

liminary studies on the toxicity of nitrogen mustard (HN₂) and methotrexate (MTX) to 3 kgm dogfish maintained in a 12 foot diameter plastic swimming pool with running seawater. The study was hampered by widespread eye infections, leading to bleeding and death distributed equally among treated and control fish. Certain observations, however, are of interest.

HN₂ at 1.5 mg/kgm caused a pronounced leukopenia in 3 of 3 fish studied one and two weeks after intravascular injection.

MTX at 0.1 mg/kgm daily for five days to 3 fish did not cause leukopenia. Five daily injections of 0.3 mg/kgm of MTX caused borderline leukopenia in 3 animals. In 2 dogfish, 1.0 mg/kgm MTX x 5 was a leukopenic dose. These observations should provide a background for future studies.

1969 #30

PARTIAL REVERSAL OF CHEMICAL CLEAVAGE INHIBITION BY MICROMANIPULATION

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"Anaesthetic" compounds of the older cytological literature referred to agents that would reversibly block cell division when applied in low concentrations. Many substances are unable to block division if applied after metaphase but later block the subsequent division cycle. E. B. Wilson observed that many of these agents reduce the size of the mitotic apparatus. Since the cleavage mechanism is established at or near the cell surface under the influence of the mitotic apparatus, it seemed worthwhile to explore the possibility that some part of the anaesthetic may be attributed to inability of the reduced mitotic apparatus to affect the cell surface.

Ethyl urethane, 0.52 M, in filtered seawater completely blocks cleavage in sand dollar (Echinarachnius parma) eggs and reduces the size of the living mitotic apparatus. In several experiments the reduced mitotic apparatus of treated eggs were artificially brought closer to the surface. When eggs are constricted by being partially extruded through the fertilization membrane shortly after fertilization, the mitotic apparatus frequently develops straddling the constriction. In this circumstance, the distance from the mitotic apparatus to the surface is reduced. In treated, constricted eggs, slowly forming, permanent furrows appear when the mitotic apparatus straddles the constriction. Furrows only appear between the asters. They do not appear in spherical eggs in the same medium or in constricted eggs where the mitotic apparatus fails to straddle the constriction. By using the side of a glass needle, the reduced mitotic apparatus was pushed and held closer to the cell surface where it elicited a furrow. Furrows formed in this way, however, were usually slower to form and eventually regressed.

Hexylene glycol enhances the size of the mitotic apparatus (Rebhun & Bernstein, J. Cell Biol. 35:111A, 1967). When sand dollar eggs are treated with 0.52 M urethane plus 0.25% hexylene glycol about half of them form furrows. About half of the furrows are permanent. Hexylene glycol does not appear to increase the distance over which the normal mitotic apparatus can act on the surface. Neither can it convert the normally unilateral furrow of cleaving Hydractinia echinata egg to a symmetrical furrow by increasing the size of the mitotic apparatus.

It would appear that the blocking effect of ethyl urethane can be partially reversed by mea-

tures which restore the normal geometrical relation between the mitotic apparatus and the surface.

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1969 #31

THE MICROCIRCULATION IN THE TAIL FIN OF Fundulus heteroclitus

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As a continuation of the work started in the summer of 1968, the microcirculation of the tail fin of Fundulus heteroclitus was studied in vivo by means of light microscopy.

The fish was mounted on the microscope stage in a special lucite cradle, designed in such a way that the gills were submerged and the tail resting horizontally in a shallow pool of circulating seawater. An ordinary Zeiss microscope was used for the observation, and the objective lenses used were 10x, 25x, 40x, and 100x oil immersion. In the case of the oil immersion, the seawater was drained from the tail, and drops of immersion oil were applied directly on the tail. This provided an excellent image of the vasculature.

Attached to the microscope was a television camera GPL 990, a videotape recorder Ampex VR 5100, and a television screen TV Setchell Carlson Model 2100 SD. While the various parts of the tail fin microvasculature were observed, an instant recording of the field was obtained, and at the end of the experiments, an instant replay of the recording was possible. The findings are presently being analyzed.

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1969 #32

THE CIRCULATION OF THE SPLEEN IN Squalus acanthias

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The structure and ultrastructure of the circulatory system of the dogfish spleen has not been investigated before. In order to gain some knowledge of the vascular system in this particular organ, particularly in view of some recent investigations by this author of mammalian spleens, the spleens of several dogfish were preserved for electron microscopy. A specially designed technique of perfusion was utilized. The dogfish was anesthetized with Nembutal, using a dosage of 20 mg per kg dogfish, and given intravenously. The abdomen was opened and a cannula inserted into the splenic artery. The perfusion of glutaraldehyde, followed by osmium tetroxide was started at the same time as the splenic vein was opened in order to obtain an immediate drainage of the spleen. The glutaraldehyde perfusion washed out the splenic blood, and the subsequent osmium tetroxide blackened the splenic tissue, indicating the success of the perfusion. Specimens are now being analyzed with the aid of the light and electron microscopes.

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