

THE RELATION OF WHOLE-BODY I<sup>131</sup> UPTAKE TO THYROID  
ACTIVITY IN THE DEVELOPING DOGFISH,  
SCYLIORHINUS CANICULA (L.)

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Inhibition of thyroid activity by various types of goitrogens is commonly ascertained by the presence of histological changes in the gland, such as hyperplasia, hypertrophy, and vacuolization of colloid, which are attributed to increased secretion of thyrotrophic hormone (TSH). Nevertheless, there are species among amphibians, *e.g.*, *Diemyctylus viridescens* (Adams, 1946; Dent and Lynn, 1958; Lynn and Dent, 1961), and elasmobranchs, *e.g.*, *Scyliorhinus canicula* (Tinacci, 1947; Olivereau, 1952; Dent and Dodd, 1961) and *Squalus suckleyi* (Pritchard and Gorbman, 1960), which have apparently a very low secretion rate of TSH or a very slow response of the thyroid to the pituitary hormone, for histological effects from administration of antithyroid substances are minimal. Studies with a radioactive isotope of iodine, I<sup>131</sup>, indicate, however, that the common goitrogens are just as effective in inhibiting organic binding of iodine in these species as in those in which marked histological changes are produced (Olivereau, 1952; Dent and Lynn, 1958; Pritchard and Gorbman, 1960), and that TSH administration stimulates iodine-binding and, in high concentration and after prolonged treatment, enlargement of thyroid follicles also (Dent and Dodd, 1961).

Class experiments with tadpoles of *Rana pipiens* which had been immersed in 0.01%, 0.03%, or 0.05% thiourea solution for two weeks had revealed a higher whole-body I<sup>131</sup> uptake than occurred in the controls. This phenomenon was ascribed to the presumed hyperplastic condition of the thyroid gland in the experimentals, although no histological examination was made (Bileau, 1956). Questions arose as to whether one could correlate whole-body iodine uptake with thyroid activity in animals in which goitrogens bring about thyroid inhibition only, without producing goiter, and also whether one could distinguish between different types of thyroid inhibitors by such means. The goitrogens commonly employed interfere with two mechanisms, *viz.*, the concentration of iodine (thiocyanate, perchlorate) and its organic binding (thiourea and its derivatives) (Pitt-Rivers and Tata, 1959).

Another aspect of thyroid activity which is of current interest is its relation to temperature in poikilotherms. Reports on teleost thyroid activity at both high and low temperatures are conflicting (Leloup and Fontaine, 1960; Olivereau, 1960). Possibly the only report on elasmobranch response is that of Dent and Dodd (1961), who found that for hatchlings of *Scyliorhinus canicula*, the amount of iodine bound by the thyroid is greater at a higher temperature.

The present study is concerned with the relation of whole-body I<sup>131</sup> uptake to organic binding of I<sup>131</sup> by the thyroid and to the histological condition of the

gland, upon treatment with thiourea or perchlorate at different temperatures. The form chosen was the embryo of the spotted dogfish, *Scyliorhinus canicula* (L.). The thyroid in this species, which lacks a larval stage, in common with that of amniotes active at birth or hatching (Dimond, 1954; Mitskevitch, 1957), shows signs of activity comparatively early in development (Vivien and Rechenmann, 1954).

#### MATERIALS AND METHODS

Twenty-five dogfish embryos, all in the latter half of development, were obtained in July and August, 1960, from an outdoor tank at The Laboratory, Plymouth, England. The mature egg cases had been removed surgically from captured females, and the embryos were developing in running sea water.

For this work the specimens were removed from the egg cases and kept, each one separately, in fingerbowls with approximately 100 ml. sea water (salinity 33.3‰) which had been taken at a depth and was considered equivalent to filtered water. Thiourea or potassium perchlorate was added to the sea water of the experimentals to make a concentration of 0.05%. Series I was kept at room temperature, which varied from 18° to 23.5° C. In Series II and III, however, both controls and experimentals were divided into two temperature groups, one at room temperature and the other at 8° C. Of the six initial specimens of Series I, only two were alive after 11 days in the same medium, probably because of heavy bacterial growth in the water. A third living specimen (1C), taken from the aquarium, was removed from its egg case and added to the series just at the time of iodine administration. For Series II (10 specimens) and III (8 specimens), the medium, including the plain sea water of the controls, was renewed once a week for the fish kept in the cold and twice a week for those at room temperature. Only one fish in each of these last series died, both of them from the cold group.

Several of the larger fish were measured at the beginning of the experiment. It was difficult to keep them extended, however. Since I did not wish to run the risk of injuring them by removing them from the water, most of the fish were left unmeasured. Final measurements were made on the preserved specimens.

After 11, 13, or 21 days of goitrogen treatment, the specimens were exposed to a solution of  $\text{NaI}^{131}$  in carrier-free  $\text{Na}_2\text{S}_2\text{O}_3$  diluted in plain sea water or drug solution to give an activity of 3  $\mu\text{c.}/\text{ml.}$  for Series I, and 1.8–1.85  $\mu\text{c.}/\text{ml.}$  for Series II and III. After 39 to 50 hours the fish were washed and placed in fresh goitrogen solutions or sea water. Then 24 hours later, after another washing, each fish was put into a 75- or 100-ml. beaker of sea water in a gamma Geiger well counter and a count of whole-body radiation made, 10 minutes for Series I, and three one-minute periods for Series II and III. The gamma Geiger detector consisted of a glass well about one-fourth inch thick, surrounded by five Geiger-Muller tubes, with the whole apparatus encased in heavy lead shielding. Its resolution time limited accuracy of counting to about 50,000 counts per minute. Background averaged 243 counts per minute.

In Series I, after the whole-body counts had been made, the yolk sac was tied off and severed from the body and counts made of the fish without yolk and of the yolk alone.

Immediately after the counting, the embryos of Series I and II were killed by immersion in Bouin's solution. Those of Series III were replaced in the same solutions they had been in since the cessation of the  $I^{131}$  treatment, and were counted again the next day. The specimens at 8° C. were then killed, whereas those at room temperature were held for three days longer at which time they were counted and preserved. The thyroid glands were then dissected out, embedded in polyester or ester wax, sectioned at 4–6  $\mu$ , and mounted serially on slides with 0.1% amylopectin solution (Steedman, 1957). Alternate slides were stained, the greater number of them with Gomori's chrome alum hematoxylin phloxin, and mounted, and the others were covered by Kodak Autoradiographic Stripping Film AR.10. After one to eight days the film-covered slides were developed and allowed to dry at room temperature. Later they were either stained with gallocyenin and metanil yellow (Bowie and Edmonson, 1960), and dried, then mounted with Xam and covered, or were mounted and covered without staining.

TABLE I

*Series I. Whole-body uptake and thyroid binding of  $I^{131}$  in developing Scyliorhinus canicula, after immersion in 0.05% thiourea solution for 11 days prior to exposure to 3  $\mu\text{c.}/\text{ml}$ .  $I^{131}$  for 43 hours and washing for 24 hours at room temperature (18–23.5° C.)*

Protocol number	Total length in mm.	Thyroid autoradiography*	Counts per minute, corrected for background		
			Entire fish	Body alone	Yolk sac alone
C4	86	++	53,663	53,391	4,798
1C	53	++	10,267	6,818	4,135
T4	80	—	5,633	5,671	643

\* Two plus signs indicate a dense autoradiogram with stripping film; one plus sign, a faint autoradiogram; and a minus sign, no autoradiogram.

## RESULTS

Tables I, II, and III summarize the greater part of the pertinent information on the effects of the antithyroid drugs and temperature. The whole-body  $I^{131}$  count cannot bear statistical analysis because of the different stages of development and probably also of thyroid activity, the small number of specimens in any one group, and the varying lengths of time between treatment and fixation. Also, since the autoradiographic film was exposed for different periods of time, no quantitative comparison of the amount of blackening over the thyroid gland is possible. Nevertheless, some patterns emerge which seem to deserve comment.

First of all, though, explanations of certain individual points are needed for clarification. The fish had a large yolk sac connected to the body by a cord (Figs. 1, 2) except for specimen 3P (Figs. 3, 4), which would probably have made its way out of the egg case about the time it was exposed to  $I^{131}$  (Ford, 1921). Five specimens (1C, 13P, 18C, 19P, and 20T) had still functional external gills at the termination of the study (Fig. 1), whereas for eight others (9C, 10P, 11T, 12C, 14T, 15C, 16P, and 17T) the gills atrophied during the experiment before the time of  $I^{131}$  administration (Fig. 2). The other individuals were

TABLE II

*Series II. Whole-body uptake and thyroid binding of  $I^{131}$  in developing Scyliorhinus canicula, after immersion in 0.05% thiourea solution for 13 days prior to exposure to 1.8  $\mu\text{c./ml.}$   $I^{131}$  for 39 hours and washing for 24 hours at either room temperature (18–23.5° C.) or 8° C.*

Protocol number	Total length in mm.		Temperature	Goitrogen treatment	Thyroid auto-radiography*	Counts per minute, corrected for background
	Initial	Final				
7C	70	85	Rm.	none	++	40,504
5P**	70	87	Rm.	perchlorate		5,272
6T**	70	88	Rm.	thiourea		11,358
9C	50	63	8° C.	none	+	1,390
8P	65–70	70	8° C.	perchlorate	–	1,715
10P***	50	63	8° C.	perchlorate		
19P†		43	8° C.	perchlorate	–	514
4T	75	75	8° C.	thiourea	–	1,479
14T		54	8° C.	thiourea	–	846
20T		49	8° C.	thiourea	–	603

\* See Table I.

\*\* Gland lost in processing.

\*\*\* Died between 10 and 13 days.

† Exposed to only 0.9  $\mu\text{c./ml.}$   $I^{131}$ .

further developed at the beginning of the study. The thyroid gland, nevertheless, in even the smallest and presumably the least advanced individuals, had very definite follicular structure with colloid more or less vacuolated, and cuboidal cells varying in height even in a given gland. There are no striking histological differences between the controls and experimentals at 8° C. or between those of Series I and II (14 and 16 days of exposure to the goitrogens, respectively) at

TABLE III

*Series III. Whole-body uptake and thyroid binding of  $I^{131}$  in developing Scyliorhinus canicula, after immersion in 0.05% thiourea solution for 21 days prior to exposure to 1.85  $\mu\text{c./ml.}$   $I^{131}$  for 50 hours and washing for 24 hours*

Protocol number	Total length in mm.		Temperature	Goitrogen treatment	Thyroid auto-radiography*	Counts per minute, corrected for background and decay		
	Initial	Final				Initial	21.5 hrs. later	93 hrs. later
15C		80	Rm.	none	++	20,374	16,929	4,642
3P	85	98	Rm.	perchlorate	–	8,016	5,841	2,833
16P		85	Rm.	perchlorate	–	1,987	1,633	1,223
17T		76	Rm.	thiourea	–	5,488	3,725	1,375
12C**		66	8° C.	none				
18C		54	8° C.	none	+	1,084	761	
13P		60	8° C.	perchlorate	–	1,282	1,016	
11T		66	8° C.	thiourea	–	1,272	930	

\* See Table I.

\*\* Moribund and preserved at 21 days.

room temperature. Nevertheless, somewhat higher cells, larger follicles, and more mitotic figures appear in the experimentals of Series III kept at room temperature (Figs. 5, 6, 7).

Growth of the developing fish was generally slowed down in the cold (Tables II and III), but not affected to any appreciable extent by either of the goitrogens. Exceptions to this, however, were specimens 9C and 10P, both of which increased in total length from 50 to 63 mm. during the cold-treatment. Activity was also

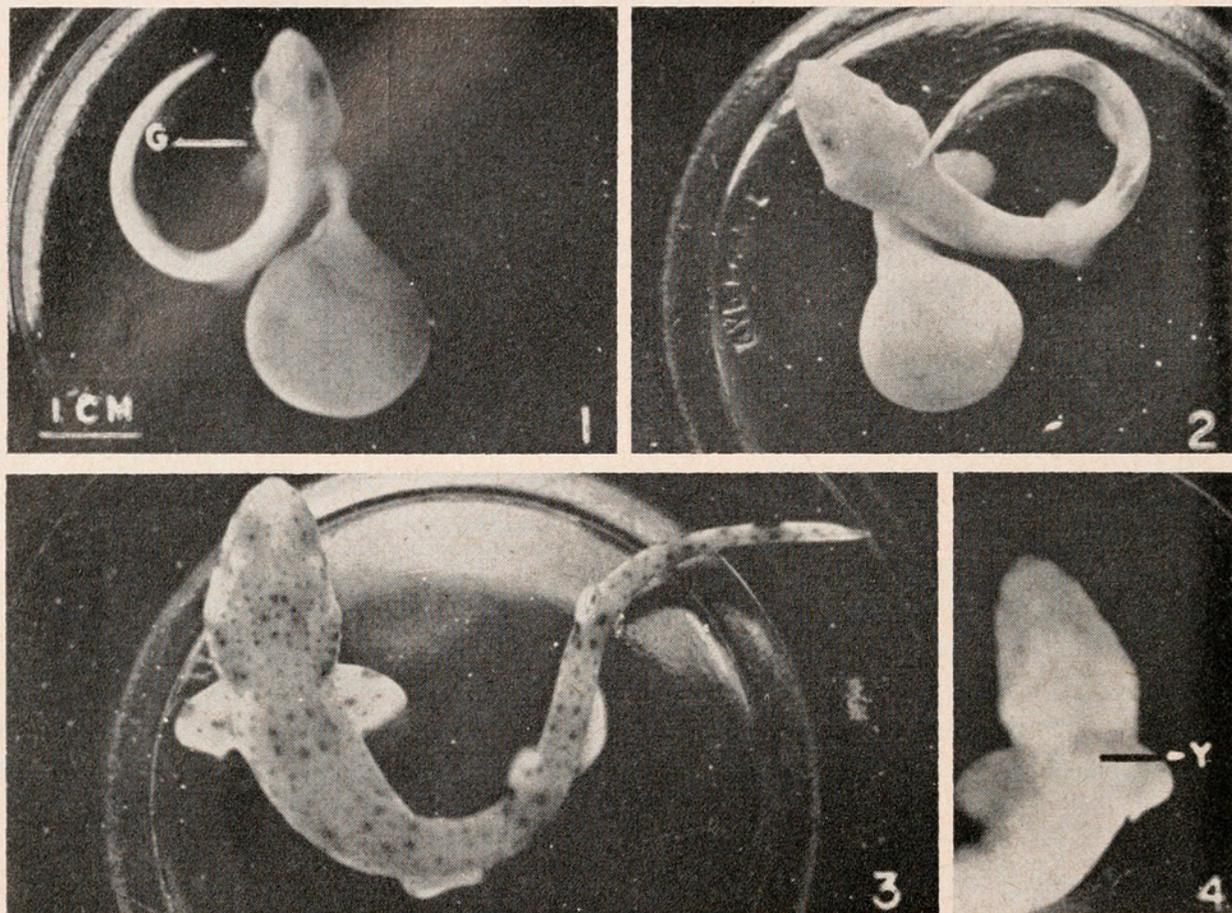


FIGURE 1. Specimen 18C, 54 mm., kept at 8° C. for 21 days, at the time of exposure to  $I^{131}$ . Notice the large yolk sac and the functional external gills (G).

FIGURE 2. Specimen 15C, 80 mm., kept at room temperature. Notice a small yolk sac, the absence of external gills and the beginning of pigmentation.

FIGURE 3. Specimen 3P, 98 mm., kept in 0.05%  $KClO_4$  at room temperature. It is probably a hatchling.

FIGURE 4. Ventral view of anterior region of specimen 3P. Notice the very small remnant of the yolk sac (Y).

diminished at a low temperature, the fish remaining very quiet as compared to those at room temperature, but there was no evidence of goitrogen influence on behavior.

In view of the size variations in the specimens, the  $I^{131}$  whole-body counts are probably not significantly different for the control and experimental animals kept at 8° C. In the case of those held at room temperature, however, an obvious pattern is indicated, for in the three sets (omitting specimen 3P, which was at least 10 mm. longer than the others and probably a hatchling at the time), the

relation of  $I^{131}$  uptake was control > thiourea > perchlorate. The thyroid gland in the goitrogen-treated fishes, although it may have concentrated iodine, did not bind it, for there was no blackening of the autoradiographic film. In the controls at room temperature, however, the colloid of the gland appears well stocked with organic iodine (Figs. 8, 9), and even in those at 8° C., there is a sparse granulation of the autoradiographic film, indicating a low degree of iodine-binding (Figs. 10, 11).

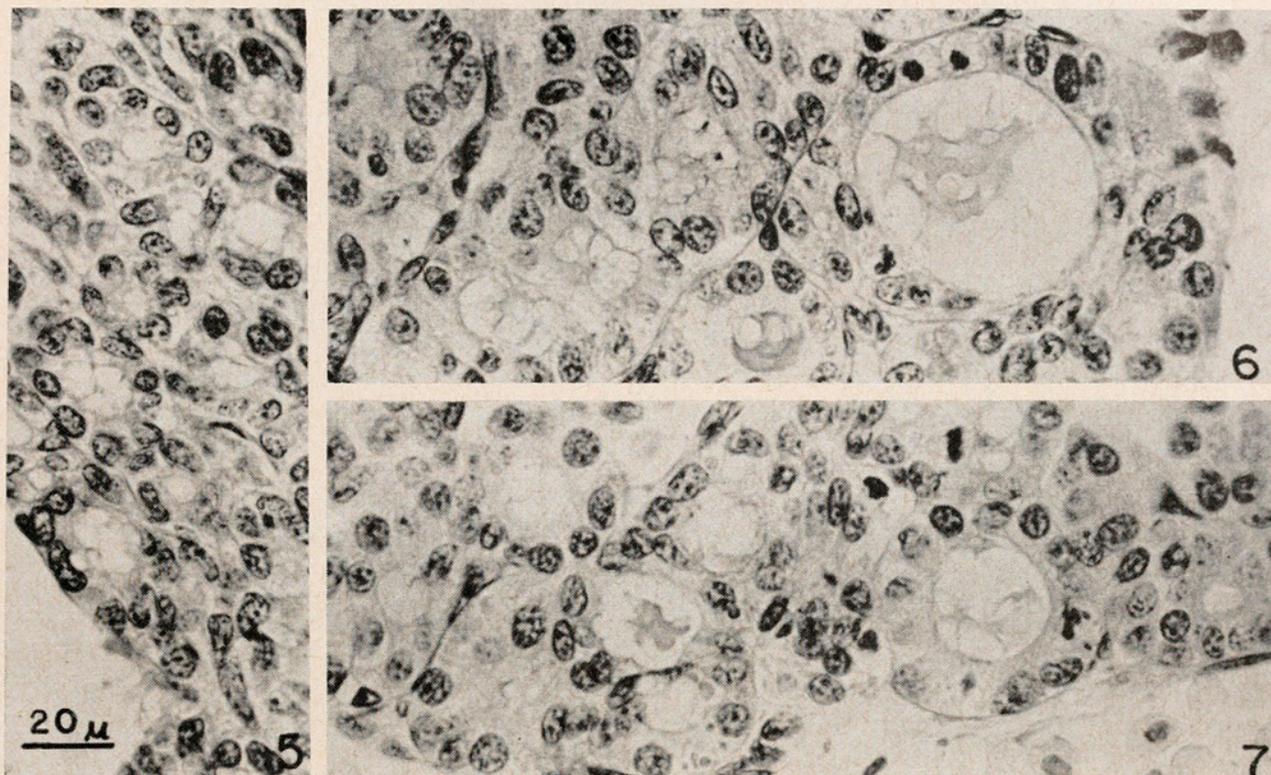


FIGURE 5. Section of thyroid gland of specimen 15C, 80 mm., kept at room temperature for 28 days. Cut at 6  $\mu$ . Stained with chrome alum hematoxylin phloxin. Compare with Figure 2.

FIGURE 6. Section of thyroid gland of specimen 16P, 85 mm., kept in 0.05%  $KClO_4$  at room temperature for 28 days. Staining and sectioning as in Figure 5. Notice the enlarged follicles, higher cells, and mitotic figures.

FIGURE 7. Section of thyroid gland of specimen 17T, 76 mm., kept in 0.05% thiourea at room temperature for 28 days. Notice the same characteristics as in Figure 6.

Finally, there is a marked difference in  $I^{131}$  excretion between the control and goitrogen-treated fish, as seen in Figure 12. Iodine removal from the control occurred at an arithmetic rate, whereas for the fishes exposed to perchlorate and thiourea the curve approached an exponential pattern.

#### DISCUSSION

Iodine-131 in a concentration of 1.0  $\mu c./ml.$  for 24 hours has been found suitable for absorption by fresh-water forms (Lynn and Dent, 1957). Since sea water contains so much iodine (50  $\mu g./kg.$ ), it was considered advisable to increase the amount of the radioactive form, and also the exposure time, in order to insure a measurable uptake. Actually, 1.0  $\mu c./ml.$  would probably have been

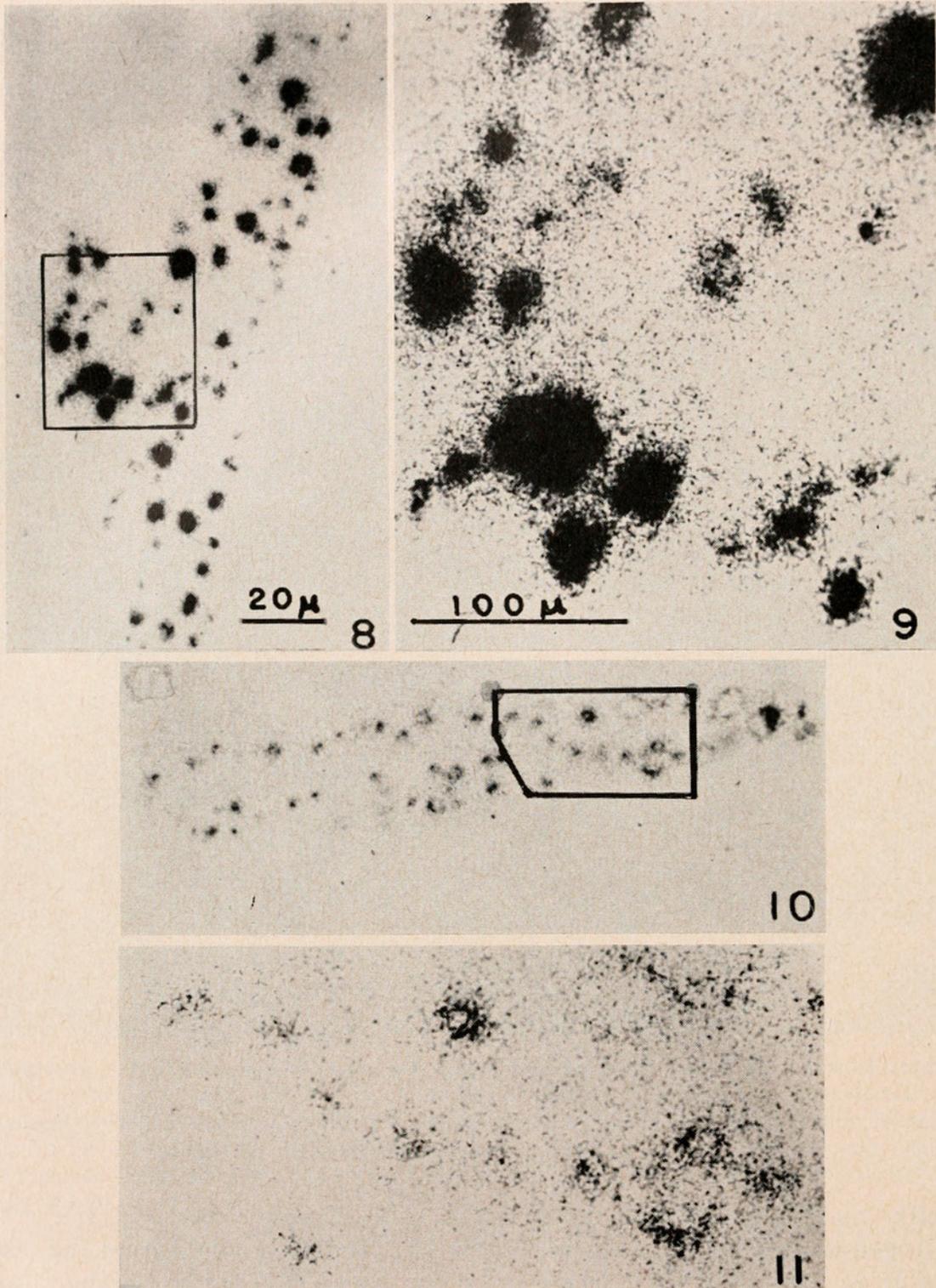


FIGURE 8. Autoradiogram from longitudinally sectioned thyroid of specimen 15C, 80 mm., kept at room temperature for 28 days. Compare with Figures 2 and 5. The animal was washed for 5 days after 50 hours' exposure to  $1.85 \mu\text{c./ml. I}^{131}$  and then fixed. Four days later stripping film was applied to the sections and, after two days of exposure, developed. Cut at  $6 \mu$ .

FIGURE 9. Enlargement of a portion of Figure 8.

FIGURE 10. Autoradiogram from longitudinally sectioned thyroid of specimen 18C, 54 mm., kept at  $8^\circ \text{C.}$  for 25 days. The animal was washed for two days between  $\text{I}^{131}$  treatment and fixation. Four days later stripping film was applied to the sections and developed the next day. Compare with Figure 1. Same magnification and sectioning as Figure 8.

FIGURE 11. Enlargement of a portion of Figure 10. Same magnification as Figure 9.

satisfactory, for specimen 19P, not only the smallest of the fish, but also one treated with perchlorate, had a count almost three times background after exposure to only  $0.9 \mu\text{c./ml}$ . The concentration of both thiourea and perchlorate (0.05%) was apparently optimal for *Scyliorhinus canicula*, for there was no evidence of toxicity effects, and the thyroid uptake of  $\text{I}^{131}$  seemed to be completely inhibited.

In *Scyliorhinus canicula*, thyroid activity begins very early in development. Vivien and Rechenmann (1954) report that at 25 mm., when the thyroid is still

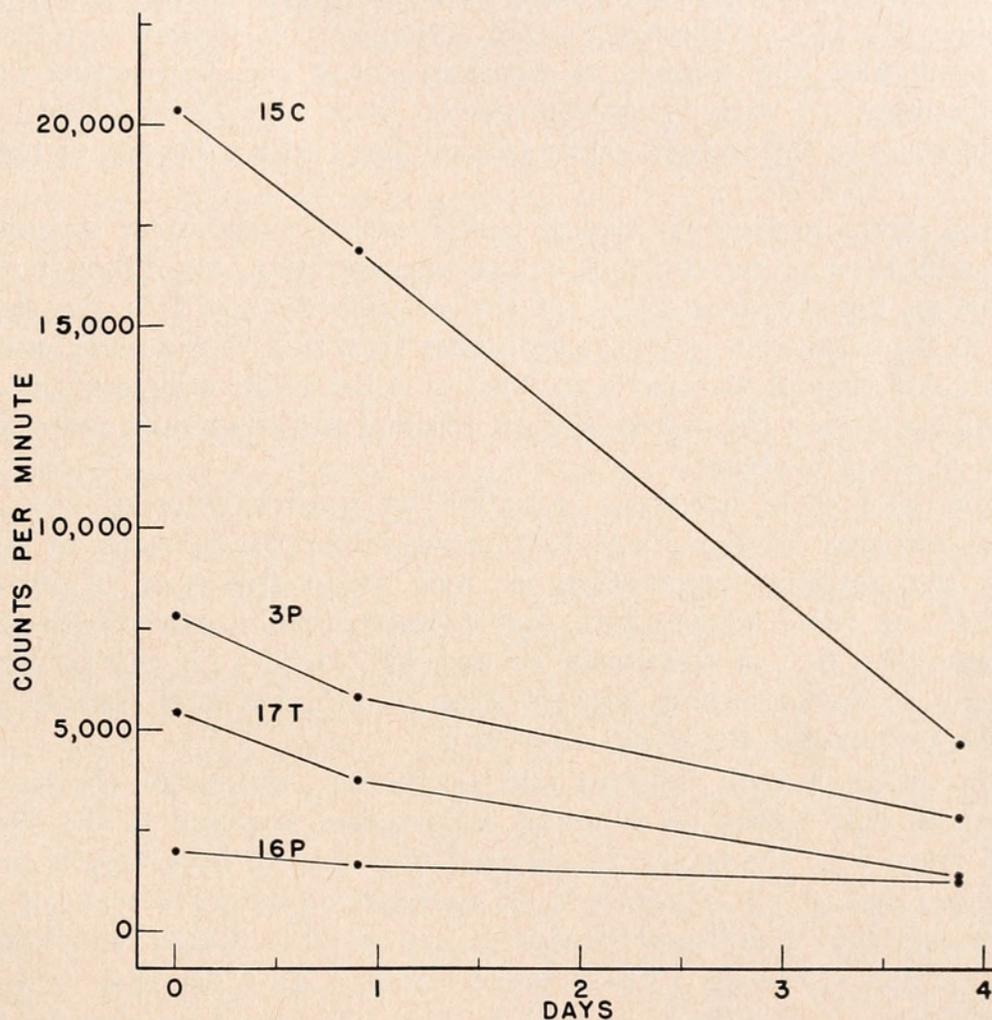


FIGURE 12. Elimination of  $\text{I}^{131}$  from four embryos of *Scyliorhinus canicula* which had been kept at room temperature for 21 days prior to exposure to  $1.85 \mu\text{c./ml}$   $\text{I}^{131}$  for 50 hours and washing for 24 hours (Series III), as seen one and four days after the initial whole-body count. Counts per minute are corrected for background and decay.

in the cord stage, it already binds iodine, but its role in embryonic life of elasmobranchs remains undetermined. Among the multiple effects of thyroid hormone during development are both gross changes, such as yolk sac retraction in teleosts and sauropsids (Dales and Hoar, 1954; Dimond, 1954), and histological changes, such as degeneration of the pronephros, as observed in amphibians (Lynn and Peadon, 1955).

In the present investigation, there were no obvious external differences between the controls and experimentals at a given temperature. Since only one specimen "hatched," no comparison can be made as to its relative speed of yolk-sac retraction,

and the histological examination was limited to the thyroid. It seems reasonable to presume, however, that some morphological and physiological differences could be found by detailed study, since the thyroid gland is obviously very active during normal development.

Two facts concerning thyroid activity in the developing spotted dogfish at a low temperature (8° C.) stand out, *viz.*, that the gland concentrates and binds iodine to a degree sufficient for autoradiographic determination, and that thyroid hormone formation is so low as to evade detection in a whole-body count (Tables II, III; Figs. 10, 11). Whether thyroid action here is limited to a low rate of hormone production and storage, or whether release and distribution to the rest of the body occur as well, is undetermined. Dent and Dodd (1961) report a four-fold increase in tail muscle iodine of hatchlings with a change of temperature from 8.6° C. to 13.6° C.

Thiourea seems to have its typical action, *viz.*, prevention of organic binding of iodine, with little or no effect on iodine concentration, for although there was no reaction on the stripping film, yet the whole-body count in the case of the fish at room temperature was markedly higher than that of the perchlorate-treated specimens. An attempt was made to determine the relative amounts of thyroidal iodide by freeze-drying the glands, but an accident to the vacuum pump prevented completion of the processing.

The rate of thyroid hormone formation by dogfish embryos, and thus the histological response of the gland to thyroid inhibitors, appears to be directly related to the ambient temperature as well as to the state of development. Specimen 1C at room temperature, for example, had a whole-body count ten times greater than that of specimens 9C and 18C at 8°. Of course, it had been exposed to a somewhat higher concentration of  $I^{131}$ , but it is doubtful that that alone could account for the great difference.

Further, although both thiourea and potassium perchlorate prevented iodine binding, yet in only Series III at room temperature, exposed to the drugs for a total of 28 days, were the typical histological responses of hypertrophy and hyperplasia visible (Figs. 6, 7). Sixteen days' exposure (Series II) was not sufficient. Apparently even an active thyroid is slow as compared to that of homoiotherms.

Pritchard and Gorbman (1960) found about a 25% increase in cell height and "vacuolization" of colloid, as well as low  $I^{131}$  uptake in thyroid glands of near-term pups of *Squalus suckleyi*, after repeated injections with propylthiouracil. Tinacci (1947) observed some thyroid hyperplasia as well as hyperemia in the thyroid of *Mustelus laevis* after 25 days of oral administration of three different goitrogens, including thiouracil. On the other hand, Olivereau (1952), who administered thiourea or thiouracil to adults of *Scyliorhinus canicula* at 20° C., reports no histological differences between the controls and experimentals, other than the presence of almost pycnotic nuclei in the thyroid epithelium after 44 injections of thiouracil.

The pituitary hormone, however, is present and active in *Scyliorhinus canicula*, for hypophysectomy of developing pups prevents  $I^{131}$  fixation (Vivien and Rechenmann, 1954), and injection of mammalian TSH increases its organic binding in both hatchlings (Dent and Dodd, 1961) and adults (Leloup and Fontaine, 1960). Dent and Dodd have further shown that injection of ventral lobe extract from

adult dogfish pituitaries over a period of three weeks results in an enhanced  $I^{131}$  binding, although the histological picture is not changed. Nevertheless, after three weeks of treatment at 13–14° C. with a very large amount of mammalian TSH, at least five times that effective in man on a weight basis, the hatchling thyroid follicles are markedly enlarged.

The results of investigations of temperature effects on reptilian thyroids substantiate the observations on elasmobranchs. Eggert (1936) observed that the thyroids of lizards kept at 6–7° C. during the summer are not activated by TSH, whereas at normal summer temperatures, the pituitary hormone is very effective. Shellabarger *et al.* (1956), working with turtles, found that TSH caused increased  $I^{131}$  uptake at 21–23° C., but not at 2–3° C. The body temperature of birds and mammals, whose thyroids are more responsive, is much above even the peak temperature in all these instances.

Of course, other factors have to be considered once one leaves the realm of development, such as light intensity and periodism, osmotic conditions, seasonal cycles, degree of maturity, sexual activity (Eggert, 1936; Bileau, 1956; Shellabarger *et al.*, 1956; Hickman, 1959; Leloup and Fontaine, 1960; Olivereau, 1960). The absence of thyroid hypertrophy and hyperplasia that Olivereau (1952) reports for adult *Scyliorhinus canicula* at 20° C. after prolonged treatment with thiouracil could be due to normal lack of utilization of thyroid hormone at the season of the year (autumn) or stage of life, or even under the prevailing conditions of light. Tinacci's (1947) work with *Mustelus laevis* was carried on during the summer, which seems to be a time of high thyroid activity in poikilotherms (Eggert, 1936; Bileau, 1956; Baggerman, 1957).

It may be objected that Dent and Dodd (1961) observed thyroid response to TSH in the spotted dogfish during the winter. Their specimens, however, were hatchlings. Zezza (1937) points out that the thyroid gland of *Torpedo ocellata* presents a much more active histological picture during growth than in adulthood, and the same thing probably holds true for *Scyliorhinus canicula*. At any rate, the gland is very active during development.

The rate of  $I^{131}$  removal in Series III at room temperature shows an excellent correlation with both whole-body uptake and thyroid autoradiography. Specimen 15C, with an arithmetic decrease in  $I^{131}$ , must have had a constant turnover rate of thyroid iodine and consequent active excretion after exposure to an excess of iodide. On the other hand, in the three goitrogen-treated specimens (3P, 16P, and 17T), diffusion seems to have been the principal mechanism, for iodine removal occurred almost geometrically. Probably the deviation from exact exponential change is due to the fact that the medium remained unchanged and  $I^{131}$  could diffuse back into the animals. Specimen 16P, with the lowest uptake, would be most affected by re-entrance of  $I^{131}$  from the medium, and would reach equilibrium sooner. Unfortunately, water samples were not counted.

The selachian embryo is a very satisfactory experimental animal, as Vivien (1954) has pointed out. Not only the oviparous forms, such as *Scyliorhinus canicula*, but also the ovoviviparous series, *e.g.*, *Squalus suckleyi* (Pritchard and Gorbman, 1960) can be reared in the laboratory.

Many problems concerning thyroid activity in poikilotherms remain unsettled. Perhaps the study of the embryo, which only gradually develops its homeostatic

mechanisms, would reveal the basic function of the thyroid gland, which is then modified as development proceeds and environmental response becomes more complex.

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#### SUMMARY

1. Embryos of *Scyliorhinus canicula*, removed from their cases and kept at either room temperature or 8° C., were treated with 0.05% thiourea or perchlorate, or they were untreated, for 11, 13, or 21 days previous to addition of I<sup>131</sup> to their medium. After two days of exposure to I<sup>131</sup> and a day of washing, whole-body counts were made. The thyroid glands were removed within four days and prepared for autoradiography.

2. At 8° C., although the film indicated the presence of a slight amount of bound iodine in the controls, there was no marked difference in whole-body count in the three groups.

3. At room temperature, the controls had very high counts and well-blackened autoradiograms, whereas the two experimental groups had low counts and no sign of organic iodine.

4. Only the experimentals which were exposed to the goitrogens for 28 days showed histological responses, such as enlarged follicles and very numerous mitotic figures.

5. Growth was inhibited at the low temperature, but apparently unaffected by either of the goitrogens at either temperature.

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