Thyroxine-Induced Regression of Tadpole Lymph Glands[1] (37311)

H. B. RIVIERE[2] AND E. L. COOPER
(Introduced by C. H. Sawyer)
Department of Anatomy, School of Medicine, University of California, Los Angeles, California 90024

The lymph gland is a lymphomyeloid structure located bilaterally in various anuran larvae (1). Along with the thymus and spleen, the lymph glands comprise the major, if not the only, organs of the tadpole immune system (1–3). The gland is strictly a larval structure, disappearing just after metamorphosis in bullfrogs and leopard frogs (1, 2). The larval lymph gland may function in much the same way as adult bone marrow in restoring transplantation immunity (4, 5). With the advent of metamorphic climax, functional bone marrow appears. No longer needed, perhaps the lymph gland, like other larval structures, regresses under the influence of thyroxine. The purpose of this preliminary investigation was to determine if thyroxine has any direct observable effect upon the larval lymph gland *in vitro*.

Materials and Methods. Lymphglandectomy. Bilateral lymph glands were removed from anesthesized *Rana catesbeiana* tadpoles (stages 10 to 12) under aseptic conditions according to previously described methods (6, 7). One lymph gland from each larva served as an experimental gland while the other gland was utilized as control. Each gland was rinsed in three changes of sterile amphibian Ringer's and placed on an organ culture grid in an organ culture dish (Falcon Plastics, Los Angeles, CA).

Cultures. All cultures were maintained initially in 1 ml of medium consisting of 70 ml Lebowitz, L-15 medium without glutamine, 1 cc L-glutamine (200 m*M* solution), 12 ml fetal calf serum, 100 units penicillin/ml 100 μg streptomycin/ml, and 15 ml sterile distilled water. The cultures were incubated at 15°. After 24 hr, the medium was changed (8). Experimental glands from each tadpole were cultured on medium containing 10 μg/ml D-, L-thyroxine (Nutritional Biochemicals Corp.) while the contralateral control glands were cultured on the medium without hormone. Thereafter, the media were changed every three days.

Histology. Control and experimental glands were fixed in 10% neutral buffered formalin at four-day intervals or at four, eight, and twelve days of culture. Tissues were embedded in paraffin, sectioned at 8 μm and stained with hematoxylin and eosin.

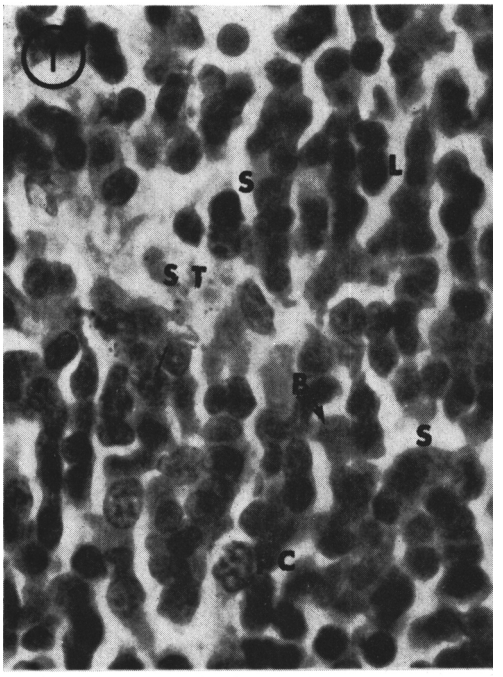
Results. Normal lymph glands. The larval lymph gland is a sinusoidal organ and has been described previously (9). The stroma is made up of collagen fibers, fibroblasts, and primitive reticular cells. The sinusoids are lined with phagocytic cells, and a few granulocytes, and plasma cells are present. Stem or blast cells are found in the center of cords of lymphocytes.

Cultures of control lymph glands. Organ culture of control lymph glands caused a collapse of the sinusoidal structure (Fig. 1). However, upon careful inspection, the phagocytic reticuloendothelial cells lining the collapsed sinusoids were still present. The parenchymal cells appeared normal, however, lymphocytes were fewer compared to normal noncultured glands. Up to eight days, granulocytes, plasma cells, and stem cells were not affected by the culture conditions. After eight days of culture, control glands showed some signs of deterioration; therefore, the results presented here are from

[1] Supported by: NSF Grant GB17767, two grants from The California Institute for Cancer Research, and a grant from The Brown–Hazen Corporation.

[2] Post-doctoral trainee in anatomical sciences. Supported by Grant GM616.

FIG. 1. Normal lymph gland at eight days of culture. The sinusoids (s) are collapsed but evident. The stroma (st) is hardly visible under the numerous parenchymal cells. Plasma (P.C.) lymphocytes (L) and blast cells (B) can be seen. (743×)



glands cultured up to eight days.

Experimental cultures. Lymph glands maintained in medium containing thyroxine showed altered sinusoidal structure just as control glands. However, unlike untreated control glands, by four days of culture, the integrity of the parenchyma was disrupted. Instead of the normal arrangement of lymphocytes around blast cells in cords separated by lining cells and sinusoids, the parenchyma was overgrown by fibroblasts. This resulted in fibrosis and loss of lymphoid cells, plasma cells, and granulocytes (Fig. 2). Numerous cell fragments and cells with pycnotic nuclei were present. Therefore, thyroxine administered *in vitro* to the larvel lymph gland results in destruction of the normal lymphomyeloid structure of the gland.

Discussion. We have shown that thyroxine, administered directly to the larval lymph gland *in vitro,* causes degenerative changes in the gland and thereby demonstrated a direct effect of the hormone upon a lymphoid organ. While this study is admittedly preliminary, it is important from the standpoint of ontogeny and phylogeny of the immune response. Amphibians are the first vertebrate class to develop true bone marrow. In the anurans, which have both larval and adult stages, it is possible to observe precise developmental alterations in certain organs due to hormonal influences on metamorphosis. This study was of interest, therefore, in determining changes that might occur in tissues responsible for generating cells that mediate immune responses.

A considerable amount of work has been done toward defining the role of the lymph gland, thymus, and spleen in the immune processes of *Rana catesbeiana* larvae and adults (10). Hormonal control of amphibian growth and metamorphosis has likewise been investigated intensively. Yet virtually no studies have emerged relating the progressive ontogeny of any immune response in anuran

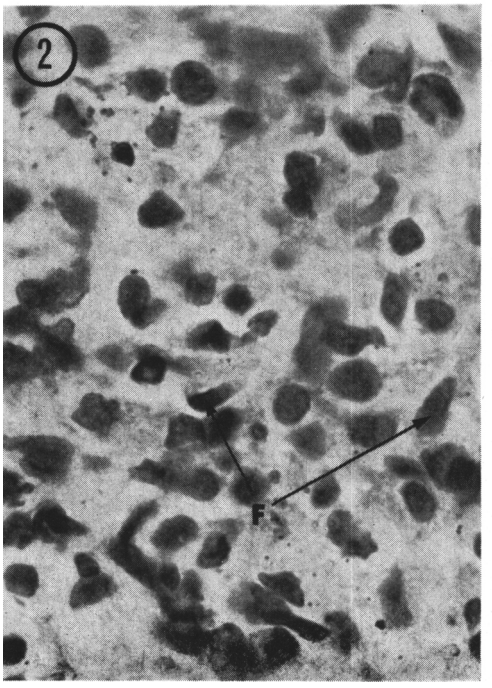


FIG. 2. Lymph gland treated with thyroxine at eight days of culture. Most of the cells of the parenchyma are absent. Fibroblasts (F) are the major cellular component. Virtually no sinusoidal structure or lymphoid cells remain. (743×)

amphibians to thyroxine or any other hormone.

Having demonstrated a morphological influence of thyroxine on the lymph gland *in vitro,* the questions arise: what is the role of the larval lymph gland *in vivo* on the tadpole immune system and what is the influence of thyroxine upon this role? The lymph gland is capable of partial restoration of allograft immunity in irradiated tadpoles (5). Thyroxine might be expected, then, to have an effect on allograft rejection. Bovbjerg (11) found essentially no difference in survival of skin allografts in normal, hypophysectomized, or thyroxine-treated larvae. Furthermore, Hildemann and Haas (12) found no difference in survival of skin allografts in *Rana catesbeiana* larvae and adults of from two months to two years of age. The level of circulating thyroxine should increase as the larvae approach metamorphic climax, and then decrease. A natural fluctuation in median survival time of skin allografts might be expected if thyroxine had any influence on allograft rejection. Such fluctuations have never been observed.

After removal of the lymph glands, larvae are incapable of synthesizing antibodies to bovine serum albumin (7). Recently, plaque-forming cells have been found in larval lymph glands after immunization with SRBC (3). Its ability to partially restore transplantation immunity and peripheral lymphocyte levels under conditions of heavy irradiation and the presence of plaque-forming cells suggest that it produces immunologically competent cells capable of mediating cellular and humoral responses. The interrelationships between lymph gland, thymus, and spleen remain unknown.

Hormones control the growth, development, and homeostasis of most living organisms. If we are to understand fully the ontogeny and phylogeny of immunity we must define the possible influences of hormones upon immune responses. The tadpole is unique in offering a suitable animal for such research.

Summary. This preliminary investigation was designed to determine if thyroxine has any direct observable effect upon the larval lymph gland *in vitro.* Both lymph glands were aseptically removed from *Rana catesbeiana* larvae. One gland from each tadpole was maintained *in vitro* in medium containing 10 μg/ml of L-thyroxine. The contralateral glands served as control cultures and were maintained on the same medium without hormone. After eight days of culture, hormone-treated glands showed signs of degeneration as evidenced by fibrosis and loss of lymphoid cells, but control glands remained intact. Thyroxine, therefore, appears to have a direct effect upon the larval lymph gland *in vitro.*

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Received Jan. 16, 1973. P.S.E.B.M., 1973, Vol. 143.