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THYROIDECTOMY OF THE DOGFISH, *SCYLLIUM CANICULA* (L.), AND THE EFFECT OF DOGFISH THYROID UPON THE OXYGEN CONSUMPTION OF RATS

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(Text-fig. 1)

The function of the thyroid gland in fish is still obscure in spite of the numerous attempts to elucidate it by means of the administration of thyroid extracts, thyroxin, thyrotropin and antithyroid drugs, as well as by the carrying out of direct histological and cytological observations upon the gland at various seasons of the year and phases of the life cycle (Goldsmith, 1949; Hopper, 1952). The influence of this gland upon the metabolic rate of higher vertebrates is well established, but, despite the recent demonstration that the teleost thyroid contains a principle which raises the oxygen consumption of mammals (Smith & Brown, 1952), most attempts to influence the rate of oxygen consumption in fish themselves by the use of thyroid stimulants or depressants have been negative (Root & Etkin, 1937; Etkin, Root & Mofshin, 1940; Hasler & Meyer, 1942; Smith & Everett, 1943), the only positive result being that of Smith & Matthews (1947), who injected extracts of the Bermuda parrot fish into the Bermuda white grunt.

Studies of the growth and maturation of thyroid-treated fish have also been contradictory (Hoar, 1951; Hopper, 1952; Smith, Sladek and Kellner, 1953), and while chemical thyroidectomy appears to inhibit growth (Goldsmith, Nigrelli, Gordon, Charipper & Gordon, 1944; Nigrelli, Goldsmith & Charipper, 1946) and gametogenesis (Barrington & Matty, 1952), the possibility remains, as the latter authors point out, that these results may be due to some action of the drug which is not mediated by the thyroid gland.

Despite the fact that surgical removal of the glands has proved an invaluable procedure in endocrine research, thyroidectomy has not been carried out in teleosts, which have been mainly used in these studies, probably because the gland is usually diffuse and intimately related to the ventral aorta. Thus a lack of information regarding the influence of the absence of their own gland upon the oxygen consumption of fish remains a serious gap in our knowledge, and it has seemed essential to attempt to fill this by devising a procedure for surgical thyroidectomy in elasmobranchs in which group the distinct and encapsulate nature of the gland presents a more promising situation, as was realized by Vivien (1941). The present paper describes such a

procedure, and presents the results of observations upon the oxygen consumption of fish after thyroidectomy. For comparison with these results the influence of elasmobranch thyroid on the oxygen consumption of male white rats has also been studied.

Materials and Methods

Operative Technique

Dogfish of lengths ranging from 60 to 70 cm and of both sexes were used in all the operations, the animals being first anaesthetized by placing them in a 1·5% solution of urethane in sea water for 15 min. After washing in tap water they were placed ventral side uppermost in an operating cradle.The head was strapped down over the oro-nasal grooves on to the operating board and the lower jaw held back by retractors, thus exposing the floor of the mouth. A half-inch transverse incision was made through the mid-line in the fold of loose skin lying immediately behind the teeth of the lower jaw. After loosening the connective fascia between the coracomandibular and coracohyoids these muscles were held apart by small stainless steel retractors in order to expose the thyroid gland. This narrows anteriorly into a long 'thyroglossal cord' which terminates in the region of the symphysis of Meckel's cartilages. The anterior end of the 'thyroglossal cord' was first severed from its connective tissue attachment and the intact gland removed in an anterior-posterior direction, the region of the anterior attachment and also the region of the posterior attachment of the gland to the muscle lying dorsal to the coracohyoids being cauterized locally with an ophthalmic cautery in order to destroy any stray follicles that might have been left by the operation. The duration of the operation varied from 20 to 30 min; there was no appreciable haemorrhage, and the animals recovered consciousness almost immediately after being replaced in sea water. After some operations the sinus left by removal of the gland was packed with absorbent sterilized gelatin sponge. Nylon thread was used to stitch the skin incision, and the normal aseptic operating conditions were employed.

After a few days all the operated animals began to feed upon strips of fresh squid muscle. An autopsy was performed on every thyroidectomized fish after the completion of the respiration experiments; in no case was thyroid tissue identified macroscopically. The mortality rate was satisfactorily low, no deaths having occurred in the experiment, to be described later in detail, in which the oxygen consumption of operated animals was determined. Operated dogfish have, in fact, been kept alive for a period of at least 72 days after the complete removal of the gland.

A vertical approach through the throat was attempted in some preliminary experiments, but this method was abandoned when it was found to result in nearly a 100% mortality within a week or so.

Determination of Oxygen Consumption of Dogfish

As a result of the experience gained from some preliminary work carried out in the summer of 1952, using the Winkler technique for the determination of dissolved oxygen, it was decided to use the polarographic technique in the definitive experiments, in order to make possible the handling of a large number of fish. The respiration apparatus was of the continuous flow type similar in principle to that described by Hall (1929). Each dogfish was placed separately into a respiration chamber consisting of a bitumen-painted compressed asbestos pipe, 15 cm in diameter and 76 cm long. The ends of each chamber were made of Perspex plates which could be removed for inserting the fish, and through which it was possible to observe the movements of the animals in order to investigate the possibility of activity influencing the oxygen consumption during the metabolism experiments. No appreciable activity was in fact observed. A constant flow of water maintained at 400 ml./min was allowed to pass through the chamber containing the animal for 8 h. Four chambers were used simultaneously. The temperature of the tank of water surrounding the respiration chambers, which varied during the experiment from 16·2 to 17·6° C, was recorded daily.

As mentioned above, oxygen content of the water was measured by the polarographic method, the apparatus used being of the manually operated type with a dropping mercury electrode (Milton & Waters, 1949; Giguère & Lauzier, 1945). Samples of the inflow and outflow water, taken between 5 and 7 p.m. each day, were run directly into 20 ml. polarographic cells, and their oxygen contents measured at 16·5° C. (The sampling error of duplicate samples of water was always less than 1·0%.)

Determination of Oxygen Consumption of Rats

Twenty adult male albino rats were used in this study, their weights ranging from 185 to 260 g. Four rats were used as untreated controls, their oxygen consumption being measured every day for 6 days. Four more control rats were injected intra-peritoneally with 2·0 ml. of 0·9% sodium chloride in distilled water, whilst in a further group of eight each animal was given a single injection of 100 mg of mammalian thyroid powder. The remaining four were each given a single injection of 95 mg of dried dogfish thyroid. This dogfish thyroid was prepared by placing the fresh gland in acetone at 4° C and then washing several times in acetone to remove the fats. Finally the glands were washed in alcohol, dried in a desiccator and ground up into a fine powder.

The four rats which received saline injections were at a later date each given a single injection of a suspension of 100 mg dried trout muscle.

The oxygen consumption measurements were made using the method of Grad (1952), except that 9,000 ml. desiccators were found to be more convenient than the original stoppered jars as respiration chambers.

All determinations were made between 9.30 and 11.30 a.m. at a temperature of $28 \pm 0{\cdot}5^{\circ}$ C. During the experiment between the periods in which they were in the respiration chambers, the animals were kept supplied with abundant food and water.

Results

Oxygen Consumption of Thyroidectomized Dogfish

The oxygen consumption of thyroidectomized dogfish was measured individually on three male and three female animals from the 1st to the 42nd day after the operation, at 3-day intervals. Six animals, three male and three female, given sham operations consisting of the complete operative procedure except for the removal of the gland, were used as controls, their oxygen consumption being also measured at 3-day intervals. Both of these groups of fish were measured before the operations in order to determine the normal oxygen consumption of untreated fish. The results expressed as the mean oxygen consumption of the six fish in each of the two groups are given in Table I. They show that there is no significant decrease in the oxygen consumption of either group for a period of 42 days after the operation, nor is there any significant difference between the oxygen consumption of the two groups when these are compared every 3rd day.

Table I. The Effect of Thyroidectomy on Oxygen Consumption of *Scyllium canicula*

Six fish in each group (* Five fish only in group). In the first column a minus (−) sign indicates days before operation, a plus (+) sign days after operation.

	Thyroidectomy		Sham operation		
Days	Mean O_2 consumption (ml./g/h)	S.E.	Mean O_2 consumption (ml./g/h)	S.E.	't'
− 5	47·8*	3·02	48·0*	1·00	0·056
− 2	49·3	3·96	47·5	2·97	0·644
+ 1	48·3	3·33	42·5	1·52	1·446
+ 4	48·3	3·52	42·8	3·85	0·962
+ 7	45·8	2·70	43·7	1·92	0·578
+10	47·0	3·07	44·2	3·00	0·596
+13	44·2	3·44	47·2	3·76	0·537
+16	51·0	3·98	55·2	2·98	0·784
+19	51·6	3·54	52·0	3·50	0·073
+22	49·8	4·57	50·0	3·16	0·033
+26	49·3	3·86	45·8	2·38	0·704
+31	46·5	3·55	—	—	—
+36	47·7	2·84	—	—	—
+39	47·2	2·86	44·3	1·87	0·775
+42	44·2	3·73	—	—	—

The Effect of Dogfish Thyroid upon the Oxygen Consumption of Adult Rats

The results given in Fig. 1 are expressed as average percentage change in oxygen consumption. The values of oxygen consumption for the various test

series before injection gave a total mean of 1·37 ml./g/h (S.E. ± 0·039) which is seen to be in close agreement with the mean oxygen consumption of 1·33 ml./g/h (S.E. ± 0·039) determined on a completely separate series of twenty-four animals. The mammalian thyroid preparation caused a maximal increase in oxygen consumption of 26·1%, 5 days after the injection; whilst the dogfish thyroid preparation gave a maximal response of 24·8%, 4 days

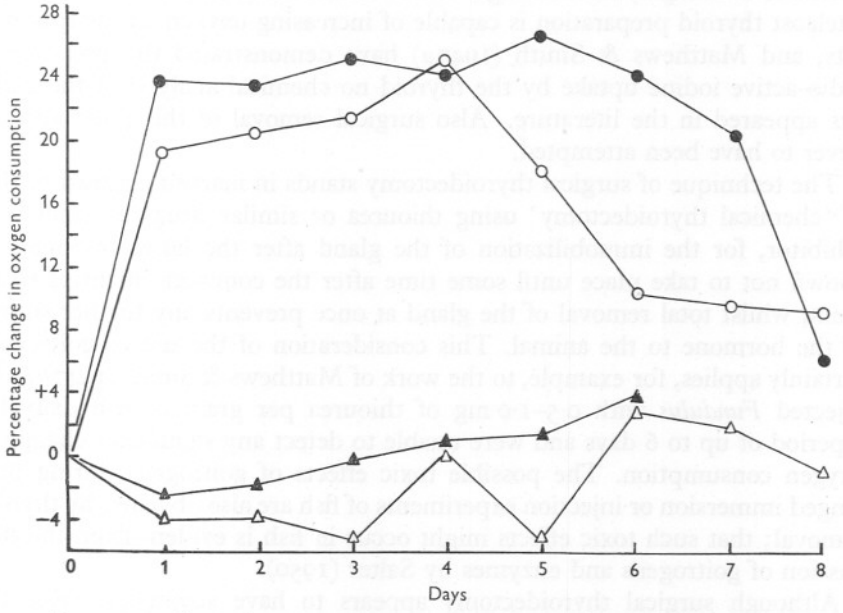

Fig. 1. Percentage change in oxygen consumption of rats injected intraperitoneally with thyroid preparations. △, normal controls; ▲, injected with 2·0 ml. 0·9% NaCl; ●, injected with 100 mg mammalian thyroid powder; ○, injected with 95 mg dogfish thyroid.

after the injection. Since the degree of response is about the same for approximately the same level of dosage it is probable that both preparations are of similar strength.

The saline-injected rats showed no increase in oxygen consumption either before or after injection with dried trout muscle.

Discussion

Gorbman, Lissitzky, Michel & Roche (1952), from radio-active iodine chromatographic studies on *Scyllium* thyroids, have shown that di-iodotyrosine and thyroxine are formed in the thyroid gland of these animals whilst the present experiments have demonstrated that dried dogfish thyroid gland when injected intraperitoneally into rats is capable of increasing their oxygen consumption. These investigations indicate clearly that there is present in the

dogfish thyroid a thyroxine-containing hormone similar to that found in mammals. Furthermore, as thyroidectomy of the dogfish has been shown above to cause no change in the oxygen consumption of the whole animal for a period of up to 42 days it may be concluded that although the thyroid hormone is present it has no influence over the immediate oxygen consumption of the dogfish. In the teleosts the evidence for a thyroxine-containing thyroid hormone is meagre, for although Smith & Brown (1952) have shown that a teleost thyroid preparation is capable of increasing oxygen consumption in rats, and Matthews & Smith (1947*a*) have demonstrated the presence of radio-active iodine uptake by the thyroid no chemical analysis of the gland has appeared in the literature. Also surgical removal of the gland appears never to have been attempted.

The technique of surgical thyroidectomy stands in marked contrast to that of 'chemical thyroidectomy' using thiourea or similar drugs as a thyroid-inhibitor, for the immobilization of the gland after the latter treatment is known not to take place until some time after the commencement of treatment, whilst total removal of the gland at once prevents any further supply of the hormone to the animal. This consideration of the use of goitrogens certainly applies, for example, to the work of Matthews & Smith (1947*b*) who injected *Fundulus* with 0·5–1·0 mg of thiourea per gram of fish daily for a period of up to 6 days and were unable to detect any significant change in oxygen consumption. The possible toxic effects of goitrogens during prolonged immersion or injection experiments of fish are also obviated by thyroid removal; that such toxic effects might occur in fish is evident from the discussion of goitrogens and enzymes by Salter (1950).

Although surgical thyroidectomy appears to have advantages over the chemical thyroidectomy approach for the investigation of thyroid function both suffer from the fact that the investigations so far reported, including the present work, may not have had the experiments prolonged for sufficient period for delayed effects on oxygen consumption to become evident. Thyroidectomy or goitrogen treatment of mammals results in a significant decline in oxygen consumption within some 5 days, but does not generally produce a maximal depression of basal metabolic rate until 2 or 3 weeks after the commencement of treatment (Siebert & Smith, 1930; Barrett & Gassner, 1951), and as the rate of oxidative metabolism in poikilotherms is known to be lower than that of homoiotherms the rate of response in the former might be expected to be substantially slower. Moreover, the fact that the temperature of poikilotherms is generally lower than that of homoiotherms might also effect the speed of response, for it has been demonstrated in fish that the thyroid gland shows cellular hypertrophy during treatment with thiourea more rapidly at high than at low temperatures (Fortune, 1953). Another possibility to be taken into account in evaluating the present results is the fact that the animal may be able to synthesize extra-thyroidally a similar

hormone to that produced by the thyroid. Preliminary experiments have shown the presence of protein-bound radio-active iodine in the plasma of dogfish which have been thyroidectomized for at least 6 weeks (Matty, unpublished). It might be supposed that if the extra-thyroidal hormone maintained the normal oxygen consumption level in thyroidectomized dogfish then treatment of such animals with anti-thyroid drugs for some period might prevent any production of the hormone and so cause a fall in the oxygen consumption, assuming, of course, that these drugs also inhibit the extra-thyroidal synthesis. Until the results of such further work are reported the possibility of extra-thyroidal hormone supply is not eliminated but, as in the higher vertebrates where the conditions of complete thyroxine deficiency after thyroidectomy is difficult to obtain (Leblond & Eartly, 1952), such investigations initially depend on surgical thyroidectomy.

Having established the presence of a thyroxine-containing hormone in dogfish which does not have any immediate effect on the oxygen consumption of the whole animal the search for identifying the functions of the thyroid must be directed elsewhere. According to Waring, Landgrebe & Bruce (1942), thyroids removed from the dogfish hypophysectomized a month previously afford no evidence for pituitary control of thyroid activity. There is some indication of a correlation between reproduction and thyroid activity in teleosts (Barrington & Matty, 1952; Robertson & Chaney, 1953; Scott, 1953), and there is evidence also that the activity of the gland is increased in elasmobranchs during the breeding season (Guariglia, 1937; Zenza, 1937). Some observations during the present work have been in conformity with these latter reports, but unfortunately there is as yet no direct experimental evidence for a relationship between the thyroid gland and the reproductive activity of elasmobranchs.

Another possible function of the thyroid is that it is concerned with the early stages of growth and maturation of fish. Experiments on teleosts have shown that on immersion in goitrogens growth is retarded (Hopper, 1952; Smith *et al.*, 1953), but as Smith *et al.* have pointed out the effect may be a non-physiological toxic one, and, as already mentioned, efforts to accelerate growth of teleosts by thyroxine, thyroid or thyrotropin treatment have given most equivocal results. The situation in elasmobranchs is even more obscure, for no studies on the possible relationship between the thyroid gland and growth in these animals seem to have been reported. It is, however, hoped that the use of surgical thyroidectomy, as described in the present work, will provide a useful basis for investigating some of these outstanding problems of thyroid relationships in the elasmobranchs.

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SUMMARY

Surgical thyroidectomy has been successfully performed on the dogfish, *Scyllium canicula*, and has been shown to have no signficant effect on the animal's oxygen consumption over a period of 6 weeks.

The injection of extracts of dogfish thyroid into adult male white rats results in an increase in the animal's oxygen consumption of the same order as that produced by mammalian thyroid preparations.

The significance of these results is discussed in the light of recent investigations of thyroid function in fish.

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