SOME EFFECTS OF THIOURACIL IN THE GERMAN BROWN TROUT

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The goiterogenic action of thiourea in fish has been demonstrated by Goldsmith, et al. (1944). In experiments on viviparous hybrids of Platypoecilus maculatus x Xiphophorus hellerii, these workers found that the drug inhibited growth and development and brought about thyroid changes similar to those described in the higher vertebrates. The present study was undertaken to determine the effects of the related compound, thiouracil, on the eggs and fry of the German brown trout (Salmo trutta fario).

MATERIALS AND METHODS

Five hundred developing trout eggs were obtained 16 days after fertilization from the State Fish Hatchery at Madison, Wisconsin. Approximately half of the eggs were placed on a tray constructed of plastic screen and suspended in a 12-liter battery jar containing a 0.033% thiouracil solution. The remaining eggs, serving as controls, were similarly suspended in a jar of water. Continuous aeration was supplied by an aquarium pump. The liquids in both experimental and control containers were changed twice weekly, the water used having been drawn from the tap at least three days prior to any given change. In order to maintain the low temperatures necessary for trout development, the battery jars were placed in a large galvanized tank through which cold tap water circulated. During the course of the experiment (from mid November until early May) the water temperatures varied from 8 to 15 degrees centigrade. Temperature changes from day to day were small and were the same in both battery jars. The effects of thiouracil on egg mortality and hatching time were noted.

Five days after hatching, 50 fry from thiouracil-treated eggs were transferred to 3-liter jars containing 0.033% thiouracil solutions. On the same day 100 fry from control eggs were placed in 3-liter jars of water. The two experimental and four control jars each contained 25 young trout. Twenty-six days later the

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water in two of the control jars was replaced by 0.033% thiouracil. Thus, two groups of thiouracil-treated fish were under observation, Series I having been immersed in the solution from the 16th day of embryonic life and Series II from the 31st day after hatching. The procedures for aeration, changing of liquids, and maintenance of low water temperatures were the same as described for the eggs. Beginning 25 days after hatching, the young trout were fed finely chopped fresh beef liver three times weekly. At intervals during the experiment, eggs and fry were preserved in Bouin's fluid for histological study. Transverse serial sections of the head and anterior body regions were cut at 10 micra and stained with Delafield's hematoxylin and eosin.

RESULTS AND DISCUSSION

Egg mortality and hatching: In Figure 1, a graphic comparison is made between experimental and control eggs as to mortality and hatching time. Of the eggs developing in thiouracil, 18% died before or during the hatching period. The corresponding figure for the control eggs was 9%. Thiouracil, therefore, had only a slight toxic effect.

![Graph showing egg hatching percentage over time](image-url)

**Figure 1.** Percentages of thiouracil-treated and control trout eggs hatched between the 35th and 41st days after fertilization.
Hatching in the experimental jar took place between the 36th and 41st days after the eggs were fertilized. The greatest incidence was on the 39th day when 39% of the eggs hatched. In the control jar, the process began on the 31st day and continued until the 40th day with a peak of 52% of the eggs hatching on the 38th day. Thus, thiouracil produced a slight but definite delay in hatching. These results are confirmed by an earlier unpublished experiment (Warner, 1948) in which trout eggs developing in 0.033% solutions of thiourea and thiouracil were retarded about 24 hours in their hatching as compared to eggs developing in suspensions of carp pituitary powder and in water. Similarly, Grossowitz (1946) reported delays up to ten days in the hatching of hen's eggs after treatment with varying doses of thiourea. Although thiouracil treatment brought about a delay in the hatching of trout eggs, histological examination revealed no differences between experimental and control thyroids in fish preserved at that time. Therefore, it cannot be stated with certainty that the delay was the result of thiouracil-induced hypothyroidism.

Later development: For nine weeks after hatching the young trout appeared to be in good health, but between the 67th and 77th days a period of excessive mortality ensued. Inadequate diet and overcrowding were two of the more probable factors involved in the large number of fatalities. The incidence of death was highest among the Series I fry, only three individuals surviving until the 84th day when that portion of the experiment was terminated. At that time 12 Series II fry and 16 controls remained alive and in good condition. With the exception of specimens preserved at intervals for histological study these fish were observed until they attained an age of 154 days.

Certain external effects of thiouracil treatment became apparent during the post-hatching period. A delay in yolk-sac resorption was observed among the experimental fry of Series I. Six weeks after hatching this structure was still prominent in 50% of the thiouracil-treated individuals but was conspicuous in only 15% of the controls. Since the thyroids of the experimental fry were markedly enlarged at this time, the delay can be attributed to a hypothyroid condition brought about by thiouracil treatment. In the Series II fish no thyroid changes had occurred and there was no delay in yolk-sac resorption.

After immersion in thiouracil for 85 days (64 days post-hatching) bright reddish areas appeared in the ventral pharyngeal and pericardial regions of all Series I fry. The same situation developed in the Series II fish after 93 days of thiouracil treatment. This condition seemed to be due at least in part to an
engorgement of the ventral aorta and its tributaries associated with enlargement of the thyroid gland.

Between the 3rd and 44th days after hatching, the average body length of 25 Series I trout selected at random increased from 14.7mm to 27.1mm while over the same period an equal number of control fry similarly selected increased from 15.7mm to 27.8mm. Subsequent measurements on the 55th and 68th days after hatching showed no further gain in average body length among either experimental or control fry. It should be pointed out that this cessation of growth coincided roughly with yolk-sac resorption and immediately preceded the period of heavy mortality. It is possible, therefore, that the fry could not adapt themselves to the changeover from a yolk-sac type of nutrition to a liver diet. Overcrowding may also have been an important factor in growth stoppage and subsequent death. Among the Series II fry and their controls, some growth occurred during the later stages of the experiment. At 154 days after hatching, five thioracil-treated fish had attained an average length of 33.9mm while six controls of the same age averaged 35.8mm. The foregoing measurements reveal no striking impairment by thioracil of growth in length in either series of experimental fish.

Other more subjective changes in body proportion, coloration, and behavior were apparent in both Series I and Series II fry toward the end of the periods of thioracil treatment. Several of the experimental fish presented a slightly humpbacked appearance. The head was relatively short and blunt and the gill region from a ventral view was abnormally broad. The body was comparatively thin in many cases although this may have been due in part to a lack of food in the alimentary canal. The vertical bands of dark pigment cells along the flanks were smaller and less well defined in a greater proportion of thioracil-treated fry than in the controls. The experimental fish were for the most part more sluggish in their movements and less voracious in their feeding habits than the untreated fry.

**Thyroid Histology:** Histological studies of control thyroids (Figures 3 and 5) were made in conjunction with similar observations on both series of thioracil-treated fish. As a result some aspects of normal thyroid development were noted which seem worthy of mention. They are summarized as follows:

1. Small thyroid follicles containing colloid were first seen in 27 day embryos.

2. In embryonic and early post-hatching stages the thyroid follicles were found in two separate groups close to the ventral aorta near the junctions of the first and second branchial arteries. In older fry, follicles were seen in almost all pharyngeal
FIGURE 2. Thyroid gland of 63-day thiouracil-treated fry showing follicular hypertrophy, hyperplasia and loss of colloid. Transverse section at level of second branchial artery. C, cartilage of lower jaw; A, ventral aorta; F, thyroid follicle. X258.

FIGURE 3. Thyroid gland of 63-day control fry. Section at level of second branchial artery. F, thyroid follicle. X258.

FIGURE 4. Thyroid gland of 84-day thiouracil-treated fry showing loss of follicular organization. Section at level of second branchial artery. X258.

FIGURE 5. Thyroid gland of 84-day control fry at level of second branchial artery. X258.
sections at the levels of the 1st and 2nd gill arches with a few extending into the 3rd gill arch region. They were more diffusely arranged along the aorta than in earlier stages, but the heaviest concentrations were still near the bases of the first two pairs of branchial arteries.

(3) An increase in number of follicles occurred during the first two months after hatching. As an example, in a newly-hatched fry, 12 were counted, whereas in a fish 63 days old at least 100 were present. No marked increase beyond this number was noted in later stages.

(4) Considerable variation in follicle size was apparent in all stages studied. The extreme range in maximum diameters of those measured was between 10 and 65 micra. Relatively greater numbers of large follicles were present in older fish.

(5) Colloid was present in all follicles except those of extremely small size, and the amount per follicle appeared to increase somewhat with age. In most cases the colloid was slightly drawn away from the follicle cells.

(6) In young follicles the epithelium was cuboidal in nature while in later stages low cuboidal and squamous types predominated.

The thyroids of Series I experimental fish were studied in 16- and 27-day embryos and in 11 stages of fry between the ages of one and 84 days. Up to and including the time of hatching, no effects of thiouracil treatment were evident. The first change in thyroid histology was observed in an eight-day fry which had been immersed in thiouracil for 29 days. Here, a loss of follicular colloid was accompanied by a moderate cellular hypertrophy. At 25 days posthatching, marked thyroid enlargement was apparent. Colloid was almost completely absent and in several instances the lumens of the follicles were difficult to distinguish. In 63 day fry the thyroid had enlarged so as to fill almost the entire connective tissue space around the aorta (Figure 2). Measurements of several representative follicles showed a range in maximum diameters between 30 and 170 micra. Studies of successive sections revealed a folding or lobulation of the wall in follicles of extreme size. The lumens in most cases were slit-like and contained little or no colloid. Measurements of cells in the columnar type epithelium averaged 16 micra, about four times the height of cells in control thyroids of the same age. Distinct hyperplasia had occurred in 77- and 84-day thyroids, but many of the follicles, particularly in the latter stage, appeared to have lost their organization and follicular boundaries were not easily recognized (Figure 4). The cells for the most part were smaller than at 63 days although considerable variation in size was
apparent. This atypical picture may have been the beginning of thyroid exhaustion, but the specimens studied were undoubtedly in poor health, having been preserved during or just after the period of widespread mortality. Therefore, factors other than thiouracil may have been involved in changing the histology of these glands.

In the second experimental series, nine stages of fry between the ages of 42 and 154 days were studied. Loss of colloid and cellular hypertrophy were first observed in two 50-day specimens. These fish had undergone thiouracil treatment for 19 days, a period of immersion ten days shorter than that required to produce hypothyroid symptoms in the Series I fry. The thyroids of month-old fry, therefore, appeared to be somewhat more susceptible to the effects of thiouracil than were those of 16-day embryos. These results may have been brought about by (1) greater sensitivity of the older thyroids to thiouracil, or (2) greater production or more effective utilization of thyrotrophic hormone from the hypophysis in the older fish.

Typical hypertrophy and hyperplasia occurred in the later Series II stages. The greatest enlargement was observed in a 154-day fish which had been under the influence of thiouracil for 123 days. Here, the thyroid formed a continuous compact mass around the ventral aorta throughout the gill arch region. Some of the follicles extended dorsally to the pharyngeal epithelium and laterally to the gill cartilages and gill arch muscles. The vascularity of the gland was more pronounced than that observed in younger specimens. Thus, the thyroids of Series II fry showed a progressive increase in size and number of cells with no indication of exhaustion in the later stages.

The picture of hypothyroidism brought about by thiouracil in the German brown trout resembles in several respects the syndrome of goiter in hatchery brook trout (Salvelinus fontinalis) described by Marine and Lenhart (1910). Pharyngeal reddening, lack of growth inhibition, sluggishness, and lowered viability were common to both species. The changes in thyroid histology of loss of follicular colloid, hypertrophy, hyperplasia, and migration of follicles to outlying areas also followed the same general pattern. The brook trout goiters were more extreme, many of them being observable externally. This greater development may be attributed to the more advanced age of the fish involved and to the much longer period of exposure to the goiterogenic factor. Another point of quantitative difference is the earlier and more complete loss of colloid observed in the thiouracil-treated glands of the German brown fry. The main causative agent of goiter in the brook trout was believed to be the diet consisting of hog liver
and heart (Marine, 1914). It is of interest to note that the beef liver diet to which the German brown trout were subjected produced no histological symptoms of hypothyroidism.

Effects of thiouracil in young trout were in general the same as those obtained by Goldsmith and co-workers with thiourea in viviparous tropical hybrids. However, marked growth inhibition occurred in the latter fish but not in the former. Aside from species and drug differences, another factor involved in this discrepancy may have been the lower environmental temperatures to which the trout were exposed. As a result, the effectiveness of thiouracil in lowering metabolic rates may have been reduced, thereby causing less retardation of growth. The onset of histological symptoms of hypothyroidism in the trout occurred from 20 to 30 days earlier than that reported for the tropical fish. This would indicate a greater sensitivity of the trout thyroid.

It can be stated that the action of thiouracil in trout, particularly in its effect on thyroid histology, is comparable to that reported for birds and mammals.

SUMMARY

Treatment of German brown trout eggs with thiouracil produced a slight delay in hatching time. Immersion of newly-hatched fry in thiouracil solutions for periods up to 154 days delayed yolk-sac resorption and decreased activity and viability, but caused little inhibition of growth in length. Marked thyroid enlargement occurred in fish exposed to thiouracil for more than 30 days.

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REFERENCES
