THE RAPIDITY OF THE INVOLUTION OF ACTIVE THYROID HYPERPLASIAS OF BROOK TROUT FOLLOWING THE USE OF FRESH SEA FISH AS A FOOD.*

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In a recent paper (1) the effect of certain foods on the production and prevention of goitre in brook trout (*Salvelinus fontinalis*) was described and it was shown that a continuous diet consisting only of hog's liver and heart[1] quickly (in one to two months) induces well marked thyroid hypertrophy and hyperplasia, and that when hashed fresh sea fish alone was substituted, further hyperplasia was prevented, and any existing thyroid hyperplasia involuted to its colloid or quiescent state.

In the present communication will be given data concerning the rapidity of the involution following the substitution of the fish diet, and the time limits within which it is completed, and also, for comparison, the time limits of the involution induced by other means in Lake Erie pike, in brook trout, and in mammals (dogs).

The fish used in these observations were taken from the regular stock hatched about January 15, 1913, and distributed to the ponds about July 1. They had, therefore, in common with the entire 1913 hatch, been fed with finely divided hog's liver for the first few months (four to five), and after that with a mixture of heart muscle and liver. I arbitrarily chose fish from pond 8,[2] since complete examinations made in August of all the 1913 hatch, both those living in the troughs and those in the ponds, showed approximately the same degree of thyroid hyperplasia.

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[1] The food is but one of a complex of interacting factors, among which are overcrowding, and relatively insufficient water supply, with its diminished oxygen and excess of excreta contents. The food, however, is believed to be the major single factor.

[2] For the plan of the hatchery see Marine, D., *Jour. Exper. Med.*, 1914, xix, 71.

The experiments were conducted as follows: On September 28, 1913, the food of all the fry of 1913 was changed to fresh butter fish (*Stromateus triacanthus*) given once daily except for the weekly fast day. Eight fish were taken from pond 8 on this date as controls, and four fish were removed every seventh day for sixty-three days following. The data are presented in the following tabulation.

The eight fish taken as controls at the beginning of the experiment have uniformly hyperplastic thyroids listed as "marked hyperplasias," comparable and similar in all essentials to those present in the fry of corresponding ages in 1909, 1910, 1911, and 1912. The strain of fish and the food (hog's liver and heart) have also remained constant during these years. The distribution of the thyroid tissue is approximately the same in all the forty-four fish used. In all cases the thyroid follicles have filled the thyroid area about the aorta and between the 1st and 3d gill arches, have extended up to the pharyngeal mucosa, have invaded the bone and muscles, and in most cases have extended through to the skin adjacent to the ventral attachments of the 1st and 2d gill arches.

Specimens taken seven days after the feeding of fish was instituted show slight but distinct changes in the thyroid toward involution. The follicular spaces are more distinct and contain some stainable colloid. The epithelium is slightly changed, and the stroma and vascularity appear unmodified, and, according to the arbitrary standards I have used, such a histological appearance would be designated "colloid-moderate-marked hyperplasia."

The fourteen day specimens are listed as "colloid-moderate hyperplasias," *i. e.,* the stainable colloid is more abundant, the follicular spaces better defined, and the epithelium has shrunken from high columnar to low columnar.

The twenty-one day specimens show still further accumulations of colloid in the follicles, and the lining epithelium has shrunken to the cuboidal form. These specimens are listed as "colloid-early hyperplasias."

Specimens removed twenty-eight days after the fish feeding was instituted show still further shrinking of the epithelium, and the colloid content is now nearly uniform and homogeneous. Vacuoles

TABLE I.

Pond No.	Age in months.	Date taken.	Food and length of time fed.	Condition of thyroid.		Remarks.
				Extent of overgrowth.	Classification.	
8	8+	Sept. 28, 1913	Hog's liver for 1st 4 mos. of life; then hog's liver and heart	Entire subpharyngeal area infiltrated, including bone and muscle	Marked hyperplasia	This is an example of 8 controls. All have approximately the same degree of hyperplasia.[3] Colloid absent.
8	8+	Oct. 5, 1913	Whole butter fish only, beginning Sept. 28, 1913, 1 wk.	Entire subpharyngeal area infiltrated, including bone and muscle	Colloid-moderate-marked hyperplasia (beginning involution)	This is an example of 4 specimens examined. All have approximately the same thyroid condition. Colloid appears in follicles.
8	8+	Oct. 12, 1913	Butter fish only for 2 wks.	Thyroid area filled, extending to pharyngeal mucosa, in bone and muscle	Colloid-moderate hyperplasia (involuting)	This is an example of 4 specimens examined. All have approximately the same thyroid condition. Colloid in fair amounts in all follicles. Epithelium low columnar.
8	9	Oct. 19, 1913	Butter fish only for 3 wks.	Entire subpharyngeal area filled, extending to pharyngeal mucosa and skin and gills, in bone and muscle	Colloid-early (involuting)	This is an example of 4 specimens examined. All have approximately the same thyroid condition. Colloid in all follicles fairly homogeneous. Epithelium cuboidal.
8	9+	Oct. 26, 1913	Butter fish only for 4 wks.	Entire subpharyngeal area filled, extending to pharyngeal mucosa, bone, and muscle	Colloid-early (approximating pure colloid)	This is an example of 4 specimens examined. All have approximately the same thyroid condition. Colloid dense, nearly uniform. Follicles more separate and stroma more prominent, due to shrinkage of blood supply and size of follicles. Epithelium cuboidal.

[3] Complete examinations of all the fry at the hatchery were made in August, and all were found to have thyroid hyperplasia (Marine D., *Jour. Exper. Med.*, 1914, xix, 70).

Pond No.	Age in months.	Date taken.	Food and length of time fed.	Condition of thyroid.		Remarks.
				Extent of overgrowth.	Classification.	
8	9+	Nov. 2, 1913	Butter fish only for 5 wks.	Entire subpharyngeal area filled, extending to pharyngeal mucosa, to bone, muscle, and gills	Colloid goitre (complete involution)	This is an example of 4 specimens examined. All have approximately the same thyroid condition. Colloid dense, uniform. Follicles separate. Epithelium low cuboidal.
8	9+	Nov. 9, 1913	Butter fish only for 6 wks.	Entire subpharyngeal area filled, extending to pharynx mucosa, bone, and muscle	Colloid goitre (complete involution)	This is an example of 4 specimens examined, all showing same thyroid condition.
8	10	Nov. 16, 1913	Butter fish only for 7 wks.	Entire subpharyngeal area filled, extending to skin, pharyngeal mucosa, bone, and muscle	Colloid goitre (complete involution)	This is an example of 4 specimens examined, all showing same thyroid condition.
8	10+	Nov. 23, 1913	Butter fish only for 8 wks.	Entire subpharyngeal area filled, in bone and muscle	Colloid goitre (complete involution)	This is an example of 4 specimens examined, all showing same thyroid condition.
8	10+	Nov. 30, 1913	Butter fish only for 9 wks.	Entire thyroid area filled, extending to pharyngeal mucosa, to bone, and muscle	Colloid goitre (complete involution)	This is an example of 4 specimens, all showing same thyroid condition.

are still present at the colloid-epithelium junction. By the use of less rigid standards than I have adopted, these thyroids could be classified as completely involuted rather than as "colloid-early hyperplasias." By comparing the follicles with those of normal fish or with those seen in the subsequent specimens of this series, however, it is evident that the involution is not complete. The specimens taken after thirty-five days of fish feeding are listed as "complete involutions"; that is, the lining epithelium is low cuboidal. The stainable colloid is homogenous, and nearly uniform in all follicles. The follicles are smaller and more widely separated from each other, while the stroma is correspondingly more prominent. The capillaries about the follicles are less prominent. The distribution of the follicles is, of course, the same as that attained during the growing actively hyperplastic stage, *i. e.,* in bone and muscle with extension to the skin and pharyngeal mucosa.

The specimens examined on the 42d, 49th, 56th, and 63d days respectively are nearly identical with those of the 35th day. There is probably some further involution in the last specimens, as evidenced by a slight increase in the staining density of the colloid and in the flattening of the lining epithelium. This is also true of normal thyroid follicles, and has occasioned much discussion as to what is the normal type. In a tissue like the thyroid epithelium, which is capable of such marked hypertrophy and hyperplasia, as well as marked involution, one must adopt somewhat arbitrary groups, within which variations between certain narrow limits are not taken into account.

The time in which complete involution of these relatively mild degrees of thyroid hyperplasia occurs, following the use of butter fish in this particular environment and strain of brook trout, is about thirty-five days. As was shown in a previous paper (1), the thyroid gland, once involuted, remains in this colloid or involuted stage as long as the fish are fed with this food. No further growth of the thyroid takes place under these conditions. Thus the thyroids of the three and two year old brook trout at this hatchery have the same amount of thyroid tissue with the same distribution of follicles that was attained during the first ten months of their lives, at which time the change of food was instituted.

Iodin involutes the hyperplasias more rapidly than butter fish. In Lake Erie pike with mild degrees of thyroid hyperplasia it was found (2) that iodin involuted them in from sixteen to eighteen days.

In the experiments of 1909 (3) it was found that the thyroid of twenty months old brook trout with extensive hyperplasia involuted in from twenty to twenty-five days following the daily addition of traces of iodin to the water. In the iodin experiments of 1910 (4) it was found that involution took place in about forty days in twenty-nine months old brook trout with large and often slightly ulcerated goitres, while in forty-one months old emaciated brook trout with extensively infected and ulcerated external goitres healing and involution were complete at the sixty-second day. In mammals the involution with iodin is of course more rapid (5). In young dogs with uncomplicated simple hyperplasias, the involution is usually complete in from fourteen to eighteen days.

As the whole butter fish contains appreciable amounts of iodin[4] one may ask whether this effect of fish as food is merely the well known iodin action, and the somewhat longer involution time only the manifestation of a very dilute solution. In the absence of any data against this view, I prefer tentatively to consider it an iodin effect rather than to postulate another explanation in favor of which there are at present no direct data. Experiments are now in progress which it is hoped will shed some light on this question.

[4] Iodin in fish, birds, and mammals is, for the most part, contained in the thyroid glands, although traces of iodin have been found in certain fish oils, as, for example, cod liver oil, where presumably contamination with thyroid was avoided. Using the modified Baumann and the Hunter methods, I have not been able to recognize traces of iodin in whole amphioxus and in butter fish (*Stromateus triacanthus*) and weak fish (*Cynoscion regalis*) from which the thyroid areas had been removed. A. T. Cameron (*Biochem. Jour.*, 1913, vii, 466; *Jour. Biol. Chem.*, 1914, xvi, 465) has published the finding of relatively large amounts of iodin in the thyroids of rays and dog fish. Thus in *Raia clavata* he found the mean iodin content to be 0.438 per cent. of dried gland, while in *Scyllium canicula* he found a mean content of 1.16 per cent. of dried gland. I have made the following iodin estimations in butter fish:

(1) With whole butter fish in 1 gm. amounts = possible trace.
(2) With whole butter fish in 5 gm. amounts = trace, unmeasurable.
(3) With butter fish, exclusive of the thyroid area, in 1 gm amounts = no trace.
(4) With butter fish, exclusive of the thyroid area, in 5 gm. amounts = no trace.

BIBLIOGRAPHY.

1. Marine, D., Further Observations and Experiments on Goitre (So Called Thyroid Carcinoma) in Brook Trout (*Salvelinus fontinalis*). III. Its Prevention and Cure, *Jour. Exper. Med.*, 1914, xix, 70.
2. Marine, D., and Lenhart, C. H., On the Occurrence of Goitre (Active Thyroid Hyperplasia) in Fish, *Bull. Johns Hopkins Hosp.*, 1910, xxi, 95.
3. Marine and Lenhart, Observations and Experiments on the So Called Thyroid Carcinoma of Brook Trout (*Salvelinus fontinalis*), and Its Relation to Ordinary Goitre, *Jour. Exper. Med.*, 1910, xii, 311.
4. Marine and Lenhart, Further Observations and Experiments on the So Called Thyroid Carcinoma of the Brook Trout (*Salvelinus fontinalis*), and Its Relation to Endemic Goitre, *Jour. Exper. Med.*, 1911, xiii, 455.
5. Marine and Lenhart, Effects of the Administration or the Withholding of Iodin-Containing Compounds in Normal Colloid or Actively Hyperplastic Thyroids of Dogs, *Arch. Int. Med.*, 1909, iv, 253.