Radiothyroidectomy of Larval Steelhead Trout.* (30414)

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As in the majority of teleosts, the thyroid gland of the steelhead trout (*Salmo gairdneri* Richardson) consists of separate follicles scattered along the ventral aorta. The majority of the follicles are located in the vicinity of the first and second aortic arches. Since surgical extirpation is impractical, various attempts at radiothyroidectomy of teleosts utilizing I-131 have been described(1-10). Although many of these studies entailed repeated injections of radioiodine, complete thyroidectomy was often not achieved. The method described below is a modification of the technique used by Baker *et al*(2). This relatively simple procedure can be employed for the preparation of large numbers of young, thyroidectomized fish and can be applied even before resorption of the yolk has been accomplished.

Methods. Steelhead alevins were obtained from the South Tacoma Hatchery, Washington State Department of Game, and were maintained in the experimental hatchery operated by the College of Fisheries, University of Washington.

The apparatus for radioiodine treatment is shown in Fig. 1. The animals were placed within the 5-gal disposable plastic square vessels in solutions of carrier-free I-131 in lake water. Tap water was circulated through the larger plastic containers to maintain the temperature of the radioiodine solution near that of the hatchery water (Table I). A 10-hour artificial day length was controlled by an automatic timing device. The radioiodine solutions were aerated constantly during treatment.

Five experimental groups were treated with radioiodine by immersion in lake water initially containing 12.5 or 25 μc I-131/cc for 4 or 8 days (Table I). In Groups 1 and 2, two animals were sacrificed every 12 hours during the treatment. In Groups 3-5, two animals of each group were sacrificed at the end of treatment. These animals were fixed in Bouin's fixative and sections were prepared for autoradiography. At biweekly or monthly intervals after termination of I-131 treatment 2 experimentals from each group and 2 controls were injected intraperitoneally with tracer doses of I-131 (5-10 μc/animal). They were sacrificed 24 hours later and prepared for histological examination and autoradiography. Slides were stained with one of the following: hematoxylin and eosin, aqueous P.A.S. and hematoxylin, Masson's or Mallory's trichrome.

Complete absence of recognizable thyroid tissue and lack of accumulation of tracer doses of I-131 in serial sections were used as criteria for radiothyroidectomy(9).

Results. Using autoradiography of the thyroid gland as a qualitative criterion, there was little radioiodine uptake after 12 hours of immersion. Some radioiodine concentration was observable in the melanophores of the skin and the pigmented layer of the eye. By 24 hours the thyroid tissue was well labeled and by the end of treatment (4 or 8 days) it contained more radioiodine than any other structure. Tracer doses could not be utilized effectively for 6 weeks after treatment due to radioactivity of residual I-131 in the thyroid region.

Actual necrosis of thyroid epithelia was not observable for 2 to 5 weeks following treatment. As the follicles broke down large numbers of lymphocytes and macrophages appeared in the thyroid region. Vascularity of the region increased and there was a tendency for connective tissue elements to proliferate. These features were exaggerated in the older groups. Large cyst-like masses of connective tissue were found among the aortic arches in a few animals in Group 5.

Complete thyroidectomy was achieved in Groups 1 and 2 in 7 to 8 weeks. All specimens examined from Group 5 indicated thyroidectomy was complete in 12 to 14 weeks.

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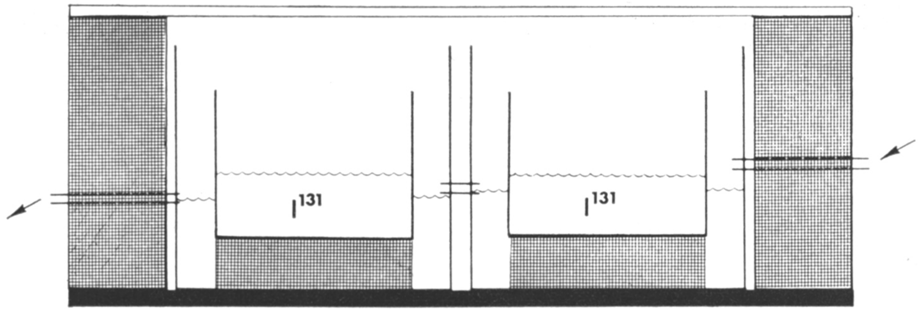

FIG. 1. Cutaway diagram of apparatus for radioiodine treatment, consisting of two 5-gal disposable square plastic vessels which contained the radioiodine solutions. Each was seated on solid concrete blocks within a similar 15-gal container. Tap water was circulated through the larger containers as indicated by arrows. Entire apparatus was surrounded by solid concrete blocks for shielding.

No regeneration of thyroid tissue was observable in these groups up to 10 months after radioiodine treatment.

Although some animals of Groups 3 and 4 were completely thyroidectomized in 12 to 14 weeks, thyroidectomy was not complete in others. The radioiodine treatment was considered to have been insufficient to produce total thyroidectomy in these groups.

Discussion. The immersion technique for radiothyroidectomy as described here has several advantages over the administration of radioiodine by serial injections as described by La Roche and Leblond(1) and subsequently used by others(3,5,8,10). It allows for the preparation of large numbers of thyroidectomized small animals with a minimum of handling. Even if only one injection of a large dose of radioiodine is used(4,6,7) larger animals must be used and preparation of large numbers of animals is tedious. The use of alevins in this experiment (Groups 1 and 2) eliminates the necessity of feeding the animals during radioiodine treatment without upsetting normal nutrition. Furthermore, radiothyroidectomy is complete in a relatively short time, the younger animals requiring only about 2 months for total abolition of thyroid function. Older animals are more refractory to equivalent doses, but thyroidectomized fry can be obtained in about 3 months. Finally, thyroidectomized fish can be made available for detailed study soon after hatching, and they can be maintained in the laboratory for months.

Although degenerative histological changes were apparent in the thyroid region, the animals were not otherwise adversely affected by radioiodine treatment in a readily apparent way, and survival was very good.

However, as in any of the procedures using radioiodine for thyroid destruction, the possible role of whole body beta and gamma radiation in the post-radiothyroidectomy effects remains to be completely evaluated. In experiments (unpublished) with young salmon and trout, we have attempted to minimize, in part, whole body radiation as an unknown variable between radiothyroidectomized and control fish. This was done by keeping the control fish in a plastic vessel immersed within the same I^{131}-containing vessel as used for the thyroidectomy but not mixing with it.

TABLE I. Description of Experimental Groups.

Group No.	No. fish	Age (wk)	Initial I-131 (μc/cc)	Treatment Time (days)	Treatment Temp (°C)	Hatchery temp (°C)
1	64	4	25	8	10	8
2	35	5	12.5	8	10	8
3	20	10	25	4	11-12	11
4	45	11	12.5	4	13-14	13
5	450	18	25	8	14-16	15

The brief description we have provided here of histological alterations in lower jaws of radiothyroidectomized trout differs in no important way from the more extensive descriptions already published by others(9,11, 12,13). These changes characteristically have been found to include: (a) progressive thyroid follicular epithelial pycnosis and cytolysis whose rates depend upon doses of the radioiodine, (b) leucocytic infiltration and removal of debris, (c) fibrous replacement and scar formation, and (d) sclerotic changes in the walls of adjacent blood vessels.

Summary. A simplified procedure for radiothyroidectomy of large numbers of juvenile steelhead trout (alevins or fry) is described. Complete destruction of thyroid tissue was achieved 8 to 14 weeks after immersion in a solution of radioiodine in lake water (initial concentration: 12.5 or 25 μc I-131/cc for 8 days).

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Effect of Erythropoietin and Oxygen Tension on *in vitro* Synthesis of Hemoglobin A and F by Adult Human Bone Marrow.* (30415)

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The hemoglobin found in the peripheral blood of the normal human adult is heterogeneous in structure. In addition to normal adult hemoglobin there are 2 other minor fractions, hemoglobin A_2 and hemoglobin F. The latter, which is the predominant hemoglobin in the newborn, falls to very low levels by the end of the first year of life and only in certain pathological conditions is it found increased. Pathological conditions include Thalassemia, some hemoglobinopathies, aplastic anemia, some cases of acute leukemia and some cases of vit. B_{12} deficiency. The factors, genetic and environmental, which control the rate of synthesis of the various hemoglobins are poorly delineated. Allen and Jandl (1), using reticulocyte suspensions obtained from the cord blood of newborns, found that conditions of hypoxia or glucose deficiency induced a *relative* increase in the synthesis of fetal hemoglobin (HbF) as compared with adult hemoglobin (HbA). Thomas *et al*(2), using various hematopoietic organs obtained from fetuses, reported essentially similar findings irrespective of the organ used. They also demonstrated that hematopoietic tissue

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