rupt the pattern of new surface formation. Rinsing fertilized sand dollar, Echinarachnius parma, eggs in calcium and magnesium free sea water and also in acidulated calcium free sea water makes the surface sticky. Their adhesion to glass and plastic surfaces is so strong that they cannot be separated without tearing the cell. By fastening cells to flat or concave or cylindrical surfaces different regions and proportions of the surface were constrained immediately after the furrow's first appearance. Cells divided despite bilateral constraint and distortion of the polar and subpolar surfaces. However circumferential constraint near and parallel to the cleavage plane stopped division even when only one side was affected. Constraint of large portions of the egg surface does not interfere with cleavage as long as an area on either side of the furrow is left free. Cleavage does not require the normal pattern of new surface formation. It seems likely that normal regional differences in surface stretching are due to the operation of physical forces originating outside the region.

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1977 #29

CHANGES IN THE ULTRAVIOLET LD_{50} DURING THE CLEAVAGE CYCLE IN ZYGOTES OF Echinarachnius parma

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Resistance to ultraviolet, using survival as the criterion, was studied in zygotes of E. parma during the first cleavage cycle. The purpose was to compare such a survival curve with the previously reported curves for sensitivity of cell division delay. (Rustad, Photochem. and Photobiol. 3:529, 1964).

Fertilized eggs were subjected to a series of dose levels of heterochromatic ultraviolet (UV) irradiation at various times during the first cleavage cycle. The source of UV was a Hanovia high pressure mercury arc with an interposed Vicor filter which eliminated wavelengths below 260 mu. The criterion for survival was the ability of the zygotes to gastrulate successfully. The dose level needed to obtain 50% non-gastrulation was noted as the LD_{50}.

The LD_{50} at ten minutes post-fertilization is 2.1×10^2 ergs/mm^2; this increases to 1.26×10^4 ergs/mm^2 by the time of first cleavage. The increase in the LD_{50} is exponential during the first hour post-fertilization at 16° C. At this point a short plateau (8.4×10^3 ergs/mm^2) of 20 min appears. After this 20 min the LD_{50} again increases to reach the maximum at 90 min post-fertilization. It remains constant during the process of cleavage. Thus it requires sixty times the energy for an LD_{50} just before cleavage as is needed immediately post-fertilization. The significance of the plateau between 60 and 80 min post-fertilization is not clear, but it is interesting to note that it begins at the same point as insensitivity to UV with regard to cleavage delay. The big contrast is that cleavage delay insensitivity remains constant until cleavage begins, whereas, the LD_{50} shows another increase.

Photoreactivation illumination (PR) will increase survival of zygotes at each LD_{50} during the cell cycle. When the irradiation is applied during the first 15 min post-fertilization the PR must be given during the immediately following period before fusion of the sperm and egg pronuclei. The damaging lesion becomes non-reversible after the completed fusion of the pronuclei.

Also, the non-surviving fraction develops with unequal and asynchronous cleavage when zygotes are subjected to the LD_{50} during the prefusion period. This PR and cleavage response is similar to the response when sperm are irradiated with an LD_{50}. However, in sperm irradiation one cannot rule out selectivity in fertilization related to the degree of primary damage.

When zygotes were exposed to LD_{50} irradiation between the fusion of pronuclei and cleavage, development would continue normal but delayed until gastrulation. At this point about 50% would fail to gastrulate. This is the same pattern as the response of irradiated unfertilized eggs which has been previously reported (Bull. Mt. Desert Island Biol. Lab. 5:36, 1965).

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ENERGETICS AND SODIUM TRANSPORT IN ERYTHROCYTES OF THE SPINY DOGFISH, Squalus acanthias

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The relationship between cation transport and energetics in the dogfish erythrocyte has not yet been fully elucidated. Previous observations from this laboratory have demonstrated a brisk O_2 consumption by the dogfish erythrocyte that can be partially inhibited by cyanide or antimycin (Bull. M.D.I.B.L. 5, 2:40, 1965). It has also been shown that these cells maintain an unusually steep K^+ gradient between plasma and red cell water suggesting high cation transport rates (J. Cell. & Comp. Physiol. 64:409, 1964). In addition, studies by Bricker (Bull. M.D.I.B.L. 5, 2:4, 1965) and ourselves (Bull. M.D.I.B.L. 5, 2:39, 1965) have indicated: (a) that Na^+ transport rates in the dogfish erythrocytes are substantially higher than found in mammalian erythrocytes; and (b) that Na^+ transport was unaltered by inhibitors of oxidative phosphorylation. These inhibitors included cyanide, antimycin, and 2-4 dimitrophenol. These findings were consistent with Na^+ transport deriving energy from two possible sources, namely anaerobic glycolysis or non-cytochrome dependent O_2 utilization.

Exposure of dogfish erythrocytes to approximately 100% N_2 leads to moderate depression in red cell Na^+ efflux. However, the use of inhibitors of anaerobic glycolysis, 10^{-3} M monoiodoacetate or fluoride, lead to further striking inhibition of active Na^+ transport.

In addition, electron microscopy indicated that, while mitochondria were found in immature red cells from the spleen, mitochondria could not be detected in erythrocytes in circulating blood.

The following may be concluded:

1) Despite the existence of an oxidative pathway in the dogfish erythrocyte, the energy for Na^+ transport arises from anaerobic glycolysis.

2) The absolute rate of Na^+ transport is markedly higher than in mammalian red cells. The rate of active transport of Na^+ in the dogfish red cell at 13° C is approximately 3 times as high as the corresponding rate in human red cells at 37° C. These findings raise some intriguing thermodynamic problems.