Reversal of Chemical Cleavage Inhibition in Echinoderm Eggs [1]

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ABSTRACT Many agents will, in low concentrations, reversibly block cytokinesis without seriously affecting mitosis. Frequently such agents visibly reduce the size of the mitotic apparatus. The purpose of this investigation was to determine whether failure to divide as a result of this treatment is causally related to reduction in size of the mitotic apparatus. Ether and ethyl urethane were used as blocking agents. *Hemicentrotus pulcherrimus* eggs fail to cleave when placed in either 0.75% ether or 0.06 M ethyl urethane in filtered sea water 30 minutes or more before cleavage time. Both agents reduce the asters. When eggs are constricted by partial removal of the fertilization membrane, the mitotic apparatus and surface are brought closer together. Constricted eggs developed furrows in concentrations of blocking agents that prevented cleavage in spherical eggs. Furrows were permanent when they coincided with the plane of artificial constriction. Cleavage in *Echinarachnius parma* eggs is blocked by 0.052 M ethyl urethane. The behavior of constricted, treated eggs is similar to that of *H. pulcherrimus* eggs. When the reduced mitotic apparatus of the treated cell is pushed to an excentric position, well-developed but usually temporary furrows appear. Hexanediol augments the *in vivo* mitotic apparatus. When eggs are exposed to combined treatment of 0.25% hexanediol and 0.052 M ethyl urethane about half will, in favorable cases, develop furrows. About half the furrows are permanent. In otherwise normal eggs hexanediol treatment slightly increases the maximum spindle-to-surface distance that permits furrowing. It appears that the cleavage blocking effects of ethyl urethane and ether in these experiments were partially reversed by measures that restore a more nearly normal geometrical relationship between the reduced mitotic apparatus and the surface.

Studies concerning the mechanism of animal cell division have frequently involved the application of agents that blocked cytokinesis without seriously interfering with mitosis. Agents whose effect is reversible were termed anesthetics (Heilbrunn, '20) or narcotics, and many experimental analyses of anesthetic effects were described in the early decades of this century. (See compilation of experimental work in Harvey, '56). In an early study E. B. Wilson ('01) observed that in etherized echinoderm eggs the size of the asters is reduced. Many other anesthetics were subsequently shown to have the same effect (Harvey, '56). The effectiveness of these agents as blocks to cytokinesis depends upon the time of application. Cytokinesis is blocked only if treatment is begun at metaphase or before despite visible diminution of the mitotic apparatus (Beams and Evans, '40; Swann and Mitchison, '53; Kuno, '54). But surface active agents suppress cleavage regardless of the time of application (Kuno, '54). Wilson ('01) correlated extent of furrowing with astral size and since then many experiments have indicated that asters play an important role in furrow establishment (Wolpert, '60; reviewed by Rappaport, '65; Rappaport, '69a). Wolpert ('63) speculated that failure of cytokinesis in anesthetized cells may originate in failure of the reduced mitotic apparatus to differentiate poles

[1] A portion of these results were reported at the 1969 meeting of the American Society for Cell Biology (Rappaport, '69b).

from the furrow region. The purpose of this investigation was to explore the relationship between furrow establishment and astral size.

It was found that the anesthetic effect of ether and of ethyl urethane were partially reversed when the surface was moved closer to the reduced mitotic apparatus. Hexanediol, which augments the size of the *in vivo* mitotic apparatus (Rebhun and Sawada, '69), can also partially reverse the anesthetic effect of ethyl urethane.

MATERIALS AND METHODS

In the sea urchin *Hemicentrotus pulcherrimus* ovulation was stimulated by replacing the coelomic fluid with 0.5 M KCl, and sperm were obtained by dissection of the testes. Gametes of the sand dollar *Echinarachnius parma* were obtained by 0.5 M KCl injection. Partially denuded eggs of both species were selected from a population that had been expelled through a hypodermic needle a few minutes after fertilization (Rappaport, '64). Manipulations were accomplished with Leitz micromanipulators and observed and photographed with an inverted microscope. The temperature of the sand dollar eggs was maintained at 19° or cooler.

RESULTS

When *Hemicentrotus pulcherrimus* eggs are immersed in a solution of 0.75% ether in filtered sea water 30 minutes before normal cleavage time, cytokinesis is blocked. In living cells the mitotic apparatus appears smaller than normal so that the distance from the outer visible limits of the asters to the surface is increased. In order to reduce this distance, eggs were artificially constricted by partially denuding them. They were distorted into a dumbbell shape as they were forced part way through an opening in the fertilization membrane. In unanesthetized eggs the mitotic apparatus shifts as it grows until it usually straddles the constriction at the time of cytokinesis (Rappaport, '64). The smaller mitotic apparatus of etherized eggs, however, often remains on one side of the constriction. Furrows often develop in constricted

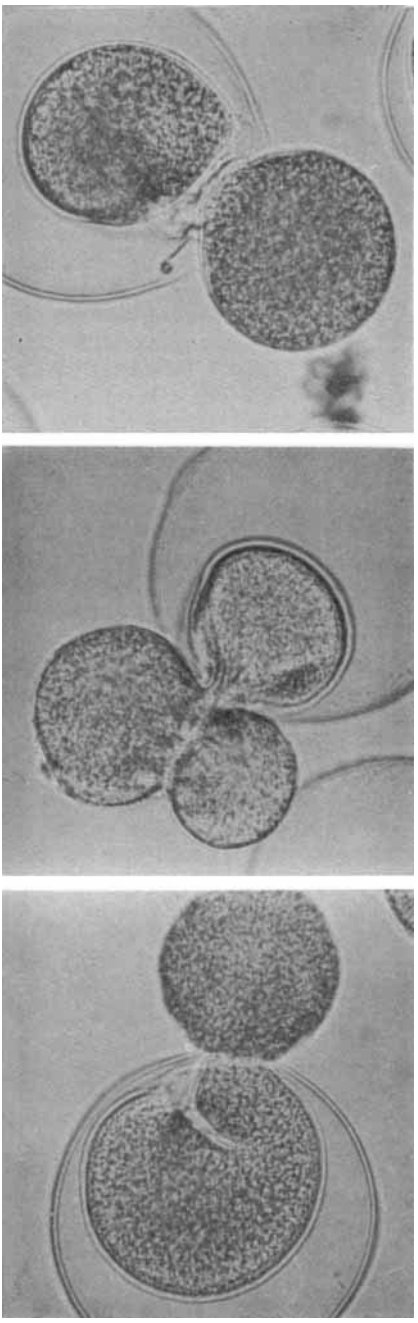

Fig. 1 Artificially constricted *Hemicentrotus pulcherrimus* eggs cleaving in 0.75% ether in filtered sea water. The furrow of the right cell was permanent, but those of the other cells receded.

eggs that were etherized 30 minutes before normal cleavage time. Furrows in anesthetized eggs appear later and progress more slowly than those of unanesthetized controls. Furrows oriented in the plane of the artificial constriction usually persist, while those that appear elsewhere usually regress (fig. 1). Spherical denuded and undenuded eggs sealed in the same ether-sea water solution with the constricted eggs did not develop furrows.

It was sometimes difficult to maintain the proper concentration of highly volatile ether during manipulations, and for this reason ethyl urethane was subsequently used as the anesthetic agent. 0.06 M ethyl urethane in filtered sea water suppresses cleavage of *H. pulcherrimus* when applied 30 minutes before normal cleavage time. The visible effect of ethyl urethane in anesthetic concentrations appears identical to that of ether. The mitotic apparatus is reduced but maintains nearly normal cyclical activity. Furrows also appear in artificially constricted eggs. As was the case in etherized eggs, permanent furrows developed only in the plane of the artificial constriction.

The minimum concentration of ethyl urethane required for suppression of furrowing in sand dollar eggs from Maine waters was 0.052 M. The mitotic apparatus of urethane treated eggs was smaller and less distinct than normal (fig. 2). When constricted eggs were placed in 0.052 M urethane they formed permanent furrows, provided the mitotic apparatus straddled the plane of artificial constriction (fig. 3). Furrows only appeared between the asters. No furrows formed when the mitotic apparatus lay elsewhere in the cell. In order to control more precisely the relationship between the mitotic apparatus and the surface, individual completely denuded eggs were manipulated with glass needles. Eggs in 0.052 M urethane solution were flattened against the bottom of the operation chamber (Rappaport, '68) with the side of a stout needle. As the needle pressed into the cell, the mitotic apparatus slid toward the margin. The operation was completed in the early amphiaster stage at least 30 minutes before normal cleavage time. Furrows developed in the operated cells (fig. 4). They appeared later than normal and their rate of progress was abnormally slow. In time, the cleavage furrow developed extensively and deepened until it reached the level of the needle where it stopped. Eventually these furrows receded. Spherical unoperated cells in the same chamber never developed furrows.

These results suggested that the suppressive effect of the anesthetics used could in part be due to the increased distance between the cell surface and the reduced mitotic apparatus. Since

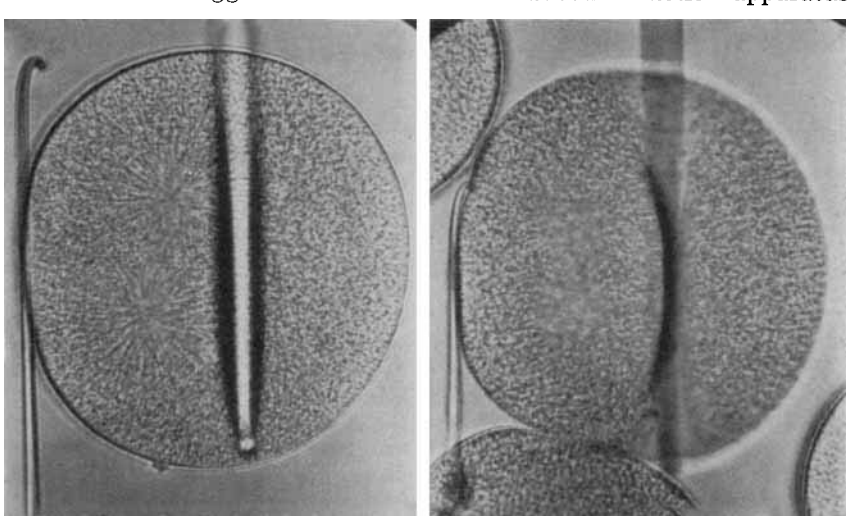

Fig. 2 Appearance of the asters in flattened *Echinarachnius parma* eggs in sea water (left) and 0.052 M ethyl urethane (right).

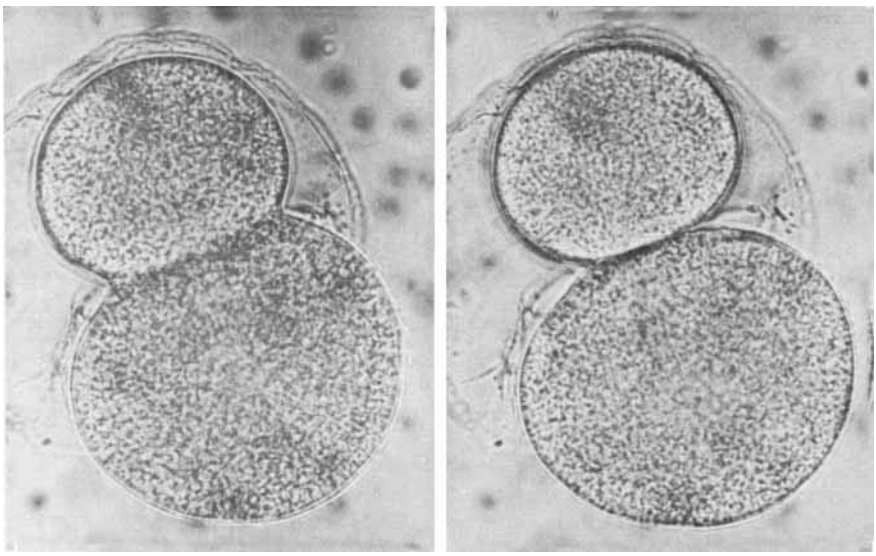

Fig. 3 Cleavage of an artificially constricted sand dollar egg in 0.052 M ethyl urethane.

hexanediol augments the *in vivo* mitotic apparatus (Rebhun and Sawada, '69) it seemed worthwhile to test its capacity for reversing the effects of urethane. A series of concentrations of hexanediol were combined with eggs in 0.052 M ethyl urethane. Eggs exposed to sea water containing 0.052 M ethyl urethane and 0.25% hexanediol showed some reversal of the cleavage block. In favorable cases about half the treated eggs developed furrows. About half of these furrows were permanent (fig. 5). The response of eggs to this treatment varied from female to female. Hexanediol alone appeared to inhibit furrowing in concentrations of 1% or more.

Furrow establishment requires a specific geometrical relationship between the mitotic apparatus and the cell surface (Rappaport, '69a). Furrows do not form when the mitotic apparatus is shifted far from the equatorial surface (Rappaport and Conrad, '63; Rappaport, '65; Rappaport and Ebstein, '65). The observations of Rebhun and Sawada ('69) and the results of experiments described above suggested the possibility that the mitotic apparatus of hexanediol treated cells may be able to establish furrows in more distant surface than the mitotic apparatus of untreated cells can. Sand dollar eggs in the early amphiaster or streak stages were flattened with the side of a stout needle oriented parallel to the spindle. The spindle-to-surface distance was controlled by raising and lowering the needle. Furrow formations at different spindle-to-surface distances were determined for eggs immersed in filtered sea water, 0.125% hexanediol and 0.25% hexanediol. Higher concentrations of hexanediol interfered with division. In all, 56 measurements were made. In both treated and untreated eggs furrows always formed when the spindle-to-surface distance was 90 μ or less. There were no furrows in either group when the spindle-to-surface distance exceeded 100 μ. When the spindle-to-surface distance was between 90 μ and 100 μ a difference between treated and untreated cells became apparent. One hundred percent (8 of 8) hexanediol treated eggs established furrows whereas 30% (3 of 10) of the untreated eggs established furrows in this range of spindle-to-surface distances. The difference is not considered striking. The relatively large size of the sand dollar mitotic apparatus may complicate these experiments. For production of a spindle-to-surface distance great enough to prevent furrowing, cells must be flattened and subjected to considerable physical con-

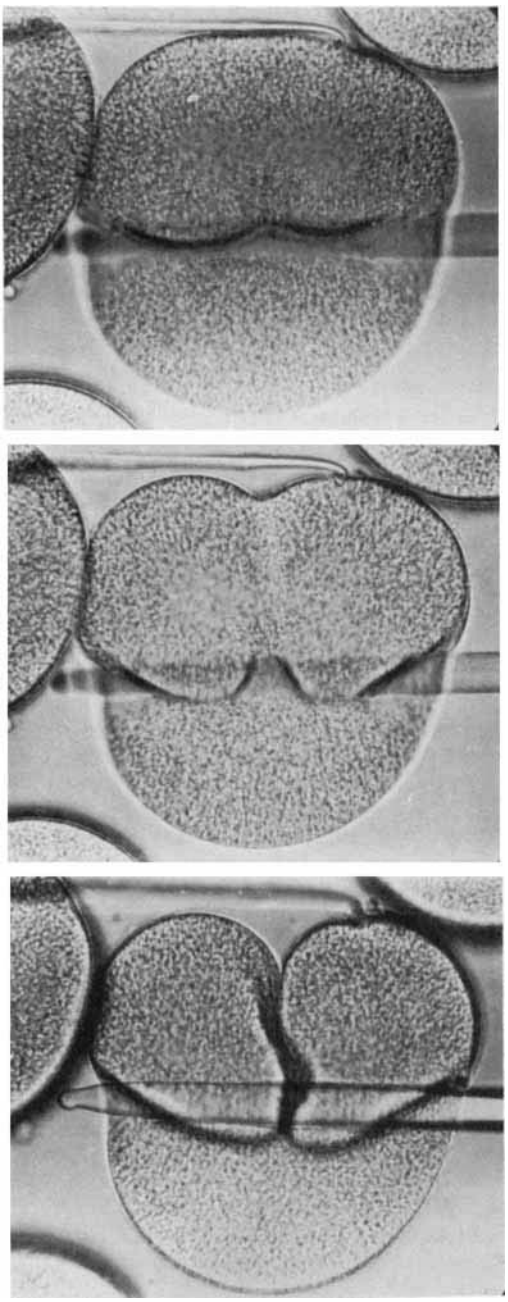

Fig. 4 Cleavage of a sand dollar egg in 0.052 M ethyl urethane. Furrows only appear when the reduced mitotic apparatus is pushed close to the cell margin.

straint. A better analysis of the hexanediol effect might be obtained by using transparent cells with a relatively smaller mitotic apparatus.

DISCUSSION

This investigation was undertaken to determine whether the cleavage blocking effect of two frequently used mitotic poisons is causally related to their visible effect upon the mitotic apparatus. These compounds were applied in the minimum concentration that completely suppressed cytokinesis. Undoubtedly higher concentrations would have had additional disrupting effects. Certainly both urethane (Cornman, '54) and ether (Swann, '54) affect cell activities other than assembly of the mitotic apparatus. There are many other mitotic poisons that suppress cleavage by interfering at different points in the cell cycle (Gelfant, '63). The results of these studies should not be construed as a general explanation of the mechanism of action of all mitotic poisons.

Cytokinesis in echinoderm eggs involves two processes. In the first process the mitotic apparatus establishes, by a stimulus whose nature is yet to be elucidated, the position of the furrow in the cell surface (Swann and Mitchison, '58; Wolpert, '60; Rappaport, '65). After a furrow is established, removal of the mitotic apparatus does not interfere with cleavage (Hiramoto, '56). In the second process, the region of the cell surface modified during the first process exerts the physical force that divides the cell. After this region is established, it can function despite drastic chemical insult (Beams and Evans, '40; Swann and Mitchison, '53; Kuno, '54). It appears that the first process is more easily disrupted than the second.

1. *Alteration of cell shape.* Constricted cells and those in which the mitotic apparatus had been pushed to an excentric position developed furrows in concentrations of ether and ethyl urethane that suppressed furrow formation in spherical cells. The metabolic activities of treated cells are assumed to be the same whatever their shape. The experiments demonstrate that one of the consequences of metabolic alteration can be partially revised by geometrical modifica-

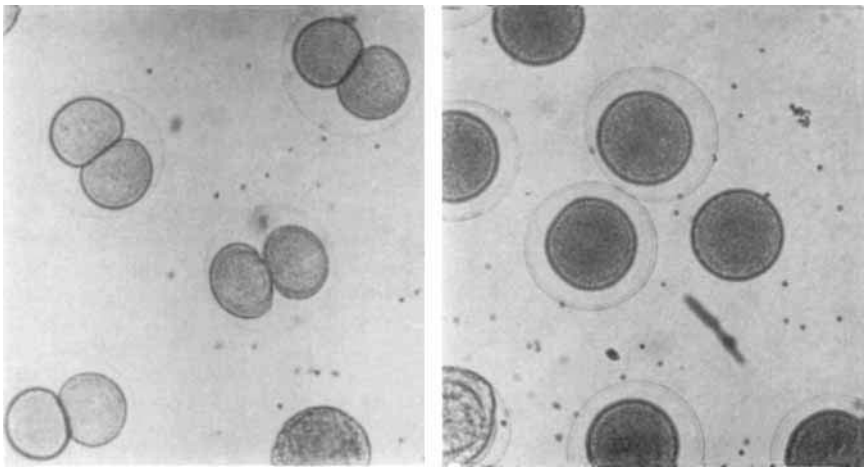

Fig. 5 Cleavage of sand dollar eggs in 0.052 M ethyl urethane plus 0.25% hexanediol (left) and 0.052 M ethyl urethane alone (right).

tion. Furrows appear in treated cells when the distance between the mitotic apparatus and the surface is shortened. The visible effect of ether and ethyl urethane was reduction in the size of the mitotic apparatus. The results of the manipulations could be construed as a restoration of some necessary geometrical relationship. It appears that the cleavage blocking effect of ether and ethyl urethane in the concentrations employed is largely due to reduction of the distance over which the mitotic apparatus can elicit a furrow in the cell surface. Beyond this it must be pointed out, however, that the occurence of abnormal and incomplete furrows suggests that the cell's capacity to carry out the cleavage process after it is initiated has also been somewhat diminished. This interpretation of the mechanism of action of ethyl urethane and ether at low concentrations is consistent with early correlations between asters and furrows (Wilson, '01; Wolpert, '60; Rappaport, '65) and later quantitative studies (Rappaport, '69a). Cells exposed to mitotic blocking agents over several cycles sometimes form multiple furrows in conjunction with numerous asters (Wilson, '01; Gray, '31; Kobayashi, '62). It may be that when the number of reduced asters within the cell is large, they support each other to the extent that some are held close enough to the surface to initiate furrowing.

2. *The effect of hexanediol.* Little can be said concerning the effect of hexanediol except that the partial reversal of anesthesia it accomplished was predicted by reason of its demonstrated enhancement of the *in vivo* mitotic apparatus. In their analysis of the role of this and other mitotic augmenting agents, Rebhun and Sawada ('69) reason that they affect the control system which determines the way in which microtubule precursor is partitioned between the oriented and unoriented state. Better understanding of the nature of events occurring within cells exposed to both anesthetic agents and hexanediol requires quantitative and ultrastructural study.

ACKNOWLEDGMENTS

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