EFFECT OF THYROID PREPARATIONS AND IODIDE ADMINISTRATION ON YOUNG SALMON (*Salmo salar*, L.)

by

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CHAPTER I

INTRODUCTION

GENERAL PURPOSE:

The role of the thyroid gland in fish is little understood, while it is well known in mammals, birds and amphibians. Thus, in mammals and birds this gland controls the metabolism. In many amphibians the thyroid is responsible for the metamorphosis occurring at the transition from an aquatic to a terrestrial environment. In the case of fish, however, Etkin in 1940 claimed that the gland played no role at all. This statement was challenged by others - the detail of the argument will be dealt with below.

Since the situation remained confused, it was decided to investigate the role of the thyroid in fish. The Atlantic salmon was chosen as the species to be studied because of a feature of the development of this species. The young fresh water salmon or "parr" transforms into a less pigmented "smolt" prior to passage into the sea. According to Hoar (1939) this phenomenon would be under thyroid control.

Of the two classical approaches in the studies of thyroid endocrinology - extirpation of the gland and administration of glandular extracts - only the latter was used because the diffuse thyroid gland of the salmon did not lend itself to operative removal.(1) Various types of thyroid extracts were fed to salmon parr. Nothing happened until at least a few weeks had elapsed. But after that several types of transformations showed up. The most unexpected of these was a considerable increase in the amount of connective tissue. This was detectable as a thickening of the skin in the head region which consequently was thrown into

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(1) Recently it has become possible to eliminate the thyroid gland by treating salmon with large doses of radio-iodide. No experimental results along this line are available as yet.

wrinkles. Thyroid treatment also results in an overall depigmentation, due, at least partly, to a decrease in the number of pigment cells.

In order to determine whether the changes observed following administration of crude thyroid preparations were really due to the presence of the thyroid hormone - thyroxine - in the extracts, intra-muscular injections of pure thyroxine were also administered. Such injected fish showed changes similar to those found in fish fed thyroid preparations. These studies were extended by administering thyroid preparations to younger (fry) or older (smolt) forms than the parr.

For thyroid preparations to produce clearcut effects, large amounts had to be used and, therefore, the effects obtained might not have been physiological. The availability of iodine-deficient salmon made it possible to approach this problem in an indirect manner. Administration of iodide to such animals should step up their output of thyroid hormone. Therefore, an attempt was made to determine whether the feeding of iodide would produce changes comparable to those obtained with thyroid extracts. Since this also proved to be the case, it may be concluded that the thyroid gland plays a definite role in fish.

HISTORICAL INTRODUCTION:

The thyroid function in fish has been the subject of a number of works that were mostly concerned with other species than the Atlantic salmon. These works will be presented in a chronological order, taking into consideration the relations between the various types of results.

The thyroid gland was first shown to be present in teleosts by John Simon in 1844. However, this author was unable to locate the gland in salmon and trout. Maurer (1886) reported the presence of the thyroid gland in trout. Later, Gudernatsch (1911) confirmed its presence in twenty-one species of teleosts including salmon and concluded that this gland was presumably present in all fish.

Marine and Lenhart (1910, 1911 and 1914) investigated the factors responsible for the occurrence of thyroid hyperplasia among brook trout (Salvelinus fontinalis) kept in hatcheries. These workers induced a regression of the hyperplasia by addition of iodide or other substances known to be rich in iodine. Marine and Lenhart concluded that the thyroid hyperplasia was a symptom of endemic goiter. This work, although primarily concerned with the cure of endemic goiter in trout, was also the first investigation related to the study of various environmental factors on the thyroid condition in fish. The authors realized that there was a relationship between the iodine content of water or food and goiter incidence. However, this work did not demonstrate that the secretions of the thyroid gland played a role in fish, nor what such a role might be.

Later, more direct attempts were made to see whether there were any effects of administration of thyroid preparations in fish. The results obtained have been, sometimes, contradictory or inconclusive. Etkin and collaborators (1937 and 1940) made many careful attempts to influence the oxygen consumption of goldfish (Carassius auratus) and toadfish (Opsanus tau) by administration of thyroid extracts or thyroxine but failed repeatedly. Matthews and Smith (1948) observed that treatment with fish thyroid extract produced an increase in the oxygen consumption of the parrotfish (Bathystoma). The extract was prepared from thyroid gland of parrotfish. But the data are at best inconclusive, since, of the eighteen fish treated, only seven gave a positive response. Matthews and Smith concluded that fish weighing less than 15 gm. gave a positive reaction. Examination of their data indicated, however, that four out of the eleven fish giving a negative response weighed more than 15 gm. and no explanation was given for it.

In an extensive review of the subject, Etkin (1940) concluded that the thyroid hormone had little if any effect on fish and, at any rate, did not effect the basal metabolic rate in the dramatic manner observed in warm-blooded animals.

Working along a different line of investigation Krokert (1936) made a morphological study of the effects of thyroid preparations in guppies (Lebistes reticulatus). An increase in the rate of growth was detected with an increase in the size of the fish and in the differentiation rate of the gonopod. All treated fish, males and females, showed an increase in the movements of the opercula, a fact which was interpreted as being an indication of increased oxygen consumption (?).

Although Smith and Everett (1943) failed to duplicate these results, Hopper recently (1950) confirmed some of the observations of Krokert by showing that beef thyroid preparations caused a marked increase in the growth rate of males and females, as well as an acceleration of gonopod differentiation in the male. It therefore appears that the thyroid hormone plays a role in the growth of Lebistes reticulatus. This is further substantiated by Hopper's complementary demonstration that fish, placed in 0.03 per cent thiouracil solution, showed a reduced growth rate and a slower gonopod differentiation.

A different approach to the problem of the thyroid function was to study the relation of this gland to the pigment changes occuring at some periods of development in fish. By treating yellow eels with thyroid preparations, von Hagen (1936) demonstrated that the gland was essential for the so-called metamorphosis of these fish into the silvery adult eels found in rivers. Yellow eels kept on thyroid treatment were shown to acquire the pigmentary pattern of the adult much more rapidly than the yellow eels kept as controls. It was therefore demonstrated that the thyroid gland played a role in the pigmentary transformation of young yellow eels into adult fresh water eels. This work thus became the basis for studies of the thyroid gland function on pigmentary transformations of fish.

The transformation from parr to smolt in the genus Salmo has been investigated by a number of workers. The obvious changes found to occur during this "smoltation" are a decrease in the dark pigmentation of the ventral and lateral regions and an increase in the silvering of the latero-ventral region of the body. Hoar (1938, 1939) presented an extensive embryological and morphological study on the thyroid gland of the Atlantic salmon. He showed by histological means that maximum thyroid activity was associated with the initiation of the period of most rapid growth in fresh water, i.e. at the beginning of the smolt stage. He, therefore, assumed that "smoltation" was under the control of the thyroid gland and that this transformation period could be referred to as a "metamorphosis" accompanied by typical pigmentary changes.

The pigmentary transformations accompanying "smoltation" were first thought to be due to an onset of sexual maturation in the young salmon. However, Orton and Jones (1938 and 1940) showed that the male salmon parr sometimes attained sexual maturity long before its silvering and transformation into a smolt.

The relationship between the pigmentary changes of metamorphosis and the thyroid activity of fish was then studied by several workers. The method adopted was to observe the effects produced by various thyroid treatments. Langrebe (1941) studied the effects of injections of beef thyroid extracts and beef anterior pituitary extracts on the pigmentation of Salmo salar and brown river trout (Salmo trutta). He used only two salmon parr and four young river trout, but clearly showed that crude beef thyroid extracts caused, in both types of fish, a decrease in the intensity of dark pigmentation and a silvering of the body. The conclusion was drawn that the thyroid hormone stimulated smoltation of salmon and trout. Furthermore, a crude beef anterior pituitary extract produced a silvering of the salmon, although not in the trout. This effect on salmon was attributed to an activation of the thyroid gland by the pituitary thyrotrophic hormone. Langrebe's conclusions that the effects observed were due to the thyroid and thyrotrophic hormones respectively could not be considered final since he used non-purified extracts. It should also be kept in mind that his results were obtained on a small number of fish.

Robertson (1949) repeated Langrebe's experiments with the rainbow trout (Salmo gairdnerii) using the thyroid hormone, thyroxine. He also observed a decrease in pigmentation which he attributed to a contraction and disintegration of the melanophores, and a silvering which he attributed to a deposition of guanine on the scales. Robertson obtained the same results with powdered thyroid extracts and iodinated casein, but not when potassium iodide was used. He believed, like Langrebe, that the effect of thyroid extracts and thyroxine was to produce the same pigmentary changes as those occuring in smoltation and concluded that the thyroid gland controlled this phenomenon.

THE DEVELOPMENT AND LIFE CYCLE OF THE ATLANTIC SALMON:

In the present work the pigmentary changes described by Langrebe and Robertson were confirmed and, in addition, striking transformations in the connective tissue were observed in the Atlantic salmon as a result of the administration of thyroid preparations. Most of the young salmon used in these experiments were at the parr stage. In a few cases, fry and smolt were used. To facilitate an understanding of the nature of these stages a brief account of the life cycle of the Atlantic salmon will be given.

Salmon are born and spend the first few years of their life in the fresh waters of the rivers or brooks which have characteristic features. The rivers flow swiftly and are formed of an alternation of rapids or falls and quiescent pools. The water of the rivers proper is clear and remains relatively cold even in the warmest days of summer.

The rapids are used as spawning grounds. Here the female builds a rough nest of large stones and lays her eggs. The male places himself close to the female and simultaneously fertilizes the eggs with a shedding of his milt. The fertilized eggs are then left to incubate in the river. The period of incubation extends from November to the end of February and varies depending on slight fluctuations of temperature of the water between 1^{o} and $5^{o}C$. In the course of incubation the egg differentiates and, after 160 days, gives rise to a small vesiculated fish approximately one centimeter in length which is called an "alevin". The alevin hides among the rocks for a period of about six weeks, during which it nourishes itself by slowly resorbing its vitelline vesicle or yolk sac. After the yolk sac has been completely resorbed the young fresh water salmon passes through three well recognized periods of development known as the "fry", the "parr" and the "smolt" stages.

The Fry:

The fry stage extends from the time the six-week old alevin has completely resorbed its yolk sac until the end of the first summer, at which time the fish is approximately seven months old and about 5 cm. in length.

On both sides of the body, the fry have seven to nine accumulations of pigment which are described as "lateral bars". In the dorsal region, a single row of six to eight similar "dorsal bars" may be seen. Upon microscopic examination of the skin two distinct layers of pigment cells can be demonstrated. The deepest layer being located close to muscle, cartilage or bone, is called the "subdermal" layer. The surface layer is located against the inner surface of the basement membrane of the epidermis and is referred to as the "epidermal" layer. Some of the subdermal pigment cells agglomerate to form the lateral and dorsal bars. The pigment cells of the epidermal layer are dispersed so as to give an appearance of uniform dark pigmentation over the body surface. They are, however, more abundant in the upper than in the lower half of the body. Thus the ventral region contains only a few scattered pigment cells and therefore appears almost white. In addition to its dark pigmentation the fry has orange or red spots along the lateral line. These show considerable variations and are believed to be due to factors in the diet. (Lindsay, personal communication).

The Parr:

The parr stage extends from the end of the fry stage until the fish are preparing to migrate to the sea. This takes place when they are about two to three years of age. The parr attains a maximum length of 15 cm.

The lateral and dorsal bars persist throughout the parr stage. At the beginning of this stage, however, an additional number of pigment cells, located in the subdermal layer, appear between the dorsal bars. These pigment cells gradually increase in number and spread over the dorsal region so as to eventually obscure the dorsal bars at the end of the parr stage. By this time the pale areas between the dorsal portion of the lateral bars have also been invaded by the pigment cells. As a result, the upper portion of the lateral bars fuse into a continuous dark area.

In the parr the ventral region is even whiter than in the fry. At the end of the parr stage there is a deposition of a silvery material along the latero-ventral portion of the fish; a phenomenon which progresses towards the lateral line.

The orange or red spots observed in the fry, are also found on the parr, but disappear as silvering progresses.

The Smolt:

The smolt stage extends from the end of the parr stage (as described above) throughout the last period of growth in the river. Generally the beginning of the smolt stage takes place when the older parr reach the regions of the estuaries and are about to enter the sea. The end of this stage occurs at sea and is poorly defined.

During the transformation of parr into smolt, the lateral bars are partly obscured by dark pigment cells which accumulate in the subdermal layer. It is also known that during the course of smoltation the skin of the fish thickens and hardens considerably. The further deposition of silvery material on the inner portion of the scales obscures the rest of the lateral bars. At this time the smolt stage has been reached. Thus, it is apparent that the process of smoltation may be followed by observations on the changes in the pigmentation of the fish.

After a year at sea the salmon is known as a "grilse", and after more than a year spent at sea, it is referred to as a "kelt". These adult salmon may return to spawn in the river and thereby complete the cycle. It has been shown that the Pacific salmon possesses a "homing instinct", so that it returns to spawn in the river where it was born. The Atlantic salmon, though anadromous like the Pacific salmon, may, on the other hand, return to spawn in a river far removed from its birth-place. It may be pointed out that the present work deals essentially with Atlantic salmon and most of our results were obtained in fish at the parr stage, although salmon fry and smolt were also treated to confirm the findings obtained with the parr.

CHAPTER II

INFLUENCE OF THYROID EXTRACTS ON THE YOUNG ATLANTIC SALMON

The main purpose of this work was to test the effect of various thyroid preparations on the young Atlantic salmon. The experiments were carried out mainly with fish at the parr stage. Thus parr were used in the first and second series of experiments. In the third series use was also made of younger - so-called fry - and older - so-called smolt - salmon.

1st SERIES OF EXPERIMENTS:

Methods:

This series of experiments was conducted at the Fish Hatchery of the Laurentides in St. Faustin, Quebec, from the 1st of June to the 23rd of August, 1948. The experimental parr were reared in Tadoussac Fish Hatchery. They had been kept in circulating fresh water at this hatchery until they were large enough to be used for experimentation. They were then shipped to St. Faustin.

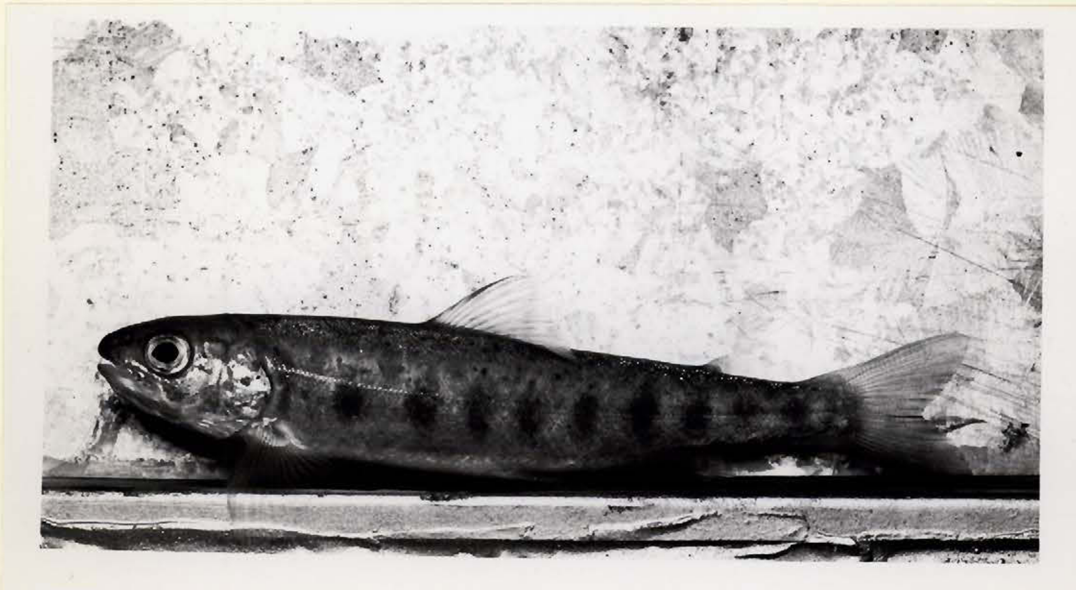
The salmon parr were a year and a half old at the start of the experiments. Their length varied from 5.0 to 15.0 cm. in length. At this stage the fish had eight to ten distinct pigmented lateral bars and six to eight dorsal bars. (Fig. 1).

The fish were kept in running water. It was necessary to accept for the experiments the conditions prevailing in the hatchery. The running water was a mixture of spring and brook water. The addition of brook water prevented the occurrence of goiter observed among the fish kept in spring water alone. Unfortunately, the proportion of the two types of water was not kept constant. Since the brook water was too warm in the summer, a mixture was used in such

PLATE I

EXPLANATION OF FIGURE

Fig. 1 - Side view of a normal parr. Note that nine lateral bars are visible. The fish is also more pigmented along its dorsal than its ventral region. The contour of the head is smooth.



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proportions as to check goiter incidence and as to maintain the temperature at 8° to 10°C.

The temperature of the water was recorded twice a day, at 10:00 a.m. and 3:00 p.m. in one of the experimental tanks (Table 1). The detail of temperatures is reported for this series as an example of possible variations. The rate of flow of the water in the troughs was maintained at approximately 16 liters per minute and was recorded daily. The troughs were 144 x 18 x 10 inches in dimensions and the depth of the water was kept at 7 inches in all experiments. The troughs we: covered by galvanized steel mosquito net in order to prevent the loss of fish by jumping. The troughs for control and treated parr in any given experiment were selected so that all the fish received an equivalent exposure to light. The trough were cleaned on Tuesdays, Thursdays and Saturdays to prevent pollution of the water by remaining food and feces.

All groups were weighed by difference once a week. For this purpose a 1,000cc. beaker half filled with water was first weighed. All the fish of one group were then caught by means of a steel dipbet and placed in the beaker, which was then covered with a watch glass to prevent any loss of water by splashing during the process of weighing. The beaker and its contents were again weighed. The total weight of each group was calculated by difference. The mean weight of a fish was obtained by dividing the total weight by the number of fish in the group.

Before the experiment, the fish were carefully sorted and distributed in comparable groups of treated and control, according to weight and intensity of their pigmentation. A few fish which did not show distinct lateral bars at the beginning of the experiment were discarded.

The control animals were fed with an excess of ground beef liver,

TABLE I

EXAMPLE OF DATA ON TEMPERATURE OF TANK WATER

- 1948 -

Date		Temperature °C	Date		Temperature °C
June	1	8.8 - 8.9	July	1	9.0 - 9.4
"	2	8.6 - 8.8	"	2	9.0 - 9.3
"	3	8.3 - 8.4	"	3	9.2 - 9.6
"	4	8.5 - 8.7	"		
"	5	8.1 - 8.5			
June	7	8.4 - 8.7	July	5	8.9 - 9.2
"	8	8.6 - 8.9	"	6	9.1 - 9.2
"	9	8.3 - 8.6	"	7	9.3 - 9.8
"	10	8.9 - 9.3	"	8	9.4 - 9.6
"	11	8.7 - 9.2	"	9	9.1 - 9.3
"	12	8.6 - 9.1	"	10	8.9 - 9.0
June	14	9.0 - 9.5	July	12	8.6 - 8.9
"	15	8.8 - 8.9	"	13	8.9 - 9.2
"	16	8.4 - 8.7	"	14	8.7 - 9.0
"	17	8.8 - 9.2	"	15	8.6 - 8.7
"	18	8.8 - 9.4	"	16	8.6 - 8.6
"	19	9.0 - 9.6	"	17	8.8 - 9.1
June	21	8.4 - 8.4	July	19	8.3 - 8.1
"	22	8.2 - 8.4	"	20	8.4 - 8.6
"	23	8.0 - 8.4	"	21	8.9 - 9.1
"	24	8.5 - 8.7	"	22	9.2 - 9.3
"	25	8.6 - 8.9	"	23	9.4 - 9.1
"	26	8.5 - 8.9	"	24	8.9 - 9.3
June	28	9.4 - 9.8	July	26	9.1 - 9.1
"	29	9.5 - 9.9	"	27	9.4 - 9.6
"	30	9.3 - 9.7	"	28	9.2 - 9.5
			"	29	9.1 - 9.2
			"	30	9.0 - 9.2
			"	31	8.9 - 9.0

August	1	9.4 - 9.7	August	12	9.0 - 9.0
"	2	9.6 -10.0	"	13	8.8 - 8.8
"	3	9.7 - 9.9	"	14	8.5 - 8.6
"	4	9.4 - 9.6			
"	5	9.2 - 9.1	August	16	8.4 - 8.5
"	6	9.1 - 9.4	"	17	8.6 - 8.8
			"	18	8.5 - 8.6
August	8	8.9 - 9.4	"	19	8.2 - 8.3
"	9	9.4 - 9.6	"	20	8.1 - 8.0
"	10	8.9 - 9.0	"	21	8.7 - 9.1

prepared as follows: Whole livers were cut into pieces that were passed through a fine meat grinder. This ground liver had the consistency of a thick fluid. When poured into the water of the trough, the material broke into numerous small pieces, which were easily ingested by the smallest fish. In this experiment the ground liver was kept on ice but was never stored for more than two days previous to feeding. The fish were fed three times a week, i.e. on Mondays, Wednesdays and Fridays.

Experimental Groups:

This series consisted only of salmon parr which were subjected to either of two types of thyroid treatments. One group received commercial thyroid extract and another dried beef thyroid. Corresponding control groups were started with each one of these experimental groups. Thus this series included a total of four groups.

The first group consisted of parr used as controls of those receiving the commercial thyroid extract treatment. The twenty fish in this group were maintained on a ground liver diet. They were taken from the stock at the same time as the corresponding treated group.

The treatment with commercial thyroid extract given to the second group consisted of feeding the fish a diet of approximately equal portions of ground beef liver, prepared as above, and of powdered Casgrain and Charbonneau's commercial thyroid extract. The liver and the thyroid extract were thoroughly mixed just prior to feeding. Twenty salmon parr were treated in this manner. This group as well as the control group was set up on June 22nd. The experiment ended on August 21st, 1948, when both control and treated fish were sacrificed.

The next group was composed of 30 normal parr, which were used as controls to the beef thyroid treated fish and were maintained on a ground beef liver diet.

The fish of corresponding, or fourth, experimental group received ground beef thyroid. These thyroids were prepared as follows: Frozen beef thyroids, purchased at Canada Packers Co. Ltd. in Montreal, were dried in an oven at 100°C for forty-eight hours. They were then further dried for six hours in a sulfuric aci desiccator under the vacuum of an oil pump. The dried thyroids were then ground into a fine powder. This powder was fed to the fish after mixing with an approximately equal portion of ground beef liver just prior to feeding. The experiment lasted six weeks, from July 9th to August 21st, 1948.

Photography:

At the end of this series of experiments representative fish of the four groups were photographed. For this purpose their movements were restricted in the following manner: The fish were transferred from their trough (temperature of about 10°C) into a glass aquarium containing water at approximately 2°C. This change in temperature caused the fish to remain motionless for a few minutes. The fish were kept close to the glass wall in front of the aquarium by means of a three-sided mobile trough. In this manner a side view picture of the fish could be taken. The top view pictures were taken under similar conditions, although in this case the fish were allowed to rest at the bottom of a 1,000 cc. beaker half filled with cold water.

An ordinary camera with a 3.6 lens was employed in this series of experiments. Two 100 Watt and two 80 Watt electric bulbs were used as a source of illumination for an exposure time of 8 seconds. Eastman Kodak "M" plates were used for all photographs.

Autopsy:

At the end of each experiment the fish were sacrificed by being placed in a 1 per cent solution of urethane for at least three minutes. Their length was then measured. At the ensuing autopsy, the ventro-caudal portion of the head was put into Bouin's fluid for histology. This region was limited caudally by the heart, anteriorly by the "tongue", dorsally by the floor of the pharynx and ventrally by the ventral skin. This region was hereafter referred to as the thyroid region.

Histological Techniques:

Tissues destined for routine histological examination were embedded in paraffin. Sections were cut at 6 μ and stained with hematoxylin - eosin or Masson's trichrome.

Results:

The control parr showed a fair degree of overall pigmentation. Denser groups of pigmented cells were present on the sides, forming what was described above as lateral bars (Fig. 1 and 2). Similar accumulations on the back were described as dorsal bars (Fig. 4). These fish showed a smooth contour of the head (Fig. 1 and 2) and a normal protrusion of the eye.

The parr treated with the commercial thyroid extract and those given the dried beef thyroid showed identical transformations and will be described together:

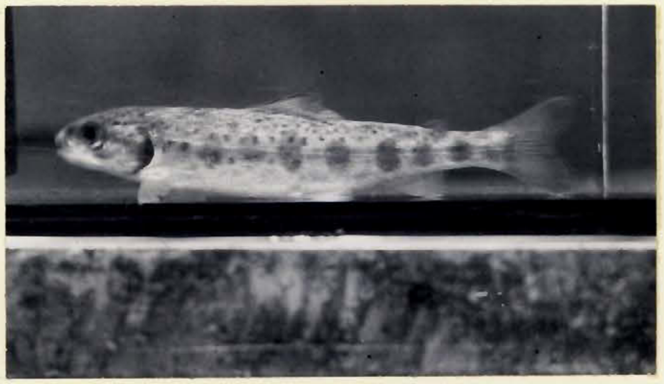
a) The pigmentation was much reduced compared with that of the control (Table 2; Fig. 3, 4 and 5). This depigmentation persisted in a few fish kept for months after the cessation of the treatment; a silvering of the ventral region accompanied these changes.

PLATE II

EXPLANATION OF FIGURES

Fig. 2 - Side view of control parr. The lateral bars are visible. The contour of the head is smooth.

Fig. 3 - Side view of parr treated with dried beef thyroid. The lateral bars are barely visible. The pigmentation is considerably reduced as compared to the control (Fig. 2). The contour of the head shows two protrusions on the top of the skull.



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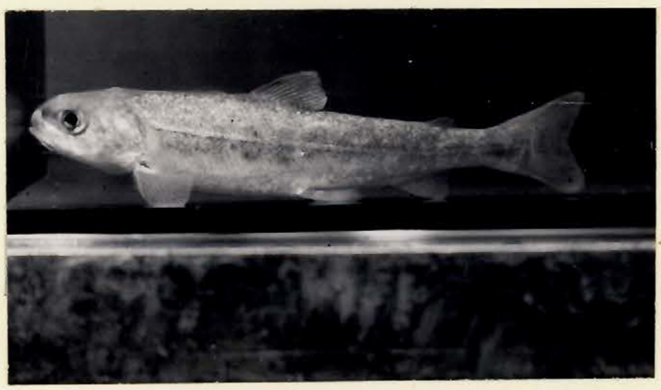

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TABLE 2

EFFECT OF FEEDING VARIOUS THYROID PREPARATIONS ON THE PIGMENTATION AND THE CONDITION OF THE EYES ON THE SALMON PARR

Treatments	Weight (gm.) Initial	Final	Weekly Increase	No. of fish at autopsy	Average intensity of pigmentation(1)	Condition of the eyes (2)
Controls	6.6	10.5	0.6	16	+++	N
Comm. thyroid extracts	6.7	9.4	0.4	14	+	E
Controls	6.9	10.7	0.6	29	+++	N
Beef thyroid	6.9	10.6	0.6	24	+	E

(1) Intensity estimated by gross examination.

(2) N - Normal protrusion of the eyes.

E - Endophthalmy.

PLATE III

EXPLANATION OF FIGURES

Fig. 4 - Top view of a control parr (below) and a parr treated with commercial thyroid extract (above).

The treated parr is much less pigmented than the control. The eyes of the treated do not bulge as much as those of the control. The opercula of the treated parr protrude more than those of the control.

Fig. 5 - Top view of a control parr (below) and a parr treated with dried beef thyroid (above).

The changes induced by feeding dried beef thyroid are similar to those obtained after feeding commercial thyroid extract as shown in Fig. 4.

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b) Typical cephalic deformations appeared a few weeks after the beginning of the treatment. They included two protrusions on the midline at the top of the head. One protrusion was located over the supraoccipital bone and the other over the dermethmoid bone (Fig. 3). When the head was examined from above, it appeared wider. This was mainly due to the lateral bulging of the opercules (Fig. 4 and 5). Finally, examination from above also showed that the eyes did not protrude as much as normally. Whether or not this was due to a true endophthalmy could not then be decided (Table 2; Fig. 4 and 5).

Incidentally, histological examination of the thyroid gland of these controls revealed that the cells were high columnar in shape and colloid in the follicles, thin and scanty. These observations indicated that the glands were rather active. Among the thyroid treated fish the activity of the thyroid gland was considerably reduced, since the follicles were lined with a low epithelium and considerable amounts of dense colloid was present.

Conclusions:

The chief observations made in this experimental series were that both commercial thyroid extract and dried beef thyroid cause the same transformation in the Atlantic salmon parr, namely a decrease in pigmentation and several changes in the size and shape of the head region.

IInd SERIES OF EXPERIMENTS:

A year after the 1st series of experiments, it was possible to repeat the work with the purpose of confirming the original observations and completing them by histological studies. This IInd series was carried out at the Fish Hatchery of the Laurentides in St. Faustin, Quebec, from the 15th of July to the 15th of December, 1949. The experimental parr used were reared in Baldwin Mills Fish Hatchery and taken to St. Faustin shortly before the experiments began. The fish were kept in circulating water at the hatchery in St. Faustin until then.

METHODS:

The salmon parr were a year and a half old at the beginning of the treatment. They measured from 5.0 to 14.0 cm. in length. The pigmentation of these fish was identical with that of the fish used in the first series of experiments. (Fig. 1).

The running water in which the fish were kept was a mixture of spring and brook water, for reasons already mentioned. In this series of experiments the mixture of water was heated by an ordinary coal furnace, stored in a large tank, and delivered by gravity to the troughs at a temperature of 10° to $13^{\circ}C$.

As in the previous series of experiments the temperature was recorded twice a day and the rate of flow of the water in the troughs was maintained at approximately 16 liters per minute. Troughs of identical sizes to those used in the previous series were used. Exposure to light was kept similar for control and treated groups. The troughs were covered with mosquito netting. Routine cleaning of the troughs was carried out on Tuesdays, Thursdays and Saturdays.

All groups were weighed by difference once a week as in the 1st series. Furthermore, at the beginning and end of the experiments the length of the fish was measured from the snout to the end of the caudal fin. For this purpose the fish were anesthetized in a 1 per cent solution of urethane (ethyl carbamate) and placed flat on their side along a graduated ruler.

In this series of experiment the basic diet was a mixture of ground horse liver and spleen instead of beef liver. The liver and spleen were ground and fed to the fish three times a week, i.e. Mondays, Wednesdays and Fridays.

Experimental Groups:

This series included one type of treatment only. One group of 30 parr received dried beef thyroid mixed with ground horse liver and spleen, while the corresponding control group of 30 untreated fish received ground horse liver and spleen only. Each group of treated and control parr were subdivided into three subgroups according to length. The first subgroup included 10 fish measuring from 5.0 to 8.0 cm. in length, the second 10 fish from 8.0 to 11.0 and the third 10 fish from 11.0 to 14.0 cm. in length. Each of the rectangular troughs containing a group of parr was divided, with fine screen wire partitions, into three zones corresponding to the three subgroups already described. The dimensions of the zones was in relation with the size of the fish. The smallest being the closest to the incoming fresh water, the medium size next and the largest last. The beef thyroid was prepared by the method already described in the first series of experiment.

In this series of experiment the effect of beef thyroid feeding on the salmon parr was observed over a much longer period of time extending from July 18th to December 15th, 1949.

Photography:

Representative fish of all subgroups were photographed while being kept still by the method already described. A Kodak Flash Supermatic camera with a 4.7 lens was used. The source of illumination was a small Synchro-Press No. 5 flash bulb with an exposure time of 1/200 of a second. Eastman Kodak "M" plates were employed for all photographs.

Autopsy:

Six weeks after the experiment had begun 5 fish in each subgroup were sacrificed, while the rest were autopsied four months and a half later.

In order to sacrifice the fish they were left in a 1 per cent solution of urethane for at least three minutes. The length of the fish was measured and the following anatomical structures were examined and fixed for histological study: 1) head; 2) caudal fin; 3) liver; 4) segment of the gastro-intestinal tract, including: stomach, pylorus, pyloric caeca and duodenum; 5) a portion including part of the small intestine as well as the spleen; 6) gonads; 7) transverse section of the body through the anterior portion of the dorsal fin.

Histological Techniques:

Tissues destined for routine histological examination were embedded in paraffin. Sections were cut at 6 µ and stained with hematoxylin - eosin or Masson's trichrome.

Since it was believed that calcium metabolism might be disturbed by thyroid treatment and deposition of calcium salts in the tissues might cause the changed appearance of the fish, an attempt was made to study the distribution of calcium in the sections by histochemical means. The tissues were fixed in a 5 to 1 solution of alcohol - formalin and embedded in paraffin. Sections were cut at 10 µ and stained by the method of von Kossa. This method consisted in treating the de-paraffinized sections by a 2 per cent solution of silver nitrate for two or three minutes. The sections were then dehydrated and mounted under Canada Balsam.

Results:

The beef thyroid preparation had no detectable effect on the weight increase of the parr (Table 3). As in the 1st series of experiments, there was a clearcut decrease in the pigmentation of the treated fish as compared to the controls. Changes in size and shape of the head were also observed (Fig. 8 and 9).

TABLE 3

EFFECT OF FEEDING DRIED BEEF THYROID PREPARATIONS ON THE GROWTH OF YOUNG SALMON PARR

Treatments	Weight (gm.) Initial	Final	Weekly Increase	Length (cm.) Initial	Final	Weekly Increase	No. of fish at autopsy
Controls	8.6	14.1	0.68	9.55	10.90	0.17	15
Beef Thyroid	9.1	14.0	0.61	9.59	11.17	0.19	15

It was further demonstrated that the degree of morphological changes occuring among the treated fish is related to the size of the fish. The smallest parr showed little outward change, while the largest showed maximum changes (Fig. 6 to 12).

Regular examinations of the fish during the course of treatment showed that the cephalic protrusions occurred early in the treated fish. Later, the whole head appeared to enlarge under the influence of the thyroid preparation. In this experiment an opacity and an increase in the thickness of the fins was noted. This had led us to suspect an increase in calcification. However, the histological sections stained by the von Kossa method showed no deposits, except of course, in the bones. At any rate, the distribution and intensity of the

PLATE IV

EXPLANATION OF FIGURES

Fig. 6 - Side view of a small-sized control parr. The fish is well pigmented. The contour of the head is smooth.

Fig. 7 - Side view of a small-sized parr treated with dried beef thyroid. The fish looks paler than the control (Fig. 6), especially in the ventral region, but the lateral bars are still prominent. The contour of the head shows a protrusion on the top of the skull.

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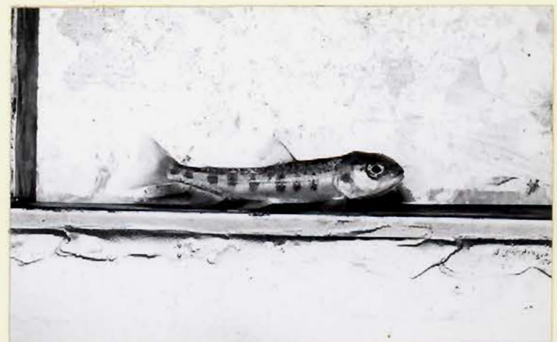
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PLATE V

EXPLANATION OF FIGURES

Fig. 8 - Side view of a medium-sized control parr.

Fig. 9 - Side view of a medium-sized parr treated with dried beef thyroid.

The lateral bars are less visible than in the control (Fig. 8); the fading was more advanced than in the small-sized treated parr (Fig. 7). The head appears enlarged, and shows a protrusion on top. The operculum covers the anterior portion of the base of the pectoral fin.

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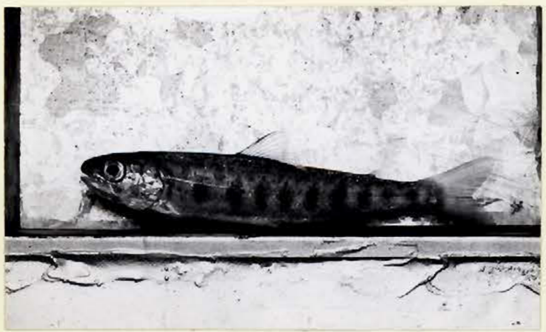

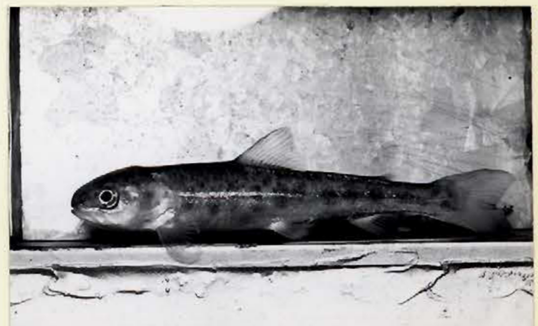

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PLATE VI

EXPLANATION OF FIGURES

Fig. 10 - Large-sized control parr above. Large-sized parr treated with beef thyroid below.

The dorsal bars are visible in the control but not in the treated. The opercular protrusions are quite prominent in the treated.

Fig. 11 - Side view of a large-sized control parr.

The lateral bars are visible. The contour of the head is fusiform.

Fig. 12 - Side view of a large-sized parr treated with dried beef thyroid.

The lateral bars are barely visible. The pigmentation is reduced as compared to the control (Fig. 11). The operculum covers part of the base of the pectoral fin. The head looks large. The fins are slightly more opaque than in the control.

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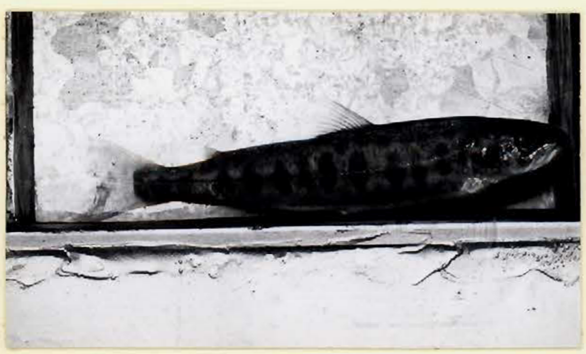

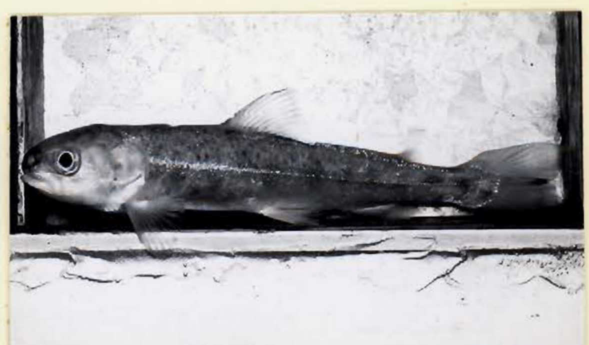

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reactions was the same in the treated and control fish.

On the other hand, sections prepared by routine histological methods showed striking changes in the treated fish, especially in the connective tissue of the skin or derma. This tissue could be examined in many of the sections, especially those of the head and transverse sections of the body through the anterior portion of the dorsal fin and the base of the caudal fin. The sections of the head also showed some connective tissue between the eyes. This was a looser type of connective tissue than that of the derma.

A brief account of the histology of the skin of the young salmon will be given before the results are described. There are two types of skin depending on whether scales are present or not. The skin which covers the head and the fins does not contain scales. Both epidermis and derma form smooth layers of fairly even thickness. The epidermis consists of the basement membrane, a basal layer of columnar cells and of stratified layers of squamous cells in which goblets cells are randomly distributed. The derma consists of intermingled loose collagenous fibers which become denser as they approach the basement membrane of the epidermis and as they reach bone or cartilage. A unicellular layer of pigment cells is found in the derma close to the inner portion of the basement membrane of the surface epithelium.

The skin which covers the rest of the body of the young salmon contains scales. The epidermis does not significantly differ from that of the head, except that the deeper layers are interrupted by scales. Underneath, there are distinct derma and hypodermis. The derma itself is composed of two separate layers. The more peripheral one is formed of loose collagenous fibers in which the scales are embedded, while the inner one is made up of very dense bundles of collagenous fibers through which transverse bundles of connective tissue run in a more or less regular manner. There are two layers of pigment cells, one close to the surface which is a continuation of that found on the head and fins, and another on the inner surface of the dense collagenous fibers of the derma. Both layers of pigment cells are one cell thick. The hypodermis is formed by a thin layer of loosely arranged collagenous fibers which separate the derma from the underlying muscles; its size varies with the location.

The effect of thyroid feeding on the connective tissue of parr is summarized in Table 4, in which the mean thickness of the derma is expressed as the number of +. The treated fish showed a pronounced increase in the thickness of the dermal connective tissue on the top and ventral portion of the head and in the caudal region as compared to the controls (Fig. 13 to 24). A slight difference could be noticed in the thickness of the derma of the body skin in the parr. These differences were quite definite in the sacrificed fish after five months of treatment. However, a few of the fish which had been sacrificed six weeks after the beginning of the treatment showed only a small increase in the connective tissue of the cephalic region, but no visible effect anywhere else. Soon afterwards the connective tissue of the fins appeared enlarged. Prolonged treatment was necessary to obtain the full effect.

TABLE 4

EFFECT OF BEEF THYROID FEEDING ON THE MEAN THICKNESS OF THE CONNECTIVE TISSUE OF THE SALMON PARR

Treatment	Average length cm.	Dermal Connective Tissue: Head – Top	Head – Ventral portion	Caudal Region	Body Skin
Control parr	10.9	++±	++±	+±	++
Treated parr	11.1	+++++±	+++++	++++	++±

Thus the connective tissue hyperplasia appeared first in the areas of skin devoid of scales, such as in the head and fin regions.

PLATE VII

EXPLANATION OF FIGURES

Fig. 13 - Cross section of the head on the dorsal surface. Control parr fed beef liver. Masson's trichrome.

From top to bottom, note the deep staining epidermis, the light staining derma (approximately 3 times as thick as the epidermis), bone, cartilage, meninges and nervous system.

Fig. 14 - Cross section of the head on the dorsal surface. Parr treated with dried beef thyroid. Masson's trichrome.

The thickness of the epidermis is the same as in the control (Fig. 13), but the thickness of the derma is almost twice that of the control. (The derma is 5 or 6 times as thick as the epidermis. No apparent change can be seen in bone, cartilage or meninges).

Fig. 15 - Cross section of the eye of a control parr:

R: anterior edge of retina (ora serrata).

L: lid (fold of skin bordering the visible portion of the eye ball). The cornea may be seen below. Masson's trichrome. Note the relative position of retina and lid.

Fig. 16 - Cross section of the eye of a treated parr. Dried beef thyroid treatment. Masson's trichrome.

Note the change in the relative position of retina (R) and lid (L) and the increase in the thickness of the derma as compared with the control (Fig. 15).

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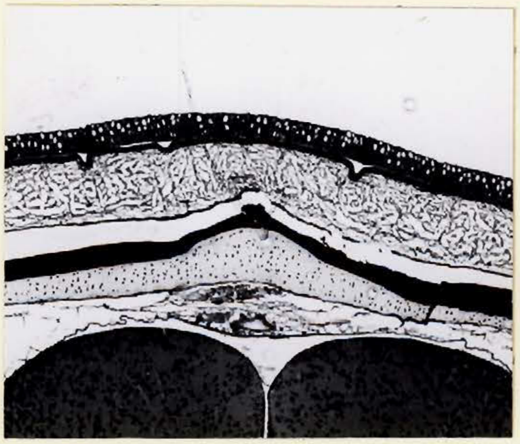

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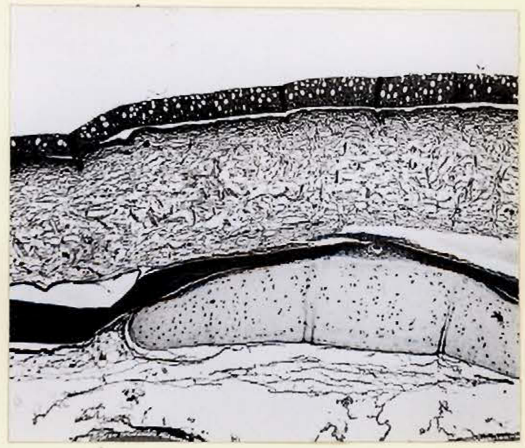

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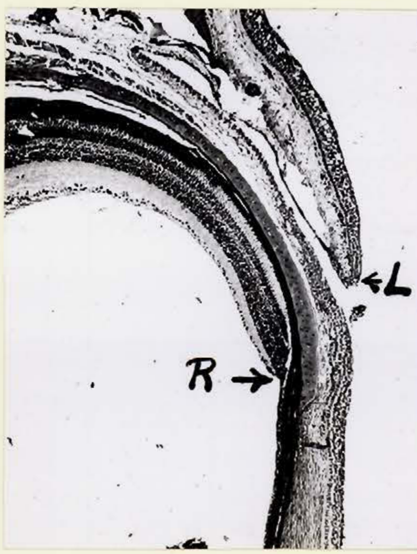

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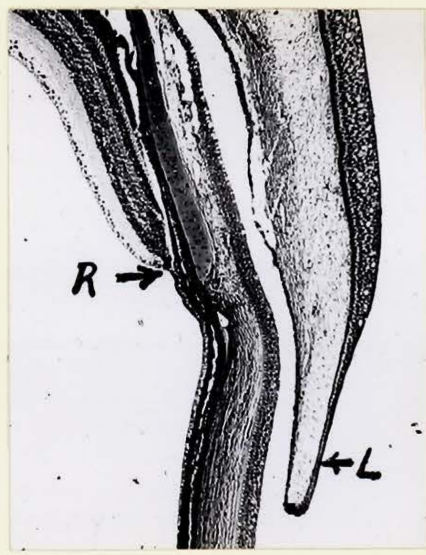

PLATE VIII

EXPLANATION OF FIGURES

Fig. 17 - Cross section through the upper jaw of control parr. Von Kossa stain for calcium, Hematoxylin-eosin counterstain.

From left to right, it is possible to see the epidermis, the derma, muscles, bone staining intense black, connective tissue and mouth epithelium.

Fig. 18 - Cross section through the upper jaw of treated parr. Dried beef thyroid treatment. Von Kossa stain, Hematoxylin-eosin counterstain.

The thickness of the derma is markedly increased but that of the connective tissue underlying the mouth epithelium seems very slightly increased.

(Slides represented in Figures 19 and 20 were stained by von Kossa's method for calcium and conterstained with Hematoxylin-eosin).

Fig. 19 - Cross section of the dorsal portion of the caudal fin in a control parr.

Fig. 20 - Cross section of the dorsal portion of the caudal fin in a parr treated with beef thyroid.

There is an obvious increase in the thickness of the derma as compared with the control (Fig. 19).

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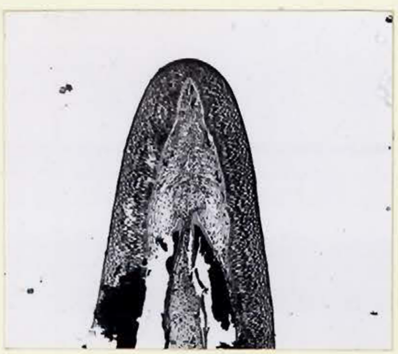

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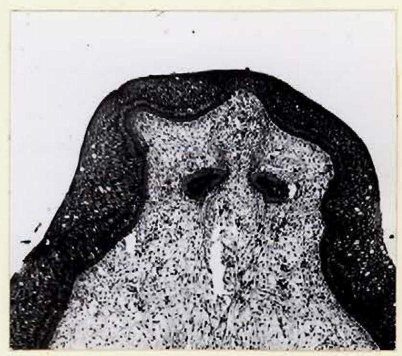

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PLATE IX

EXPLANATION OF FIGURES

(These slides were stained by von Kossa's method and counter-stained with hematoxylin-eosin).

Fig. 21 - Antero-posterior section through the middle of an operculum in a control parr.

Notice the distance between the bone (black) and the tip of the operculum.

Fig. 22 - Anterio-posterior section through the middle of the operculum in a parr treated with beef thyroid.

Notice the hyperplasia of the derma and the great increase in the distance between the bone and the tip of the operculum.

Fig. 23 - Cross section through the ventral portion of an operculum in a control parr.

The folds in the ventral region of the operculum are seen in cross section.

Fig. 24 - Cross section through the ventral portion of an operculum in a parr treated with beef thyroid.

The thickness of the derma has increased so greatly that only one fold is seen in the area previously occupied by the four folds in the control (Fig. 23).

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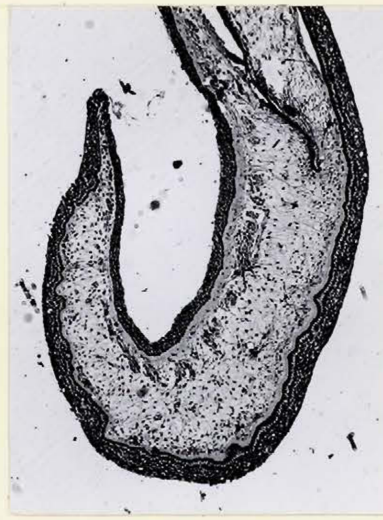

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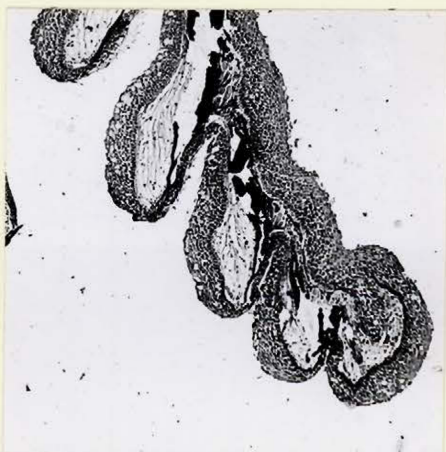

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Confirmation of the occurrence of protrusions of the head region as observed in the Ist series, was obtained. However, further observations were made on the time of appearance of the cephalic modifications. Approximately four weeks after the beginning of treatment, the parr showed a protrusion over the supra-occipital bone (Fig. 7). About a week later a second protrusion appeared along the median-dorsal line of the head at the levelof the dermethmoid bone. Simultaneously a protrusion appeared along the distal ridge of each of the preopercular bones (Fig. 10, lower fish). At this period the eyes seemed to regress by an overlapping of the lid on the eye (Fig. 16) so as to cause a condition which might be described as apparent endophthalmy. As the treatment was continued the protrusion at the level of the dermethmoid bone gradually disappeared along with an increase in the size of the head. Figures 9 and 12 show the stage at which this protrusion had disappeared. During the treatment the opercula enlarged continuously and eventuall; overlapped the base of the pectoral fins, as may be seen by a close examination of Figures 9 and 12. The caudal fin of control had the outline of a V (Fig. 8 and 11). In the treated fish this fin had acquired the outline of a U (Fig. 9 and 12) as due to an increase in the inter-fin-ray material.

Figures 6, 8 and 11 show three normal parr of the three different sub-groups used in this series, representing the small, the medium size and the largest subgroups respectively. The corresponding treated fish are seen in Figures 7, 9 and 12. It should therefore be noted again that these fish were treated for five months and that after that time the effect of thyroid feeding is slight in the small fish (Fig. 6 and 7), intermediate for the medium-sized parr (Fig. 8 and 9) and maximum for the large fish (Fig. 11 and 12).

CONCLUSIONS:

In addition to the symptoms previously described (Ist series), feeding beef thyroid preparations causes an hyperplasia of the dermal connective tissue, especially in the skin of the head and fins. This effect of the thyroid treatment is maximal in the largest and minimal in the smallest salmon parr.

IIIrd SERIES OF EXPERIMENTS:

In order to observe whether the effects of beef thyroid were specific for the salmon parr, the same type of experiment was repeated using younger (fry) and older (smolt) salmon. This series of experiments was conducted at the Fish Hatchery of Gaspe in Gaspe, Quebec, from the 27th of June to the 2nd of September, 1950. Besides salmon parr, fry and smolt, young trout were also used in this series. All these fish were reared in the Gaspe hatchery.

METHODS:

The salmon fry were five months old. They measured from 3.0 to 5.0 cm. in length. At this stage the fish had seven to nine distinct pigmented lateral and dorsal bars.

The salmon parr used in this series were a year and a half old. They measured from 5.0 to 15.0 cm. in length and were similar in every respect to those used in the previous experiments.

The smolt were between two and a half and three and a half years of age. They measured from 15.0 to 25.0 cm. in length. They exhibited the pigmentary pattern of the adult. At this stage the lateral and dorsal bars have disappeared and the dorsal region is uniformly pigmented while the ventral and lateral regions are silvery.

The young trout were approximately 6 months old. They measured from 5.0 to 8.0 cm. in length. At this stage the trout had distinct lateral bars and were considered as typical trout parr.

For this series also, the fish were kept in running water under the conditions prevailing in the hatchery. The water used was a mixture of spring and brook water. The temperature of the water was recorded twice a day and found to vary between 9° and 13°C. The troughs used were 68" long by 15" wide by 8" deep. Five inches of water were maintained in them and the rate of flow of the water was kept at approximately 16 liters per minute daily. Before each experiment was started, pairs of similar troughs were selected for corresponding control and treated groups. The troughs were covered with mosquito netting as soon as the experimental parr or smolt were put into them. The routine cleaning of these tanks was carried out three times a week, i.e. Tuesdays, Thursdays and Saturdays.

All groups were weighed once a week as already described. Fish were also measured and compared morphologically at the beginning and the end of every experiment.

In this series the fish were fed ground beef liver that had been kept on ice for not more than two days previous to feeding. They were also fed three times a week, i.e. Mondays, Wednesdays and Fridays.

Experimental Groups:

This series included one type of treatment on four types of fish. The treatment was that of thyroid feeding (half beef liver, half beef thyroid as above) and the fish used were salmon fry, salmon parr, salmon smolt and young trout hatched that year. Each group of treated fish had a corresponding group of controls that were maintained on the basic diet of beef liver.

The following groups were used:

1) 500 control fry
2) 500 thyroid-treated fry
3) 100 control parr
4) 100 thyroid-treated parr
5) 20 control smolt
6) 20 thyroid-treated smolt
7) 100 control trout
8) 100 thyroid-treated trout

Photography:

Representative fish of all the salmon groups were photographed after they had been preserved in formalin. A Kodak Flash Supermatic camera with a 4.7 lens was also used in this series. The source of illumination was two RFL 2 reflector photoflood lamps and an exposure time of 1/10 of a second was used. Again in this series Eastman Kodak "M" plates were employed for all photographs.

Autopsy:

At the end of the experiment the fish were sacrificed by means of a one per cent solution of urethane. They were measured and examined.

At the ensuing autopsy the following anatomical structures were examined and fixed for histological study: 1) the thyroid region of the head; 2) the pectoral and caudal fins; 3) the liver; 4) the heart; 5) a segment of the gastro-intestinal tract including stomach, pylorus, a portion of the pyloric caeca and duodenum; 6) a portion including the spleen and a part of the small intestine; 7) the gonads; 9) a cross-section of the body at the anterior origin of the dorsal fin and including the skin, muscle, kidney and swimming bladder of the fish.

Histological Techniques:

Tissues destined for routine histological examinations were embedded in paraffin. Sections were cut at 6 µ and stained with hematoxylin - eosin or Masson's trichrome.

The distribution of metachromatic material was studied in tissues fixed in Zenker - Formol and embedded in paraffin. Sections 6 µ thick were stained with toluidine blue according to Lison (1936).

The Counting of Pigment Cells:

In the third series of experiments pigment cells were counted along the median dorsal and median ventral regions of fry, parr and smolt. (These regions are also referred to as "crests"). For this purpose, treated and control fish were injected with a 1 to 1000 solution of adrenalin 10 minutes before sacrifice in order to obtain a contraction of the pigment cells and to thus facilitate counting. The dose given varied from 0.05 to 1.50 cc. of adrenalin solution, depending on the size of the fish. Pieces of skin were taken from the dorsal and ventral region anterior to the anal fin and posterior to the dorsal fin. Care was taken to include all layers of the skin and a thin layer of underlying muscles. The pieces of the skin were dehydrated by immersion in successive changes of alcohol from 50 per cent to absolute and then cleared in xylol. The tissues were mounted under a coverslip in cedar oil.

The slide was placed on the stage of a horizontal microscope and an image was projected onto a piece of white paper with the help of a prism adapted to the ocular (Fig. 25). All pigment cells were outlined on the paper, and subsequently counted in an area corresponding to 0.2 mm. square on the skin. Four fields were taken at random along the ventral crest and also on the dorsal crest. This estimation was carried out for four fish, thus giving thirty-two determinations per group.

PLATE X

EXPLANATION OF FIGURES

Fig. 25 - This figure shows a sketch of the projecting apparatus used in counting pigment cells:

B: Body of the microscope

C: Condenser

L: Light Source

LB: Light Beam

MS: Mechanical Stage

O: Objective

TB: Stainless Steel Case

OL: Opening in Steel Case to Adjust the Light Source

OM: " " " " " " " Microscope

PF: Projected Image

PR: Prism

S: Slide with Skin for Counting Pigment Cells

ST: Stand

W: Wire

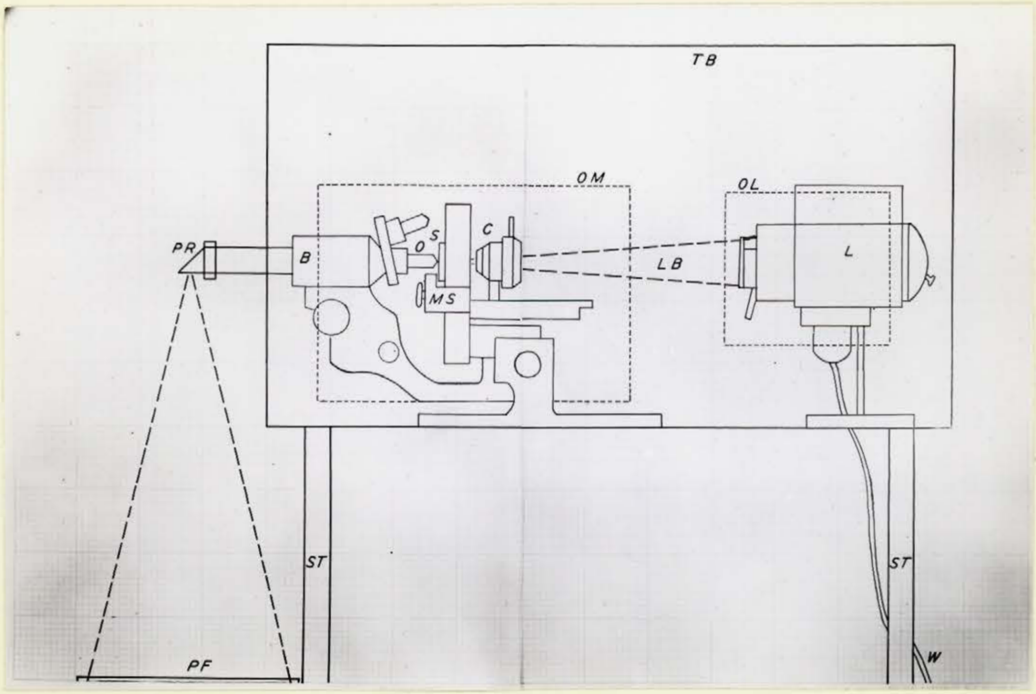

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TABLE 5

EFFECT OF BEEF THYROID PREPARATIONS ON THE AVERAGE THICKNESS OF THE CONNECTIVE TISSUE OF SALMONIDAE

Type of fish and treatment	Average length - cm.	Dermal Connective Tissue: Head, Top	Dermal Connective Tissue: Head, Ventral Portion	Dermal Connective Tissue: Caudal Region	Dermal Connective Tissue: Body Skin	Internal Conn. Tissue: Inter-orbital
Salmon fry, controls	4.9	++	++	++	+±	++±
Salmon fry, treated	4.8	+++	+++	++	+±	++±
Salmon parr, controls	8.7	++±	++±	+±	++	+++
Salmon parr, treated	9.2	++++	++++	+++	++	+++
Salmon smolt, controls	22.1	+++	+++	+	++	+++++
Salmon smolt, treated	21.9	+++++	+++++	+++	+++	+++++±
Young trout, controls	11.1	++	++	++	+++	+++
Young trout, treated	10.5	++++	++++	+++	++++	++++±

RESULTS:

The results obtained on parr in the first and second series of experiments were confirmed on the parr in this series. A gross depigmentation and an increase in the connective tissue was also observed in the treated salmon fry and smolt and in the young trout.

The effect of beef thyroid feeding on the thickness of the connective tissue of fry, parr, smolt and young trout was roughly estimated as number of + in each fish. The average figures are shown in Table 5. Thus the treated fry showed an increase in the thickness of the dermal connective tissue on the top and ventral portion of the head. The treated parr showed an increase in the thickness of the dermal connective tissue on the top and ventral portion of the head and in the caudal region. The smolt showed an increase in the thickness of the dermal connective tissue on the top and ventral portion of the head, in the caudal region and on the body skin. The treated trout showed a maximum effect. The thickness of the dermal connective tissue of all regions increased, and an increase in the interorbital region was also observed. These results on the salmon fry, parr and smolt, as well as on the young trout, were obtained after feeding beef thyroid for a period of six weeks.

The results of this experiment further confirmed our observations of the IInd series by showing that the bigger a fish was the stronger was its reaction to thyroid treatment. In this series young river salmon at different stages of development were tested and the maximum effect was again seen in the smolt (largest, Fig. 26 and 27) and the minimum in the fry (smallest, Fig. 30 and 31) with an intermediary effect in the parr (medium size, Fig. 28 and 29). The modifications due to thyroid treatment were first seen in the treated smolt (i.e. two weeks after the beginning of the treatment), then in the parr (i.e. three and

PLATE XI

EXPLANATION OF FIGURES

Fig. 26 - Dorsal view of the head of a smolt treated with beef thyroid on the right and of a control smolt on the left.

The skin of the head of the treated smolt, extending as far back as the posterior edge of the operculum is thrown into thick irregular folds. The opercula of the treated fish appear thicker and rougher than those of the control. Finally, the treatment has caused a decrease in pigmentation.

Fig. 27 - Lateral view of the head of a smolt treated with beef thyroid on the right and a control smolt on the left.

The thickening of the skin, especially around the operculum, is very striking in the treated smolt. The pigmentation is far less in the treated than in the control fish.

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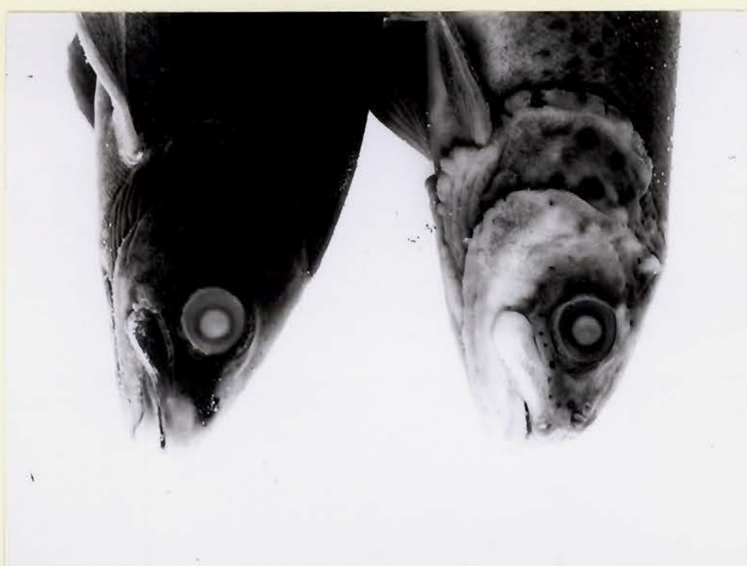

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PLATE XII

EXPLANATION OF FIGURES

Fig. 28 - Dorsal view of a parr treated with beef thyroid on the right and a control on the left.

The treated fish show a decrease in pigmentation compared with control.

Fig. 29 - Lateral view of a parr treated with beef thyroid on the right and a control on the left.

Depigmentation is especially visible in the ventral region. An enlargement of the operculum can also be observed in the treated parr.

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PLATE XIII

EXPLANATION OF FIGURES

Fig. 30 - Dorsal view of a fry treated with beef thyroid on the right and a control on the left.

The endophthalmia and protrusions on the opercula of the treated fry are visible.

Fig. 31 - Lateral view of a fry treated with beef thyroid on the right and a control on the left.

The ventral region of the treated fry is paler than that of the control. A protrusion can be seen on the head of the treated fry.

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a half weeks after the treatment had started) and finally in the fry (i.e. four and a half weeks after the beginning of treatment).

Six weeks after the treatment had started the hyperplasia of the connective tissue had reached as far as the following regions, namely: 1) in the smolt the derma over the whole body; 2) in the parr the derma of the skin that did not contain scales; 3) in the fry the derma of the cephalic region only. If a comparison is made on the relative effects of thyroid feeding between two species of salmonidae such as the young salmon and trout, the results showed that not only did the trout parr show more intense modifications than the smolt, in proportion to its size, but the treatment had extended its effect to the connective tissue of the interorbital region (Table 5).

The effect of beef thyroid feeding on the thickness of the epithelium of the skin or epidermis of fry, parr, smolt and young trout was estimated by histological examination of the skin. Observations were made on the thickness of the epidermis on the head, body and caudal region. Significant changes were observed only in the smolt and young trout. The treated fish showed an increase in the thickness of the epidermis in all three regions studied. The results are summarized in Table 6.

In order to determine whether the increase in thickness observed in the epidermis was due to an increase in the number of cells or an increase in the size of the cells, the number of metaphases in 10, 0.1 mm. square, areas of skin from the head were counted (Table 6). The beef thyroid treatment produced a significant increase in the number of metaphases in the epidermis of the smolt.

TABLE 6

EFFECT OF BEEF THYROID FEEDING ON THE THICKNESS OF THE EPIDERMIS IN SALMON SMOLT AND YOUNG TROUT

		Head	Body	Caudal Region	Metaphases per 0.1 mm. square (1)
Smolt	Control	++	++	++±	0.22
	Treated	++++	++++	+++	0.60
Trout	Control	+++	+++	+++	0.23
	Treated	+++++	+++++	++++	0.33

(1) Average results from 10 different areas on the heads of six fish (60 readings).

The administration of beef thyroid preparations produced an overall decrease in intensity of pigmentation in the fish. The decrease in pigmentation is most evident as a lightening of the dorsal region or as a disappearance of the lateral bars. A decrease in pigmentation was also observed in the ventral region. These effects were first noticed four weeks after the treatment began, and gradually increased in intensity. They persisted and were still clearly detectable three months after treatment had been stopped.

Upon gross examination of the pigmentary pattern in the young salmon fry and parr there were no striking differences between the fish at these stages. Although a close observation revealed that the dorsal bars stood out more clearly in the fry than in the parr. It is already known that the disappearance of the dorsal bars took place by infiltration of the unpigmented patches between each dorsal bar by pigment cells. This process of pigmentation had reached its climax in the smolt stage where the fish showed a uniform pigmentation of the dorsal

TABLE 7

INFLUENCE OF FEEDING BEEF THYROID ON THE NUMBER OF PIGMENT CELLS IN VENTRAL AND DORSAL SKIN OF YOUNG SALMON (FRY, PARR, SMOLT STAGES) AND TROUT

Stage	Treatment	Average Body Weights			Ventral Region		Dorsal Region	
		Initial (gm.)	Final (gm.)	Weekly Increase (gm.)	No. of Pigment Cells (1)	Value of P.	No. of Pigment Cells (1)	Value of P.
Fry	Control	0.5	0.8	0.04	6.7	<0.001*	79.6	0.4>P>0.3
	Thyroid feeding	0.5	0.8	0.04	1.2		75.7	
Parr	Control	5.1	6.3	0.15	3.2	<0.001*	89.1	0.01>P>0.001*
	Thyroid feeding	5.1	6.9	0.23	0.9		64.3	
Smolt	Control	40.3	77.5	5.3	6.7	<0.001*	91.7	0.1>P>0.05
	Thyroid feeding	38.6	62.1	3.3	1.2		82.2	
Young Trout	Control	2.9	9.8	0.77	10.0	<0.001*	102.6	0.01>P>0.001*
	Thyroid feeding	3.1	9.5	0.71	3.0		85.0	

* Indicates highly significant differences between control and treated.

(1) The number of pigment cells in an area of skin 0.2 mm. square.

region. The parr is at the intermediate stage of pigmentation between fry and smolt where the dorsal bars are visible but faded out by early pigmentation of the inter-dorsal-bar spaces. The dorsal and lateral bars of the fry are generally fewer than that of the parr. Once the dorsal portion of the lateral bars and the dorsal bars of the parr have disappeared completely through a uniform darkening of that region, and once the silvering of the ventral region has covered the remaining of the lateral bars and red dots, the fish is referred to as a smolt.

Six weeks after the beginning of the treatment, just prior to autopsy, a gross examination of their pigmentation was carried out on each of the treated groups of young salmon. It was thus found that the treated fry were slightly darker than the treated parr and these in turn darker than the treated smolt.

An attempt was made to obtain an objective estimate of the degree of pigmentation in relation to the number of pigment cells of control and treated parr. The number of pigment cells was counted in an area of skin 0.2 mm. square from both the ventral and dorsal areas. The results of this experiment (Table 7) showed that a large number of pigment cells were present in the dorsal region, while in the ventral region there were few. In both areas, however, the controls had a significantly greater number of pigment cells than the treated fish. Thus, the thyroid extract had decreased the number of pigment cells of the treated parr.

In order to determine whether thyroid preparation produced pigmentary changes at other than the parr stage, quantitative measurements were also made in fry and smolt, as well as in young trout. The results (Table 7) indicated that the beef thyroid produced a significant decrease in pigmentation in the ventral regions of fry and smolt, as in the parr. However, in the dorsal region, the differences were significant only in the parr. The trout also showed a significant decrease in pigmentation in both ventral and dorsal regions.

CONCLUSIONS:

The hyperplasia of the connective tissue produced by thyroid extracts in the salmon parr was accompanied by a depigmentation which has been analyzed and shown to be due, at least in part, to a decrease in both size and number of pigmented cells. Similar results were obtained in smolt and fry.

Beef thyroid feeding causes an increase in the thickness of the epidermis in the salmon smolt. This seems to be due mainly to a significant increase in the rate of mitosis.

In Salmonidae the effects of beef thyroid preparations are not limited to the Atlantic salmon, but can also be detected in the young speckled trout. All the observations made in the young salmon could be verified in the young trout. Upon beef thyroid feeding this fish showed hyperplasia of the dermal and intracephalic connective tissue and a significant decrease in the number of pigmented cells of the skin.

CHAPTER III

INFLUENCE OF SODIUM THYROXINATE ON THE YOUNG ATLANTIC SALMON

So far the experiments were concerned with the effects of thyroid extracts on the young salmon. It is well known that these extracts contain the protein thyroglobulin, which itself includes thyroxine held in peptide linkages. Recently it was definitely established that thyroxine is the thyroid hormone (Gross and Leblond, 1951). Therefore, to see whether similar results could be obtained with this hormone itself, d-l sodium thyroxinate, was injected into salmon parr.

1st SERIES OF EXPERIMENTS:

METHODS:

This series of experiments was conducted at the Fish Hatchery of the Laurentides in St. Faustin, Quebec, in the summer of 1948. The salmon parr used in this experiment were of the same origin as those used in the 1st series of experiments concerned with the influence of thyroid extracts on the young salmon. The fish were also kept in running water under conditions prevailing in the hatchery. The temperature was checked twice a day. The fish were fed a basic diet of ground beef liver and weighed once a week. Troughs were also cleaned three times a week.

In this first experiment the effect produced by sodium thyroxinate on the parr was examined after injection of this hormone. A solution was prepared by dissolving 20 mg. of sodium thyroxinate in 5 cc. of a 1 per cent solution of sodium hydroxide; 0.05 cc. of this solution, equivalent to 200 micrograms of sodium thyroxinate, was injected bi-weekly (Tuesdays and Fridays) using a 1 cc.

hypodermic syringe and a number 27 needle. Thus, the fish received 400 micrograms of this substance per week. The injections were intramuscular, in the region dorsal to the lateral line at the level of the adipose fin.

In order to inject solutions that were isothermic with the fish, the flasks containing the thyroxinate and saline solutions were kept for half an hour in the same water as the fish. Immediately before injection the fish were placed in a 1 per cent solution of ethyl carbamate (urethane) until they lost their equilibrium, indicating that slight anesthesia had taken place.

Experimental Groups:

The thirty control fish were injected with 0.05 cc. of a solution containing 7 gm. of sodium chloride per liter.

Thirty salmon parr were treated with sodium thyroxinate. The two groups (controls and treated) were examined throughout the eight weeks' period from July 9th to August 21st, 1948.

Photography:

The external changes produced in the treated fish of this first series of experiments were recorded in a photograph. Live fish were photographed with an ordinary camera equipped with a 3.6 lens. Two 100 watt bulbs were used as a source of illumination for an exposure time of five seconds. An Eastman Kodak "M" plate was employed for the photograph.

Autopsy:

After the experiment had ended 10 fish were removed at random from both the treated and control groups. The fish were sacrificed by placing them in a 1 per cent solution of urethane for at least three minutes. Their length was then measured. The thyroid region of each fish was removed and fixed for histological study.

Histological Techniques:

The thyroid regions were fixed in Bouin's fluid and embedded in paraffin. Sections were cut at 6 µ and stained with hematoxylin - eosin.

RESULTS:

A comparison between the control and the thyroxinate treated fish showed that thyroxine produced the same transformations as the thyroid extracts. The decrease in pigmentation can be observed in Figure 32, in which the treated fish is shown alongside the darker control fish. The average results on pigmentation are condensed in Table 8, in which reference is also made to the condition of the eyes in the fish. The protrusion of the opercula and the apparent endophthalmy of the thyroxine-treated fish are due to the hyperplasia of the connective tissue.

TABLE 8

EFFECT OF SODIUM THYROXINATE INJECTION ON THE PIGMENTATION AND THE CONDITION OF THE EYES OF SALMON PARR

Treatments	Weight (gm.) Initial	final	Weekly Increase	Average Intensity of pigmentation (1)	Condition of the eyes (2)
Control (saline injection)	8.5	12.0	0.9	++±	N
Thyroxinate (injection)	7.5	11.5	1.0	+	E

(1) Intensity estimated by gross examination (not by counting).

(2) N: Normal protrusion of the eyes.
E: Endophthalmy.

PLATE XIV

EXPLANATION OF FIGURE

Fig. 32 - Top view of a control parr (above) and a parr treated with sodium thyroxinate (below).

The changes induced by sodium thyroxinate are identical to those obtained after feeding commercial thyroid extract as shown in Fig. 3.

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Conclusions:

Injections of four hundred micrograms of thyroxine weekly to salmon parr produces the same effects (depigmentation, etc.) as feeding these fish with either commercial thyroid extract or dried beef thyroid. It is therefore concluded that thyroxine - the thyroid hormone - is the effective agent of thyroid preparations.

IInd SERIES OF EXPERIMENTS:

METHODS:

In an attempt to determine the minimal dose required to produce changes in the parr, different doses of sodium thyroxinate were administered by means of pills placed directly in the stomach. This experiment was carried out at the Gaspe Fish Hatchery in Gaspe, Quebec, in the summer 1950. The experimental fish of this experiment were placed under the same general conditions as the fish receiving the beef thyroid treatment in the IIIrd series.

The fish were measured at the beginning and at the end of the experiment and weighed every week during the course of the experiment.

The pills, 3 mm. in diameter by $1\frac{1}{2}$ mm. thick, were obtained from Ayerst, McKenna and Harrison Ltd. The filling consisted of a mixture of gelatin and glucose. Five types of pills were available containing sero, ten, thirty-three, one hundred and three hundred and thirty-three gammas of sodium thyroxinate respectively. The fish were anesthetized in a 1 per cent solution of urethane, and the pills were introduced directly into their stomachs with the help of fine straight forceps.

Experimental Groups:

Five groups of salmon parr consisting of twenty five fish each were given different doses of sodium thyroxinate using the pills described above. The experiment lasted for seven weeks extending from July 12th to August 31st, 1950, during which time the fish were periodically examined.

Autopsy:

Twenty fish were removed from each of the groups used in the IInd series of experiments on the influence of thyroxine. The fish were sacrificed by allowing them to remain in a 1 per cent solution of urethane for at least three minutes. Their length was measured, their thyroid region removed and fixed for histological examination.

Histological Techniques:

The thyroid regions were fixed in Bouin's fluid and embedded in paraffin. Sections were cut at 6 μ and stained with Masson's trichrome.

RESULTS:

The various doses of thyroxine given orally to salmon parr in this experiment produced no defnite results. Thus, no depigmentation (Table 9) nor cephalic deformations were observed. In other words, the doses of thyroxine used orally here were not effective.

Examination of the thyroid gland of the treated fish showed on the other hand a reduced activity of the gland as compared with the control parr. It was then assumed that at least a certain amount of thyroxine had been absorbed and had reached the thyroid gland.

TABLE 9

EFFECT OF DIFFERENT DOSES OF SODIUM THYROXINATE (1) ON THE PIGMENTATION OF PARR

	Average Weight Initial (gm.)	Average Weight Final (gm.)	Weekly Increase (gm.)	Average Intensity of Pigmentation (2)
Control	4.7	5.4	0.10	+++
Parr receiving 20 gammas of thyrox. weekly	5.1	5.9	0.11	+++±
Parr receiving 66 gammas of Thyrox. weekly	5.1	6.3	0.17	+++
Parr receiving 200 gammas of thyrox. weekly	4.8	6.2	0.20	+++
Parr receiving 666 gammas of thyrox. weekly	5.0	6.0	0.14	+++±

(1) Sodium thyroxinate was administered as pills and were introduced into the stomach.

(2) Intensity estimated by gross examination (not by counting the number of pigment cells).

CONCLUSIONS:

While the intra-muscular action of thyroxine produced a depigmentation and other morphological changes, the oral administration of this hormone, up to 666 µg per week, did not produce any of these transformations. No difference could be observed between controls and treated fish. Possibly pure thyroxine is not effective orally in fish.

CHAPTER IV

INFLUENCE OF IODIDE TREATMENT ON THE YOUNG ATLANTIC SALMON

It was demonstrated that thyroid extracts, as well as thyroxine itself, produced remarkable morphological changes in the young salmon. The question then raised was, could the thyroid gland of the fish synthetize enough thyroxine by itself to produce similar changes. In order to solve this problem, iodine-deficient salmon parr were used, that is to say, fish whose thyroid gland was producing low amounts of hormone, since the raw material - iodine - was lacking. This condition could be recognized by the fact that the thyroid gland of these fish was enormous. Histological examinations of these glands showed the hyperplasia of the secreting epithelium characteristic of this condition (Fig. 33 and 34). Different doses of iodide were added to the circulating water where the fish were kept, as described below. Under these conditions the thyroid gland should be able to utilize the supplementary iodide to produce its hormone. It would be expected that the amount of thyroxine produced by the thyroid gland under these conditions should be considerably increased.

In the experiment to be described, an attempt was made to discover whether iodide, added to the water of iodine-deficient fish, could produce changes similar to those already observed in fish receiving thyroid preparations.

METHODS:

This experiment was carried out at the Fish Hatchery of the Laurentides in St. Faustin, Quebec, in the summer 1948. The salmon parr used in this experiment were of the same breed as those employed in the study of the effect of thyroid extracts (1st Series of Experiments).

The fish were kept in the running water of the fish hatchery, where the temperature was recorded twice a day. In this experiment the fish were kept under the same general conditions as those on the beef thyroid feeding experiment in the summer 1948. Weighing was done once a week and groups were fed on beef liver three times a week.

Efforts were made to obtain a continuous and regular supply of iodide and the following technique was used: Two solutions were prepared containing different amounts of iodide. One contained 150.0 gm. potassium iodide and 50.0 gm. sodium iodide in 20 liters of tap water (1 per cent solution); the other contained 750.0 gm. potassium iodide and 250.0 gm. sodium iodide in the same volume of tap water (5 per cent solution).

The bottles containing these solutions were securely stoppered and inverted on a wooden stand. The solutions were delivered to the troughs by gravity through rubber tubing. The rate of flow was regulated by means of a screw clamp. The end of the rubber tubing was placed in the flow of running water so as to ensure rapid mixing.

Two troughs were arranged in series. The rate of flow of the water in the first trough was adjusted at 5000 cc. per minute. In the incoming stream of fresh water the 1 per cent solution of iodide was allowed to drip at the rate of 1 cc. per minute, thus giving a concentration of 10 parts per million (p.p.m.) of iodide in the water of the first trough. The water from this trough ran into the second trough. It was assumed that the amount of iodide fixed by the fish of the first trough was negligible. In the second trough, to this water already containing 10 p.p.m. of iodide, a 5 per cent solution of iodide was allowed to drip at the rate of 1 cc. per minute. In this manner 50 p.p.m. of iodide were introduced and the concentration of the resulting solution thus became 60 p.p.m.

Twenty parr were kept in each of these two troughs of running water containing different concentrations of iodide, and twenty parr were kept as controls in running water to which no iodide had been added. The experiment lasted from June 14th to August 21st, 1948, during which time the fish were subjected to regular examinations for changes in pigmentation.

Autopsy:

After the experiment had ended, 11 fish were removed at random from each of the treated groups as well as from the controls. The fish were sacrificed by placing them in a 1 per cent solution of urethane for at least three minutes. Their length was then measured. The thyroid region was removed and fixed for histological study. The thyroid gland of the salmon is composed of diffuse and randomly dispersed islets of thyroid tissue which are found chiefly along the dorsal surface of the ventral aorta.

Histological Techniques:

These regions were fixed in Bouin's fluid and embedded in paraffin. Sections were cut at 6 μ and stained with hematoxylin - eosin.

RESULTS:

The effect of iodide on the pigmentation of the skin was examined (Table 10). The parr kept in water containing 10 p.p.m. of iodide were slightly paler than the controls and those kept in water containing 60 p.p.m. were definitely paler. The animals of either groups were, however, not as light as the fish which, for a comparable period had been treated with thyroid extracts. Thus, iodine treatment had produced a decrease in pigmentation.

There was no detectable effect of iodide treatment on the connective tissue. Thus, the fish did not show more endophthalmy than the controls (see "Condition of the eyes", Table 10).

TABLE 10

EFFECT OF IODIDE ADDED TO THE WATER ON THE PIGMENTATION AND THE CONDITION OF THE EYES OF SALMON PARR

Treatments	Weight (gm.) Initial	Final	Weekly Increase	Average Intensity of Pigmentation (1)	Condition of the eyes (2)
Controls	5.0	10.2	0.6	+++	N
10 p.p.m. iodide in water (3)	4.7	8.7	0.4	++±	N
60 p.p.m. iodide in water (3)	5.5	8.7	0.4	++	N

(1) Intensity estimated by gross examination (not by counting).

(2) N: Normal protrusion of the eyes.

(3) p.p.m.: Parts per million.

The effect of iodide treatment on the thyroid gland of the parr was also studied. The control fish showed a highly hyperactive thyroid gland, characteristic of iodine-deficiency (Fig. 33 and 34). In this hyperactive condition the cells and nuclei were greatly enlarged and there was only a small amount of colloid in the follicles. The effect of 10 p.p.m. iodide on the thyroid gland of the parr kept in the hatchery is shown in Figure 35. The addition of iodide restored the hyperactive gland to a type more comparable to that of the normal gland of the parr living in rivers.

CONCLUSIONS:

When iodide is added, in relatively large amounts, to water in which previously iodine-deficient parr were living, it was possible to observe a decrease

PLATE XV

EXPLANATION OF FIGURES

Fig. 33 and Fig. 34 - Sections of the thyroid gland of two control parr kept under normal conditions in the hatchery. These figures show individual variations of hyperactivity. Fig. 34 shows a more hyperactive gland than that in Fig. 33.

Fig. 35 - This shows the thyroid gland of a parr kept in water containing 10 p.p.m. of added iodide. The epithelium shows a resting appearance and a relatively larger amount of colloid in the follicles than the glands seen in Fig. 33 and Fig. 34.

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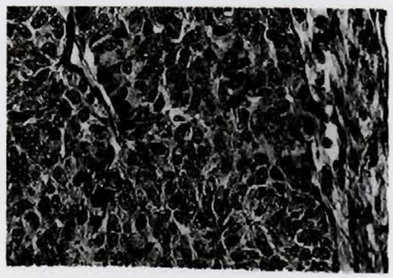

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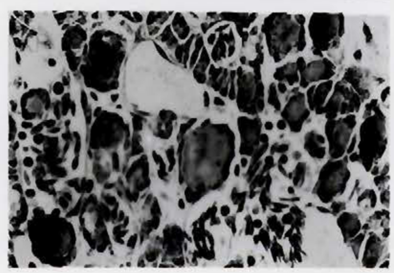

in pigmentation, which was similar to, although less intense, than that produced by thyroid extracts. The effect of iodide was greater with 60 parts of iodide per million of water than with only 10 parts per million.

CHAPTER V

DISCUSSION

Until recently the only recognized effect of the administration of thyroid preparations on salmonidae was a decrease in pigmentation. This change had been demonstrated by Langrebe (1941) and later confirmed by La Roche et al (1949 and 1950) and Robertson (1949).

The present work, however, indicated that thyroid treatment also produced a remarkable increase in the amount of connective tissue, especially of the head and fin regions.

The various thyroid products used were dried beef thyroid and commercial thyroid extract which were given as part of the diet and sodium thyroxinate which was injected intra-muscularly or given by mouth in the form of pills. All preparations, except the thyroxine - containing pills, induced a marked hyperplasia of the connective tissue and a moderate decrease in pigmentation. Since thyroxine, which according to recent work appears to be the thyroid hormone itself, was as effective as other thyroid preparations (at least when administered parenterally), it can be stated that the thyroid hormone is responsible for such changes.

CONNECTIVE TISSUE:

Gross observations of the treated fish, especially the smolt, revealed a gelatinous swelling, particularly visible on the fins and on the edges of the opercula, and a wrinkling of the skin covering the head (Fig. 26 and 27). It was obvious by examination with a dissecting microscope that the changes in the cephalic regions were due to an increase in the thickness of the skin which looked like a translucent coating hiding the normal shape of the head. The changed appearance of the fish can be demonstrated by microscopic examination to be due to dermal hyperplasia.

In all areas the special features of the local connective tissue remained unchanged despite the increase in the total amount. In other words, the respective proportions of cells, fibers and interstitial material, characteristic of any given area, was not modified. Also, there was not any apparent change in staining reactions.

The enlargement of the connective tissue seemed to extend over the whole body, a fact well demonstrated in the young trout. The intensity of the response varied, however, in various locations as if the ability to react was influenced by local factors. The maximum effect was thus found in areas where the skin was folded over, such as at the edges of the mouth or opercula (Fig. 27). The minimum response was observed in the connective tissue located between muscle masses. Obviously, the possibilities of expansion are greatest in the first and minimal in the second case. Similarly, the skin that did not contain scales was soft and plastic, while the skin of the body which contained scales was much more rigid and less liable to be influenced. Therefore, the thickening of the derma was first seen to appear in the head region before it extended to the body skin.

In this manner the thickening of the connective tissue progressed from one region to another in a definite sequence during a given period of treatment. The thickening first occurred in the derma of the dorsal portion of the head, extended to the lateral and ventral portions of the head and to the fins, then to the derma of the body and finally to the internal connective tissue in the interorbital region.

The relative differences in the connective tissue explains the different cephalic deformations appearing prior to the overall enlargement of the head of treated fish. The protrusion of the operculum (Fig. 10 and 27) was due to the fact that the posterior or distal edge of the preopercular bone was pushed away from the subjacent opercular bone by the thickening of the intervening connective tissue. In a similar manner the first protrusions to appear on the top of the head (Fig. 3 and 7) may be due to a slight increase of the periosteal connective tissue causing protrusion of the two median bones of the skull (dermethmoid and supraoccipital). Whenever the various types of connective tissue had all increased, then the protrusions of the head would gradually become less apparent and even disappear.

The thyroid treatment caused a white opaque swelling of the fins which progressed from the basal portion of the appendages and later infiltrated between their rays. This was particularly apparent at the ventral and dorsal insertions of the caudal fin (Fig. 19 and 20). Histological examination revealed that these changes in the fins were also due to an increase in the amount of connective tissue present.

There was also a difference in the rate of connective tissue enlargement with age which could be readily compared in the IIIrd series of experiment with thyroid extracts in which animals of various ages (fry, parr and smolt) were treated during the same period of time. It was thus determined that six weeks after the beginning of treatment hyperplasia appeared only in the derma of the head of the fry, in the derma of the head and fins of the parr, and in the derma of the head, the fins, and body skin of the smolt. The thyroid hormone was thus more effective in the larger fish.

In order to compare the effects of the thyroid treatment on different species a group of young <u>trout</u> parr were treated during a six weeks' period. In this case, not only did the young trout parr show an increase of the derma throughou the body, but even the intra-cephalic connective tissue increased, so as to produce a further enlargement of the head. This species showed the greatest effect observed

Was this due to the fact that young trout were obviously more voracious than young salmon and thus ingested more thyroid preparation? This hypothesis seemed likely, but measurements of food intake to prove this point were not carried out.

Krokert (1936) obtained an increase in the size of fins and gonopods in male Lebistes reticulatus treated with thyroid preparations. Hopper (1950) partly confirmed these results by showing that thyroid preparations caused an increase in the gonopod differentiation in the same species. These findings could possibly be explained by an increase in the dermal connective tissue of these appendages under the influence of thyroid treatment.

EPIDERMIS:

An increase of the epidermis was produced in the smolt and young trout (Table 6). The increase can be explained by an increased mitotic rate in the smolt Further observations indicated that, only when the derma of the body skin showed hyperplasia, the epidermis was thicker, as if the dermal enlargement resulted in growth of the epidermis. Possibly, outward pressure was produced on the epidermis by the enlarging derma and, as a consequence, the mitotic rate of the epidermis increased.

PIGMENTATION:

It should be pointed out that a decrease of pigmentation observed by macroscopic examination can be attributed to four distinct mechanisms, several of which may act simultaneously. Depigmentation can be due to, namely: 1) a decrease in the size of the pigment cells (i.e. to their contraction); 2) to a decrease in the number of pigment cells (La Roche, 1950); 3) to a masking of the deeper layer of pigment cells by a silvery material (Robertson, 1949); 4) or to a masking by an increase in the connective tissue (La Roche, 1949).

Six weeks after treatment was started, treated fry, parr and smolt were examined to see whether there was any difference in the overall depigmentation of the fish. Depigmentation was obviously greatest in smolt. The treated smolt were much lighter than the controls. This was particularly visible in the dorsal region. However, the number of pigment cells in this region did not differ in treated and control smolt (Table 7). With the same number of pigment cells after thyroid treatment than under normal conditions, some explanation had to be found for the light coat of the fish.

The first possibility was a decrease in the size of cells or "contraction". Attempts at assessing the size of the cells met with unexpected difficulties since a definite expansion was produced by the anesthesia necessary for the examination of live animals under a dissecting microscope. Similarly, histological fixation seemed to modify the state of contraction of the cells. Therefore, it is not known whether or not there is a change in the size of the pigment cells.

The second possibility was a masking of the pigment cells by the guanine which may accumulate on the scales, as shown by Robertson (1949). Since this phenomenon prédominates in the ventral region, it is doubtful that if accounts for the lightening of the back.

The last and perhaps most attractive possibility was that the thickened connective tissue may hide to some extent the pigment cells of the subdermal layer - a fact readily demonstrated by removing the connective tissue with a scalpel.

While the decrease in the number of pigment cells did not play a role in the lightening of the dorsal region of the treated smolt, there was a significant decrease in the number of cells in the ventral region. A decrease in the number of pigment cells per unit area under the influence of treatment occurs most clearly in parr in which it was significant in both the ventral and dorsal regions, Table 7. In the fry the difference between the pigment cell count of treated and controls was found to be significant in the ventral region only.

Thus, a masking of pigment cells by connective tissue increase must be the

most important factor causing depigmentation, at least in the smolt. The young trout required only a month and a half for this depigmentation to occur.

The only result which did not fit in with the rest of the observations was an experiment in which pills containing different doses of thyroxine were administered by mouth to young salmon parr. The modifications observed in the other thyroid treatments were not elicited. This may have been due to the fact that the amounts of thyroxine absorbed by the fish under these conditions were not sufficient to induce the transformations obtained with subcutaneous injections. On the other hand, the liver of the fish may have detoxified most of the ingested thyroxine and so only slight amounts of the hormone entered the systemic circulation. It would be interesting to repeat this experiment, using larger doses of thyroxine in pill form and also labelled thyroxine. In this way the fat of the hormone in the gastro-intestinal tract, as well as in the tissues of the fish, could be followed.

ROLE OF THE THYROID GLAND:

It was demonstrated that thyroid extracts, as well as the thyroid hormone itself (thyroxine intramuscularly), produced remarkable changes in young salmon. At this point the question remained whether the fish's own thyroid gland could produce enought thyroid hormone to cause similar effects to those observed in the thyroid treatment. In order to solve the problem, iodine-deficient fish, that is to say fish producing small quantities of thyroxine due to an environmental lack of iodine, were used. Iodine-deficiency can easily be recognized by the fact that the gland becomes enormous. Histological examination of the thyroid gland of these fish revealed a marked hyperplasia of the secretory epithelium, characteristic of this condition (Fig. 33 and 34). Such fish were kept in water containing different concentrations of iodide as already described. Under these conditions the thyroid gland is able to utilize the supplementary iodide to synthetize its own hormone. Thus, it is easy to understand that under these conditions the output of thyroxine by the thyroid gland is considerably increased.

In this experiment the iodide treatment produced a similar, but less pronounced, depigmentation than that due to thyroid preparations. This reduction in the overall pigmentation of the fish was also found to be proportional to the amount of added environmental iodide. Robertson (1949) failed to detect any effect of iodide on fish. This apparently negative result can be explained by the fact that the iodide was given to the fish by intra-muscular injections and not through a continuous stream in the water, and, in this manner the amount of iodide given must not have been sufficient to produce the pigmentary changes observed in the experiment described above.

From these results it appeare that the additional iodide stimulated the production of larger amounts of thyroid hormone which in turn caused the changes in pigmentation. Thus, the thyroid gland must have produced the hormone responsible for the depigmentation.

THE PROBLEM OF METAMORPHOSIS:

The adaptation to sea water in the salmon is accompanied by typical skin modifications briefly described in the introduction and somewhat comparable to those obtained with treatment of the salmon parr by thyroid preparations, namely: decrease in pigmentation and increase in the thickness of the skin, especially of the connective tissue of the derma. In nature the young salmon parr seem to start their smoltation as they approach the estuaries and come in contact with the brackish water. This water is also known to contain relatively greater amounts of iodine than the water of the river. The thyroid gland of the parr is often iodine-deficient as these fish reach the estuaries (Hoar, 1939). It is possible that the iodine available for fixation by the thyroid gland is sufficient to initiate and complete the pigmentary transformations and the thickening of the skin which are characteristic of smoltation. The thyroid treatment would have thus induced an artificial smoltation.

In conclusion it is believed that the thyroid hormone may play a role in smoltation, but further evidence on the thyroid function are awaited before final conclusions can be drawn. Among other results the effect of thyroidectomy (with the use of I^{131}) will be of primary importance in the comprehension of the role of the thyroid gland in the phenomenon of smoltation.

GENERAL CONSIDERATIONS:

Etkin (1937) could not duplicate the increase in the growth rate of guppies kept on thyroid treatment as reported by Krokert (1936). Etkin (1940) then concluded, from his work and a survey of the literature on the subject, that the thyroid gland had little, if any, effect on fish. Hopper (1950) confirmed Krokert's work by showing that thyroid extracts caused a marked increase in the growth rate of guppies and that a thyroid inhibitor (thiouracil) had an opposite effect in this fish.

In the present experiments, thyroid preparations had a very definite effect on the development of the connective tissue, the epidermis and on the pigmentation of the young salmon and the young trout. All three thyroid preparations administered to the young salmon produced an hyperplasia of the connective tissue and a marked decrease in the overall intensity of pigmentation. Since the thyroid hormone itself was one of the products administered, it can be stated that these changes are due to the action of the thyroid hormone.

In a comparison with the results obtained on mammalia, it is interesting to note that the effects of thyroid preparations on the skin of fish were the reverse of those obtained on the skin of albino rats (Eartly, 1950). A latent period (4 to 6 weeks in parr) between the beginning of treatment and the appearance of morphological changes in the fish, similar to the one observed in albino rats by Grad (1949), was found to exist here.

The effects obtained in the young salmon receiving thyroid preparations are made more significant by the results on the influence of added iodide in the environment of iodine-deficient fish. This experiment proved that the thyroid gland of these fish can utilize the iodide thus available to synthetize thyroxine and then to accelerate the depigmentation which is characteristic of the onset of smoltation.

CHAPTER VI

CONCLUSIONS

1) Thyroid preparations - commercial thyroid extract or dried beef thyroid - fed to Salmo salar of various ages (Salmon fry, parr and smolt, and young trout) for period of six weeks elicited the following effects, namely:

a. Changes in head shape and size

b. Changes in fin shape and size

c. Hyperplasia of the connective tissue

d. Increase in the thickness of the epidermis

e. Decrease in degree of pigmentation

Changes in head shape and size were due to bulging of the opercula and protrusion of the dermethmoid and supraoccipital bones - the only two superior median bones in the fish. There was also an apparent endophthalmy due to overgrowth of the eyelid, which, as a result, overlapped the superior portion of the eye.

Changes in fin shape and size: The caudal fin which initially had the outline of a V changed to a U as a result of the apparent abduction of the fin rays, which, in turn, was due to an increase in inter-fin-ray material. Finally, the whol thickness of the fins increased and thus made these structures appear more opaque.

Hyperplasia of the connective tissue: On histological examination the changes in head and fin shape and size were accounted for by a marked increase in all elements of the connective tissue. In other words, intercellular material and fibers, as well as number of cells, had increased simultaneously and proportionally. This hyperplasia did not take place uniformly but rather showed a progression from non- to scale-bearing skin in the following order: dorsal, then ventral and lateral head regions, fins, derma of the body and later intra-cephalic connective tissue (particularly the interorbital region). No change in the inter-muscular connective tissue could be detected; a fact interpretable either as insufficient treatment or unreactivity. No changes in staining reactions between control and treated fish could be detected by von Kossa, Hotchkiss or metachromatic techniques, thus ruling out changes in calcium and poly-saccharide content.

The areas of connective tissue responsive to treatment seem to be determined by degree of development of the fish. The more highly developed - i.e. the larger the fish - the more extensive the hyperplasia of the connective tissue. Thus, in time, smolt showed all the dermal connective tissue to be affected by the treatment, whereas in fry only the derma of the head regions responded to a similar period of treatment. Parr showed an intermediary response. There was also a species difference as demonstrated by the extensive reponsiveness of the connective tissue of Salvelinus fontinalis of the year. In fact, for an identical period of treatment, this species showed the most intense, as well as the most extensive, response.

Increase in the thickness of the epidermis was shown to be due to an increased mitosis of all but the basal layers, as determined by counting the number of metaphases per 0.1 mm. square area of head epidermis. A significant increase over that of the controls was found only in smolt.

Decrease in degree of pigmentation: Depigmentation consisted of an overall decrease in pigmentation. This was attributable to decrease in size and possibly number of pigment cells per 0.2 mm. square of skin and to a masking of the hypodermal pigment cells by either a deposition of silvery material (claimed to be guanine) along the inner surface of the scales or by a thickening of the overlying dermal connective tissue. Treated parr showed the greatest decrease in the number of pigment cells, while fry and smolt showed a decrease in pigment cell number in the ventral region only. However, the smolt had a greater overall depigmentation than the other treated stages.

Thus it may be concluded that thyroid preparations fed to young salmon caused a hyperplasia of the connective tissue, an increase mitotic rate of the epidermis and a decrease in the number of pigment cells.

2) Since the active principle of thyroid preparations was thought to be thyroxine, similar fish were treated with 200 µg of d-l sodium thyroxinate, twice weekly for 6 weeks, by intra-muscular injection. Identical results were obtained, thus proving that thyroxine was the effective agent.

When thyroxine was administered directly into the stomach of salmon bi-weekly in doses up to 333 µg per administration, no changes resulted after six weeks of treatment. This finding was interpreted as an unsatisfactory route of administration which probably required far more initial thyroxine to effect comparable change.

3) The next step was to see whether these fish could be induced to produce sufficient endogenous thyroid hormone if the essential raw material - iodide - was provided in their experimental environment. Thus, for 8 weeks an environment of 10 and 60 parts per million of added iodide was provided. At the end of this time no connective tissue hyperplasia was detectable, but depigmentation had occurred and was proportional to the amount of environmental iodide available.

4) The changes described are similar to those occuring during natural smoltation. It may be said that administration of thyroid preparations, thyroxine or even iodide, apparently can simulate the changes occuring during this trans-

formation. Therefore it is concluded that the thyroid hormone may play a role in smoltation.

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