ON THE RELATION OF THE THYROID GLAND TO METAMORPHOSIS IN THE LAMPREY[1]

By FRANCES M. HORTON, Ph.D.

(Zoology Department, University of Birmingham.)

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I. INTRODUCTION.

The significance of endocrine glands, particularly the thyroid, in relation to the metamorphosis of the Amphibia is now well known, and Murr and Sklower (1928) have deduced from histological observations that the thyroid is also involved in the metamorphosis of the eel. The lamprey suggests itself for study in this connection, being one of the few vertebrates outside the Amphibia to undergo a complete reorganisation during development. The condition of the thyroid gland in this animal gives special point to the investigation. In the larva or ammocoete the organ is an "endostylar" structure forming part of the feeding mechanism. It becomes constricted off from the pharynx at metamorphosis to form a small follicular body containing colloid (Marine, 1913), which, though it tends to degenerate later in life, is comparable with the thyroid gland of gnathostomes. Müller (1856) homologised the latter, by reference to the dual condition found in the lamprey, with the endostyle of amphioxus and the tunicates, and considered that *Petromyzon* displays the thyroid gland in its most primitive condition.

Jensen (1916) attempted to accelerate the metamorphosis of two ammocoetes by intra-abdominal injections of iodothyrine. Rémy (1922) immersed three ammocoetes in a solution of iodothyrine for 26 days and injected the same preparation into several others. An aqueous emulsion of 2 mg. of iodoform (a dose sufficient to provoke metamorphosis of the axolotl) was injected into the coelom of another larva. Marine (1913) kept ten ammocoetes in water containing Lugol's solution to discover whether the iodine present had any modifying effect on the endostyle, but histological examination revealed no deviation from the normal condition. No metamorphic changes were induced in any of the above experiments.

For the experiments reported below ammocoetes were collected from the River Teme, near Worcester, and kept in river water. Some of the animals metamorphosed in captivity and were identified as *Petromyzon fluviatilis*. No specific differences are known between the young of the three British species of lamprey, but since all larvae were collected from the same locality it is probable that all were the young of this same species.

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II. IMMERSION EXPERIMENTS.

Iodine, potassium iodide and iodoform have been shown to be as potent as thyroxine in stimulating amphibian metamorphosis (Swingle, 1918; Huxley and Hogben, 1922). I used these three substances and a thyroid extract in a series of experiments. The thyroid extract was a Philip Harris liquid preparation known as "Elixir," made from sheep's gland, containing 0·09 gm. of fresh gland per c.c. Ammocoetes were immersed from time to time in solutions of the above substances, being rinsed in tap water before and after the treatment, and they were then observed for signs of metamorphosis during several months. A control group was transferred to tap water during the treatment periods of the other groups. Preliminary experiments were made to find a suitable concentration of each substance: solutions with which treatment was begun were, in every case, of half the maximum concentration in which ammocoetes could survive more than 12 hours' immersion.

Iodine. A solution was prepared by grinding crystals in distilled water. Twelve animals were transferred for 1 hour every other day during 36 days to a solution made by adding 1 part iodine solution to 49 parts of river water. No sign of metamorphosis appeared at the end of that period, so the same dose was given daily for 20 more days. The animals were then divided into two equal groups, (1) being treated as before, and (2) given an iodine solution of twice the former strength. Members of (2) refused to burrow after a week and were dead within a fortnight. (1) was now given doses intermediate in strength between the two previously used. No metamorphosis had occurred after 91 days. Repetition with fresh animals gave the same result.

Potassium iodide. Twelve animals were immersed for 1 hour every other day for 36 days in centinormal potassium iodide in river water, and for 1 hour daily for the subsequent 20 days. They were then divided into equal groups (1) and (2). (1) was given $N/50$ KI for an hour daily: the animals of this group were normal and unchanged after another 3 weeks. (2) was given $N/20$ KI: the animals remained vigorous for a fortnight, but all died in the third week, 140 days after the experiment was begun. The experiment was repeated with six more animals.

Iodoform. As iodoform is insoluble in water, colloidal solutions were made. Twelve animals were transferred for 1 hour every other day to a solution prepared by adding 1 part of a saturated solution of iodoform in absolute alcohol to 1000 parts of river water. After 3 weeks the concentration of the solution was doubled. In this the animals were sluggish and they refused to burrow when returned to mud. Six weeks after the beginning of the experiment greater concentrations were given until a lethal dose was reached without any signs of metamorphosis appearing. The experiment was then repeated with six fresh animals.

Thyroid extract. The extract previously described was diluted with river water and twelve animals were at first transferred for 1 hour each day to a solution of 1 part of extract to 99 parts of river water. After a month the concentration of the solution was doubled and then gradually increased over a period of 3 months until a

lethal dose was reached, without any metamorphosis occurring. As in the previous experiments, the work was repeated with six fresh ammocoetes.

III. INJECTION EXPERIMENTS.

Injections of thyroid extract were made into the dorsal tail muscles of fifteen ammocoetes, as it was found that intra-abdominal injections caused a high mortality. Doses stronger than 0·1 c.c. of 33 per cent. thyroid extract solution (see immersion experiments) proved lethal: animals given the strongest sub-lethal dose of 0·1 c.c. of 25 per cent. solution were alive and healthy after 4 months. None of the animals treated showed any signs of metamorphosis 4 months after their first injection, though the total equivalent amount of fresh gland injected was in one experiment 2·7 mg. and in another 4·0 mg. per animal.

IV. GRAFTING EXPERIMENTS.

It is well known that the grafting of thyroid into the body cavity of frog tadpoles will induce precocious metamorphosis (Hirschler, 1922; Swingle, 1922). A simple method of investigating the relative potencies of the thyroids from various animals is therefore provided by comparing their accelerating effects upon amphibian metamorphosis. This method has already been used by Sembrat (1928) in a study of selachian and teleostian glands.

Table I. *Numbers of tadpoles beginning metamorphosis on stated days after having received grafts.*

Days after grafting	Nature of graft			
	Muscle	Ammocoete gland	Ox thyroid	Lamprey thyroid
5	—	—	1	—
6	—	—	—	1
7	—	—	3	—
8	—	—	2	2
9	—	—	4	1
10	—	—	—	—
11	1	1	1	—
12	1	2	—	1
13	3	2	—	—
14	—	3	—	—
15	1	1	—	—
16	1	2	—	—

Tadpoles selected for experiment were of approximately the same size and condition. None showed any signs of fore-limb buds. Four groups of tadpoles received implants, one of ox, one of lamprey and one of ammocoete thyroid, while the fourth received frog muscle as a control. The implant was lodged between adjacent loops of the gut and the wound protected by a film of collodion (Hirschler, 1922). In the experiments with ox and lamprey thyroids, pieces of tissue of about 2 c.mm. were

used; while with the ammocoete gland the cup-like structure which comprises nearly the whole of the endostyle was transplanted into each tadpole. The lamprey glands were kept in a refrigerator until used: the freezing of thyroid tissue does not affect its action (Sembrat, 1928).

Table I shows the time relations of the onset of metamorphosis in the surviving operated animals. Tadpoles implanted with ammocoete gland behaved like the controls which had received muscle[1], whereas lamprey thyroid and ox thyroid accelerated metamorphosis to about the same extent.

V. ANALYSIS OF THE GLAND.

The colloid substance characteristic of thyroid tissue does not appear in the lamprey until after metamorphosis, but this does not exclude the possibility that iodine is present in the ammocoete endostyle. Sixty ammocoete glands were therefore analysed for iodine by Kendall's method (1914) which is accurate for amounts as small as 0·005 mg. No iodine could be detected.

VI. THE EFFECT OF IODINE AND THYROID EXTRACT ON AMMOCOETE RESPIRATION.

Bělehrádek and Huxley (1927) showed that the oxygen consumption of the axolotl rises after an injection of thyroid extract sufficient to cause metamorphosis, and Huxley (1925) found that the respiration of tadpoles treated with iodine or thyroid solutions is increased about 100 per cent.

An investigation was next made of the effects of thyroxine and iodine on the metabolism of the ammocoete. Groups of four ammocoetes were treated with solutions of iodine or thyroid extract, replaced in water, and their respiration measured under urethane in a Barcroft apparatus before treatment and then each day subsequently for 16 days. Doses approaching the lethal value decreased the rate of respiration, but no lesser dose could be found which increased the consumption of oxygen.

VII. SUMMARY.

1. Ammocoetes were treated with iodine, potassium iodide, iodoform and thyroid extract solutions. No metamorphosis was induced.

2. Injections into ammocoetes of iodoform and of thyroid extract were made, without any metamorphic changes appearing.

3. Lamprey, ammocoete, and ox thyroids were grafted into tadpoles. Lamprey and ox thyroid were equally active in accelerating metamorphosis, but the ammocoete gland has no such effect.

4. The glands of sixty ammocoetes were analysed for iodine. They contained none.

[1] Spaul (1927) attempted to induce precocious metamorphosis of frog tadpoles by a diet of *Ciona* endostyle and obtained negative results.

5. The effect of iodine and thyroid on ammocoete respiration was tested. In no case was the oxygen consumption increased.

6. It is concluded that the thyroid gland has no significance in the metamorphosis of the lamprey.

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It has lately been brought to my notice that the oxygen consumption of frog's muscle is only increased by thyroxin at lower oxygen pressures than the partial pressure of this gas in air (v. Euler, *Klin. Wochenschr.* (1933), 671). It may be that thyroid would similarly augment the oxygen uptake of ammocoetes at low oxygen pressures.