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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1966 #30

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.

3) The existence of an electron transport chain may be inferred from the inhibition of O_2 consumption by cyanide (inhibits cytochrome a_3) and antimycin (inhibits cytochrome b), but this electron transport chain is not organized in the usual structural form since mitochondria are not present.

4) The cellular sites and energy requiring processes for O_2 utilization are not known.

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1966 #31

MICROPUNCTURE STUDY OF UREA MOVEMENTS ACROSS THE RENAL TUBULES OF Squalus acanthias

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Urea is actively resorbed in the renal tubules of elasmobranchs as shown by the fact that the urine urea concentration is considerably lower than that of the blood. In the dogfish the urea U/P is 0.1 to 0.2 while the inulin U/P is 3 to 8, thus 90 to 99% of the filtered urea may be resorbed.

The purpose of the investigation was to localize the tubular site of active transport and to determine the rate of transport, and the permeability to urea. Theoretically, this can be done in two steps:

1) A test solution (containing a non resorbable solute, such as, manitol) is introduced into the tubule between oil droplets and left until a steady state has been reached where the unidirectional fluxes in and out of the tubules will be equal. If active transport out of the tubule is present, concentration in the tubule will be lower than in the blood. I.e., the active and passive outflux will equal the passive influx.

2) The unidirectional flux is measured with labeled urea at the steady state concentration.

The dorsal surface of the right kidney was exposed in the anesthetized dogfish. Micropuncture free flow samples were collected from renal capillaries and proximal tubules. Free flow samples were also collected from the ureter by catherizing them with polyethylene tubing and from the bladder. Urea was measured by the micromethod of Marsh on nanoliter samples. The concentrations found were approximately: renal plasma, 350 mM/1; proximal tubule, 350-360 mM/1; ureter, 40 to 100 mM/1; bladder, 40-100 mM/1. It was not possible to obtain free flow samples from the distal tubules because of difficulties in visualizing them on the kidney surface. Only when the tubules were filled with the colored test solution could we see them.

To determine the stopflow concentrations a solution of 450 mM/1 mannitol, 300 mM/1 urea and 50 mM/1 NaCl was introduced into the various parts of the tubule. Stopflow fluid/plasma ratios were: ureter, 1.05; small collecting duct, 0.92 to 1.01; distal tubule, 0.92; proximal tubule, 1.00-1.10.

The free flow collections indicate that the urea resorption takes place between the proximal tubules and the collecting ducts, i.e., in the distal tubules. The stopflow determinations have failed to show a clear difference between distal tubular fluid and blood. The experiments are not finished and it is quite possible that we have failed to identify the distal tubules properly.