1966 #33

RESPONSE OF DOGFISH LYMPHOCYTES IN TISSUE CULTURE TO PHYTOHEMOGGLU-TININ

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The dogfish (Squalus acanthias) is one of the most primitive vertebrates possessing a humoral immunological system (i.e., serum gamma globulin with neutralizing activity after suitable antigenic stimulation). Our studies with dogfish lymphocytes were undertaken in the attempt to learn more about the cellular aspect of its immunological system, and with this aim in mind, some properties of these cells <u>in vitro</u> were examined.

A method was found for obtaining peripheral blood white cells in good yield and with very little red cell contamination. This consisted of suspending the packed cells of the blood in a saline solution containing sucrose. After a suitable centrifugation the red cells sedimented, while the white cells which formed a layer on top of the solution could be easily removed.

The white blood cells (roughly 75% granulocytes and 25% mononuclear cells-lymphocytes) were placed in tissue culture (see research abstract #28, M.D.I.B.L. Bulletin, 1964) and examined with regard to several parameters over a period of 3-4 days.

1. <u>Morphology</u>. The granulocytes degenerated rather quickly and after several days the predominant cell type was a large mononuclear blast-like cell probably of the lymphocytic series.

2. <u>Agglutination by Phytohemagglutinin (PHA)</u>. PHD (M) - Difco, a known mutagenic stimulant of mammalian lymphocytes, was found to cause extensive clumping and aggregation of the white cells to a final dilution of 1/250. It may also be added that red cell agglutination was observed at a final dilution of 1/20 - 1/40.

3. Incorporation of H^3 -Thymidine. In one experiment, white cells in tissue culture showed a progressive incorporation for 3 days, of H^3 -Thymidine into acid-precipitable material. However, a stimulatory effect of PHA was not unequivocally demonstrated.

Slight to moderate clumping of white cells was sometimes noted in the absence of PHA, but this was never as marked as when PHA was present. There were some suggestions that the extent of clumping (without PHA) was influenced by the type of serum present in the medium.

Attempts to obtain viable lymphocyte suspensions from the spleens of adults and secondyear embryos were unsuccessful.

As a secondary project, long term tissue culture was again attempted using dogfish firstyear embryos (<u>ibid</u>., 1964) and retinal tissue from the eyes of second-year embryos. At this writing both types of cultures are viable after two months in culture, although the decline in growth rate with time has again been observed.