SOME EFFECTS OF TEMPERATURE ON DEVELOPMENT IN THE SEA URCHIN ALLOCENTROTUS FRAGILIS

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The deep water sea urchin *Allocentrotus fragilis* occurs offshore at Pacific Grove at depths of 80 to 100 fathoms. The temperature of this environment is fairly constant at 8° C. During the past two years it has been possible, at this Station, to study the embryological development of this form under laboratory conditions. Normal development of the fertilized eggs proceeded at 7°–15° C. Lower temperatures were not tried. There was some variation at the upper limit, the eggs of some females not developing normally above 14° C. while others yielded normal embryos at 16° and 17° C. At ordinary room temperature (20° C.), however, cytoplasmic cleavage is not normal if it occurs at all. A study of this effect may throw some light on the behavior of cytoplasm and nucleus in cell division.

In order to determine whether the nucleus might be responsible for the failure of cleavage at 20° C., the viability of the nucleus was tested by putting it to develop in the hardy cytoplasm of *Strongylocentrotus purpuratus*. The eggs of this purple sea urchin develop normally at the higher temperature, and when fertilized with the sperm of *Allocentrotus fragilis* they segment and develop into normal plutei at 20° C. Similarly the eggs of the sand dollar *Dendraster excentricus* fertilized with the sperm of *Allocentrotus fragilis* segment normally at the tempo of *Dendraster*. Such hybridized eggs develop into plutei, which, in their form, show the cross to be a true one. Hence the male nucleus has taken part in development. Thus, while the cytoplasm of *Allocentrotus* is inactivated at a temperature of 20° C., the nucleus of this species, if given a proper medium, functions normally.

Visual proof of the fact that this nucleus also functions in the egg of its own species even at 20° C. is shown by putting fertilized eggs of *Allocentrotus* to develop at 20° C. Here the nuclei undergo regular division while the cytoplasm remains apparently inert, *i.e.,* undivided (Figs. 1 and 2). Now these eggs had formed normal fertilization membranes at the outset before being put at the higher temperature. Therefore there is no reason to suppose that here we are dealing with a Sugawara (1943) effect in which failure of the fertilization membrane to lift results in constriction of the egg to its original volume with the results that cleavage of the egg is inhibited but nuclear division proceeds. Eventually the eggs with which we are dealing, after many nuclear divisions, disintegrate. This experiment gives further proof that in the early stages the nucleus is more resistant to higher temperatures than its cytoplasm and carries out its divisions independently of the cytoplasm.

In the later stages of development, when the cytoplasm has become comminuted into the thousand odd cells of blastula and gastrula, the spatially relative situation of nucleus and cytoplasm is quite different. If now the advanced larvae of

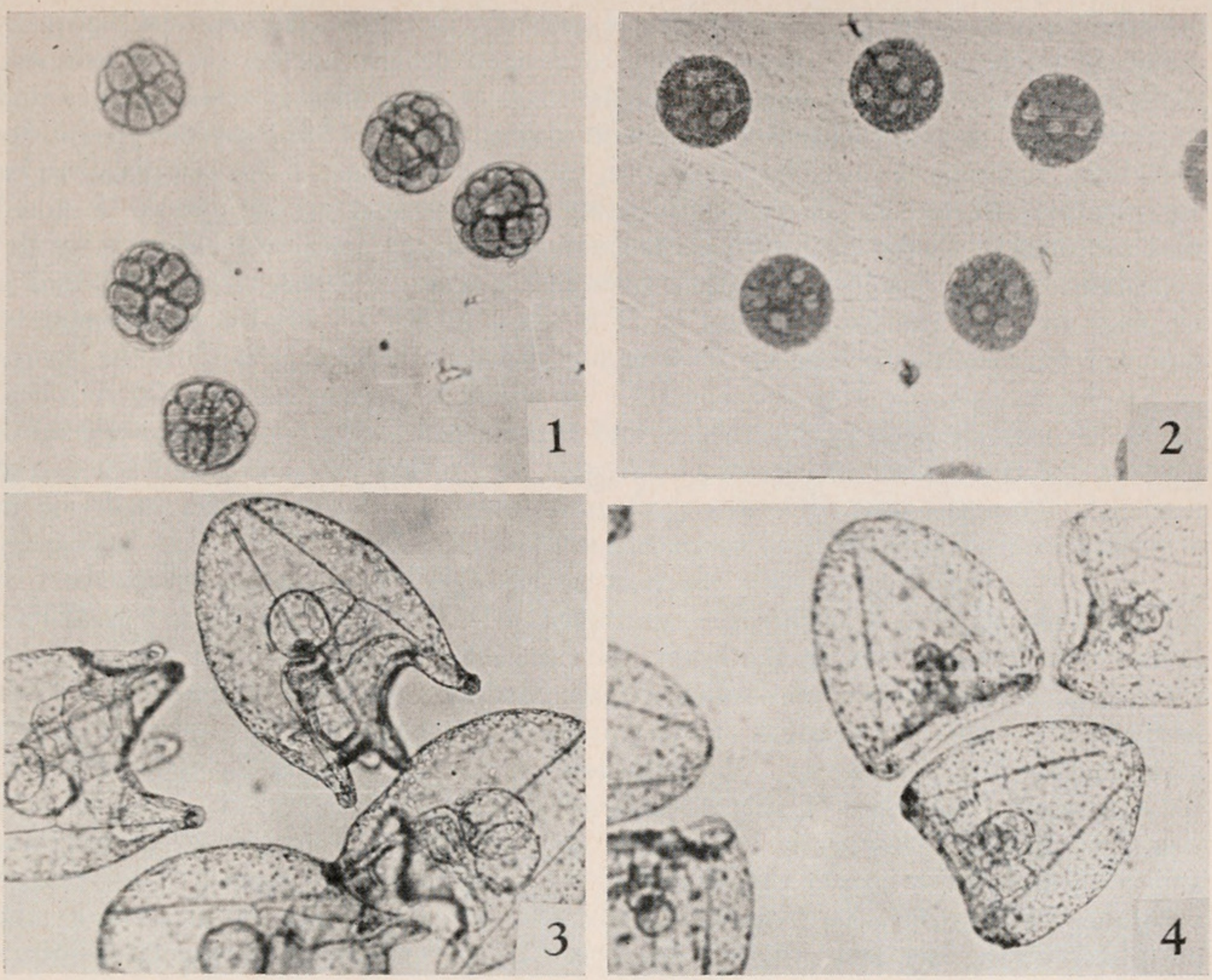

FIGURE 1. Normal 8–16 cell embryos at 15° C. FIGURE 2. Same at 20° C. FIGURE 3. Five-day pluteus developed at 15° C. FIGURE 4. Five-day plutei in which the temperature was changed to 20° C. after development to gastrulae at 15° C. Magnification 95 ×.

Allocentrotus were transferred from the cold room where they had developed to a warm room with a temperature of approximately 20° C., it was found that the higher temperature no longer exercised a lethal effect on the cytoplasm, but that such larvae reached the pluteus stage (Figs. 3 and 4). These plutei were smaller and less elaborate than the normal ones developed at the low temperature.

DISCUSSION

Generally speaking the problem of cleavage has been attacked from the point of view of colloidal behavior, the relation of cytaster formation to the constitution of the cell which is the substance of the egg. Cleavage is a process initiated by the entry of the sperm into the egg and the formation of the membrane. In artificial parthenogenesis the formation of the membrane is sufficient to initiate the development of cytasters and subsequent cleavage of the egg. A second factor has been suggested by Swann (1952) who postulates the active agents in cleavage to be catalytic substances released from the chromosomes of the sperm nucleus. These he has termed "structural agents."

In the foregoing experiments we have evidence that the differences in the interaction of nucleus and cytoplasm described depend on the nuclear-plasma relation. Thus in the early stages of development the mass of the cytoplasm compared to that of the nuclei is relatively enormous and the distance of the nucleus from the periphery, it seems reasonable to suppose, may be too great for the cytoplasm to be significantly affected by substances diffusing from the nucleus. It should be noted that the nucleus in the first division is dominated in the tempo of cleavage by the cytoplasm. This has been clearly shown in the case of *Dendraster* which has a cleavage time for the first division of 55 minutes at 20° C. If the experiment be made of enucleating the egg of *Dendraster* and then fertilizing it with the sperm of *Strongylocentrotus,* the cleavage time of which is approximately 100 minutes, the subsequent division of the nucleus and cytoplasm of the experimental egg takes place in the time characteristic of *Dendraster, i.e.,* of the cytoplasm and not of the nucleus. Thus the normally slow nucleus is forced by the cytoplasm to divide in a little more than half the time normal to it (Moore, 1933).

As to the difference in their reaction to the higher temperature on the part of the fertilized eggs of *Allocentrotus* contrasted with that of blastulae and gastrulae, it may be suggested that, in the latter, the nuclei are in such intimate contact with the cytoplasm that they confer some of their hardiness on it and are able to do this because of the close association of the two phases. Such an effect becomes understandable if we accept Swann's hypothesis as to the part played by the nucleus in the cleavage of the egg. Using Chambers' (1951) demonstration in the amoeba that the nucleus dynamically affects the edge of the cell next to it, Swann has suggested that the origin of the furrow in the first division is caused by a cleavage substance which diffuses from the chromosomes of the sperm nucleus to the periphery of the egg and initiates cleavage. In the present experiments Swann's hypothesis seems not to apply in the early stages of cleavage. In later stages, however, this hypothesis may give a reasonable explanation of the division of cells in advanced larvae at higher temperatures. Thus, while in the early stages of cleavage the cytoplasm dominates the formation of the furrow and the tempo of cleavage, the situation is altered later where successive divisions of the cytoplasm have brought the chromosomes into intimate relations with the cytoplasmic units. This would give play to the diffusion of cleavage substances from the chromosomes to the periphery as Swann has postulated. Since the effect of the higher temperature on the further development of blastulae and gastrulae is to make the resulting plutei defective, it seems reasonable to suggest that at the time of change only a part of the synthesis of structural elements has been completed and that the higher temperature to which they were subsequently exposed has inhibited the completion of structural processes essential to the form of the normal pluteus.

Summary

1. The eggs of the deep water sea urchin *Allocentrotus fragilis* develop normally to plutei under laboratory conditions at 7°–15° C.

2. At the higher temperature of 20° C. cytoplasmic division fails but the nuclei show characteristic mitotic figures.

3. The sperm nucleus of *Allocentrotus fragilis* functions normally at higher

temperatures in the eggs of *Strongylocentrotus purpuratus* and *Dendraster excentricus*.

4. Blastulae and gastrulae of *Allocentrotus fragilis* brought to a temperature of 20° C., which is lethal for the eggs and early division stages, develop into plutei of reduced size.

5. It is suggested that in the advanced larvae hardiness to the higher temperature is the result of the intimate association of nucleus and cytoplasm in the minute cells, and the synthesis of structural elements and processes at the lower temperature.

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