

Investigations of the effects of anti-mitotic agents on the homograft reaction were conducted on fishes to which ventral autografts and homografts of pigmented scales were made on either side of the linea alba. At 28°C the homografts of control fishes invariably exhibited extensive or complete melanocyte breakdown on the 3rd day after operation. Autografts survived indefinitely. Daily intraperitoneal injections (1-2 mg in 0.1 ml distilled water) of antagonists of nucleic acid synthesis (5-fluorouracil, 5-deoxyribofluorouridine and 6-mercaptopurine) prolonged survival of homografts up to 7 days by which time the fishes had died of the lethal effects of the drugs. Injections of 2 mg of 6-fluorohydrocortisone acetate and delta-1-hydrocortisone sodium succinate likewise enhanced survival of homografts, but only until the 4th to 5th days. Two antibiotics which were tested also had the effect of postponing the homograft destruction usually occurring on the 3rd day. Chloramphenicol, in doses of 10 mg per fish per day, resulted in survival of homograft pigment cells until the 5th day. Tetracycline hydrochloride (1 mg) permitted survival of some grafts until the 7th day. Administration of amino acid analogues was considerably less effective in protecting homografts from destruction. Daily injections of 5 mg of 5 different serine and phenylalanine analogues slightly prolonged homograft survival (to the 4th day), while ethionine, given in a dose of 3.3 mg per day, delayed the homograft reaction until the 5th day. These doses, however, were frequently lethal.

These results indicate that antibody production depends upon nucleic acid synthesis, where it is most vulnerable to analogue interference. Inhibition of protein synthesis by adrenal cortical hormones or antibiotics is somewhat less effectual. Administration of amino acid analogues was least effective in preventing antibody synthesis. Treatment with near-lethal doses of colchicine (0.005 mg) failed to preclude homograft breakdown, indicating that cellular proliferation is far less important in the process of antibody production than is the biosynthesis of nucleic acids and proteins.

Importance of Molecular Synthesis vs. Mitosis in Immunological Reactions

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The clonal selection theory of acquired immunity, as postulated by Burnet and augmented by Lederberg, contends that each immunologically competent cell in the mature animal is endowed with hypermutable genes each of which controls the synthesis of a particular kind of antibody. Upon exposure to antigens, certain of these cells are stimulated to multiply into clones which in turn manufacture correspondingly appropriate antibodies. It would follow that inhibition of proliferation by anti-mitotic agents might be expected to block the synthesis of specific protein antibodies.

Accordingly, adult teleosts (*Fundulus heteroclitus*) were treated

daily with intraperitoneal injections of selected antimetabolites and challenged with homografts of pigmented scales (which normally undergo melanocyte disintegration on the third postoperative day at 28° C as a result of the hosts' immunological responses). When administered near lethal doses of colchicine (0.005 mg/fish/day) or podophyllin (0.001-0.01 mg), drugs which inhibit mitosis by arresting the anaphase movements of chromosomes, host fishes nevertheless exhibited an immunological response by destroying their homografts as soon as did the controls. Thus, in the absence of overt cytokinesis, antibody formation can proceed normally.

Other compounds, unlike the foregoing, which interfere with the synthesis of nucleic acids and/or proteins, were effective inhibitors of antibody production as evidenced by delayed homograft reactions. Methyl bis-(beta-chloroethyl)-amine (Mustargen) (0.1 mg), triethylene melamine (TEM) (0.01 mg) and N, N', N'' - triethylenethiophosphoramidate (THIO-TEPA) (0.1 mg) doubled the survival time of homografts, while the folic acid antagonists, aminopterin and A-methopterin (Methotrexate), produced even more striking results when administered in doses of 0.1 mg in suspension.

Clearly, an immunological response is impossible without the synthesis of nucleic acids and proteins. Mitotic proliferation, however, is not essential to antibody formation, though it may be an inevitable consequence of antigenic stimulation especially if DNA replication is an indispensable step in communicating the specificity of the antigen to the antibody.

The Elasmobranch Thyroid-pituitary System

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Thyroid of the skate, *Raja erinacea* and *R. diaphanes*, is a brownish, compact gland located ventral to the bifurcation of conus arteriosus and carotid arteries, weighing approximately 63-112 mg. in animals with weights between 885 and 1170 gm. When its function was studied with radioactive iodide, the 24-hour uptake average (2 animals) was 0.26 percent of the injected dose and thyroid/blood ratio 94, 48-hour uptake average (2 animals) was 0.52 percent and thyroid/blood ratio 219, and 72-hour uptake average (2 animals) was 0.16 percent and thyroid/blood ratio 1.6. Peak of radioactive iodide-uptake must lie around 48 hours or earlier.

Skates survive thyroidectomy well, although the skin heals with difficulty or not at all. The operation is not difficult. Immersion in 0.1 percent solution of MS222 provokes anaesthesia in one or more minutes. Since the animals go under rather quickly, they must be watched and then removed as soon as bodily activity mostly ceases. Thyroidectomy is performed with the animal out of water, and with no special effort necessary to insure a supply of sea water to the gills although some may be squirted