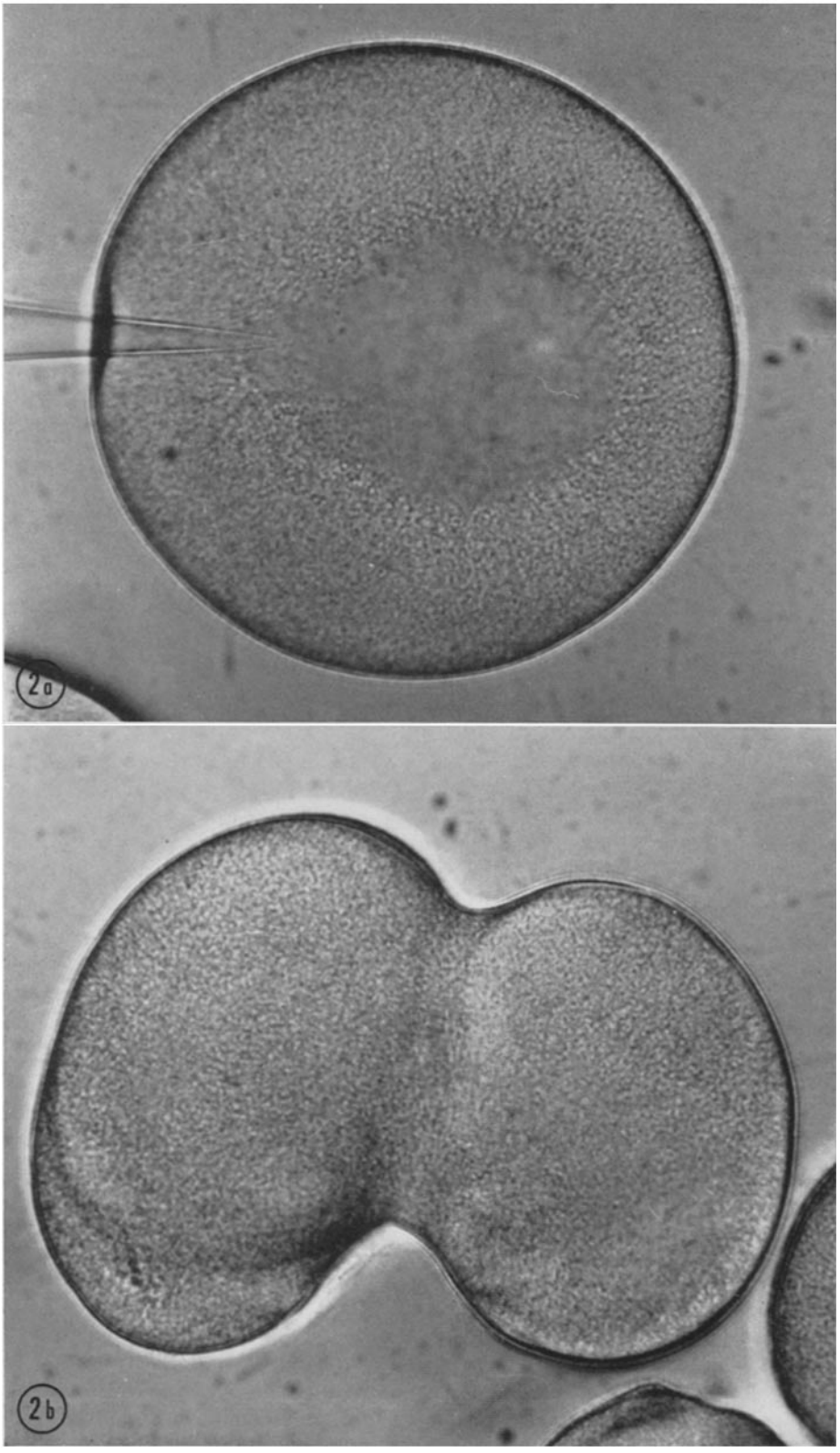
Experiment III: Microinjection of Sea Water

When sea water[2] was injected into the egg, a precipitation membrane was formed around the injected sea water, and, in consequence, the center of the cell was occupied by a large vacuole (Fig. 3 *a*). It seems likely that the injected sea water had become mixed with a part of the egg protoplasm before the formation of the precipitation membrane, because many protoplasmic granules were observed in the vacuole. The mitotic apparatus was not recognized in the protoplasm after the injection, probably because the injected sea water had caused its disintegration. Furrowing took place in most of the eggs, and they often completely divided. The position of the furrow and the mode of cleavage were scarcely modified by the microinjection. The vacuole, which had been slightly oval soon after the microinjection (the long axis being perpendicular to the cleavage plane (Fig. 3 *a*)), became more elongated when the furrowing was commenced. Then it was constricted by the advancing furrow (Fig. 3 *b*), and finally it was divided into two. When sea water was injected into the egg during cleavage, the vacuole was often dumb-bell-shaped from the beginning. This fact may suggest that the protoplasm of the mitotic apparatus is more easily disintegrated than is the rest of the protoplasm. Neither the mitotic apparatus nor the resting nucleus appeared in the egg during and after cleavage.

Experiment IV: Replacement of the Endoplasm with Sea Water

A trial was made to examine whether the cleavage could take place when the endoplasm was replaced with sea water. Two micropipettes were used, one for suction of the endoplasm, and the other for microinjection of sea water. The results were almost the same as those of Experiment III: *i.e.*, a vacuole was formed at the center of the cell and it was constricted by the advancing furrow. It was observed that the furrow advanced for a

FIGURE 2 Cleavage of a *Clypeaster* egg injected with sucrose solution. 24°C.
a. Shortly after the injection. Central part of the mitotic apparatus has disappeared, while the peripheral part is still in process of disintegration. *b*. During cleavage. $\times$ 660.


2a
2b

while in some eggs in which more than fifty per cent of endoplasm containing the spindle had been replaced with sea water before onset of cleavage, although the furrow regressed afterwards, probably owing to the injurious effects caused by the drastic operation in the present experiment.

DISCUSSION

It has been shown in the present experiments that cleavage can take place in sea urchin eggs when the central part of the cell, *i.e.* the position of the mitotic apparatus in normal cells, is occupied by inanimate materials (Experiments I, III, IV), or when the mitotic apparatus is disintegrated by the effects of injected aqueous solutions (Experiments II, III, IV) after the anaphase of mitosis. This result may, together with the result of the previous paper (3), be crucial evidence against theories of cleavage based on the mechanical activity of the mitotic apparatus. The micrographs portraying the constriction of the oil drop or the vacuole by the advancing furrow (Fig. 1 *c*, *e*, and Fig. 3 *b*) may suggest that active contraction of the furrow cortex is important as a motive force of cleavage. Recent results of Dan and Kojima (2), who observed advance of the cleavage furrow in a piece of the cortex excised out of the potential furrow region of amphibian eggs, may also indicate importance of the furrow cortex in the process of cleavage of this material.

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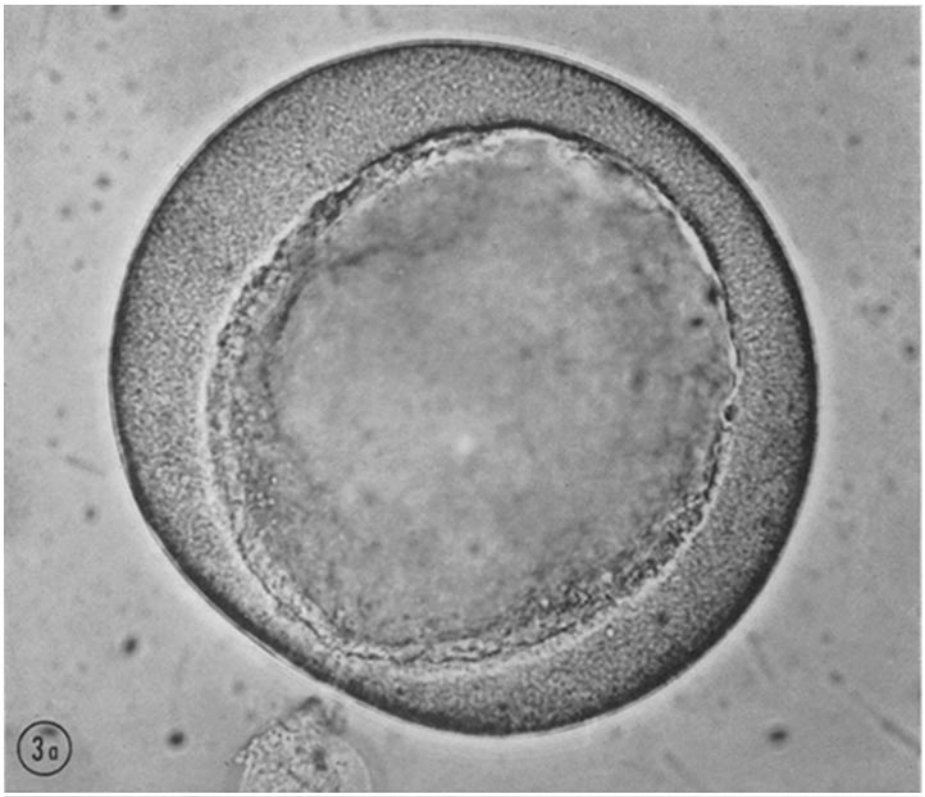
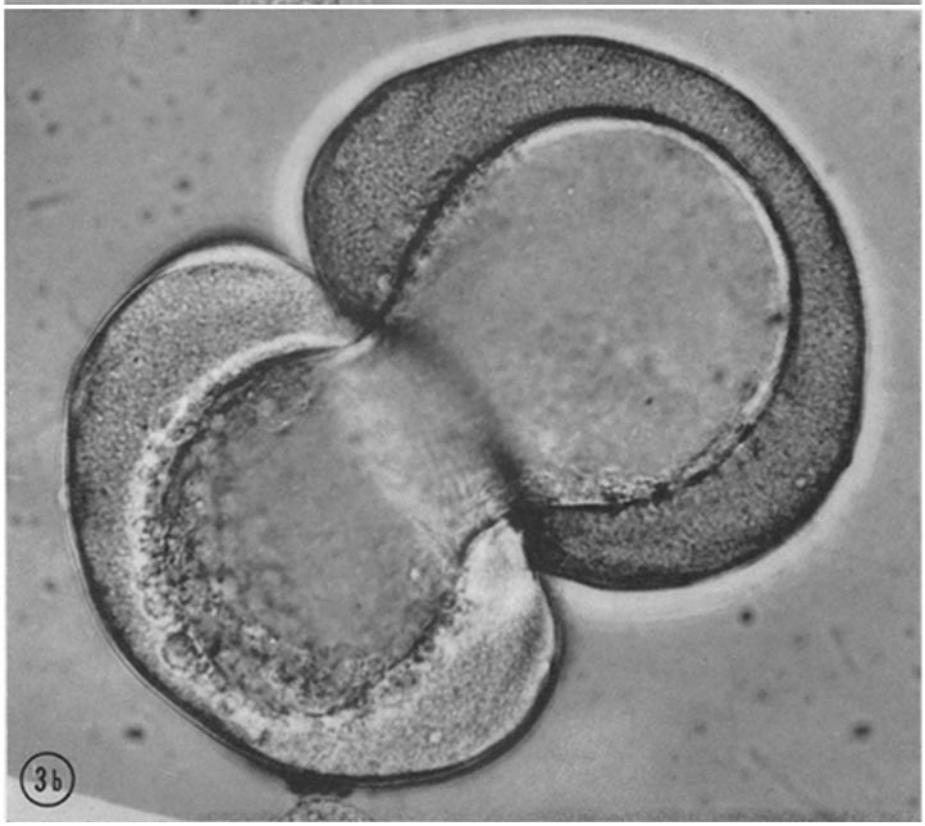
REFERENCES

1. CHAMBERS, R., *J. Cell. and Comp. Physiol.*, 1938, **12**, 149.
2. DAN, K., and KOJIMA, M. K., *J. Exp. Biol.*, 1963, **40**, 7.
3. HIRAMOTO, Y., *Exp. Cell Research*, 1956, **11**, 630.
4. HIRAMOTO, Y., *Embryologia*, 1957, **3**, 361.
5. HIRAMOTO, Y., *Exp. Cell Research*, 1962, **27**, 416.
6. HIRAMOTO, Y., *Kagaku*, 1963, **33**, 292 (in Japanese).

FIGURE 3 Cleavage of a *Clypeaster* egg injected with sea water. 26°C.
a. Before onset of cleavage. Central part of the cell is occupied by a large vacuole. The mitotic apparatus has been disintegrated. *b*. During cleavage. × 660.



3a



3b