

THE RELATION OF THYROXINE TO TOTAL IODINE IN THE THYROID GLAND*

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THE WORK of many investigators has made it clear that 90 per cent or more of the iodine in the normal thyroid gland is present in organic combination. This organic iodine, according to Harington (1933), can be completely accounted for by thyroxine and diiodotyrosine. These two compounds do not normally exist free in the gland but can be isolated after suitable hydrolysis of the thyroid protein.

Various values have heretofore been reported for the proportions of thyroxine and diiodotyrosine present in thyroid tissue. Thus, by an acid separation, Harington and Randall (1929) obtained values of 40-50 for the ratio of thyroxine to total iodine. Similar values were found by others who employed their method (Rotter and Soos, 1933; Dey, 1945). Subsequently, Leland and Foster (1932), who used butyl alcohol for the extraction of thyroxine from an alkaline hydrolysate of the thyroid, reported that only 25 per cent of the iodine of the gland is present as thyroxine. Blau (1933) by modifying the method of Leland and Foster increased the thyroxine recoveries to about 30 per cent of the total iodine.

A recent adaptation of Foster's method to small amounts of thyroid tissue (Taurog and Chaikoff, 1946a and b) made possible a study of the influence of dietary iodine upon the thyroxine content of the thyroid gland of the rat. In rats maintained on iodine intakes ranging from 2 to 440 micrograms per day, the total iodine content of the gland rose from 21 to 131 mg. per cent, but the percentage found as thyroxine remained constant (Taurog and Chaikoff, 1946c). Thus the mean value for this percentage was 31, with a standard error of 0.5. Furthermore, as profound a change in the condition of the thyroid as that produced by hypophysectomy failed to alter the percentage of the iodine present in the gland in the form of thyroxine (Taurog, Chaikoff and Bennett, 1946). These findings in the rat led us to examine the ratio of thyroxine to total iodine in the thyroids of other vertebrates.

EXPERIMENTAL

Thyroid glands were excised immediately after the animals were sacrificed, and their total and thyroxine iodine content determined by a method

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previously described (Taurog and Chaikoff, 1946b). For the larger glands, 20, cc. of 2N NaOH per gram of tissue was used for hydrolysis. When less than 50 mg. of thyroid tissue was available, one cc. of the base was used. Hydrolysis was carried out on a steam bath for 12 hours. The hydrolysates of the larger glands were diluted so that 1 cc. would contain amounts of iodine corresponding roughly to those contained in smaller glands. The butyl alcohol extraction of the hydrolysates and the subsequent iodine analyses were carried out as outlined by Taurog and Chaikoff (1946b).

RESULTS

Since no attempt was made to control the iodine intake of most of the animals recorded in Table 1, the finding of considerable variation in the iodine concentration of their thyroids is not unexpected. The lowest mean value, namely 29 mg. per cent, was observed in the shark; this is of interest since the shark lives in sea water. The high values found in the dogs reflect the high iodine content of their diets; they received 2-3 mg. of iodine per day.

TABLE 1. THE PROPORTION OF TOTAL IODINE PRESENT AS THYROXINE IN THE THYROIDS OF VARIOUS VERTEBRATES
(All animals were adult and were selected without regard to sex)

Species	No. of animals	Thyroid weight		Thyroxine iodine		Total iodine		Thyroxine as per cent of total iodine
		Mean	Range	Mean	Range	Mean	Range	
		Milligrams		Milligrams per cent		Milligrams per cent		
Shark (<i>Squalus suckleyi</i>)	8	84	37-160	8	4-14	29	17-49	27.9 ± 0.5†
Turtle (<i>Pseudemys scripta troostii</i>)	12	173	63-330	41	16-63	126	74-180	31.8 ± 1.3
Chicken (White Leghorn)	15	56	17-97	27	9-51	105	35-201	25.0 ± 0.7
Turkey	4	108	85-140	36	25-54	143	100-200	24.7 ± 0.7
Guinea Pig	8	57	45-72	12	6-24	42	25-89	28.3 ± 1.2
Rabbit	12	133	90-220	15	4-34	50	17-98	28.9 ± 1.1
Dog	8	833	461-1170	74	56-98	230	160-300	32.0 ± 0.6
Rat	15	26	18-30	12	6-18	41	19-58	29.3 ± 0.6
Horse	6	11,200	9800-12,100†	23	15-31	75	59-102	29.6 ± 1.2
Cattle	6	*	*	41	19-59	128	67-199	31.6 ± 1.0
Sheep	12	977	675-1260†	40	13-63	133	44-222	29.7 ± 0.7

* Incomplete glands were obtained.

† Weights of single lobes.

‡ Standard error of the mean

$$\sigma_{\bar{x}} = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n(n-1)}}$$

Despite the fact that among the 11 species studied the iodine concentrations in individual thyroids fluctuated from 17 to 300 milligrams per cent,—an eighteenfold variation,—the fraction of total iodine found in the form of thyroxine remained relatively constant. The values for thyroxine iodine expressed as a percentage of total iodine were found to be within the range of 25 to 32.*

* In comparing the variation of the ratios between and within species, the variance ratio, F, was found to be 6.45 (Snedecor, 1938). Although this value indicates that a statistically significant difference exists among some of the means of the ratios shown in the last column of Table 1, this difference is obviously quite small.

DISCUSSION

Several investigators have compared the thyroxine and total iodine levels in the thyroid glands of various animals. Baumann *et al.* (1942) found (by Blau's method) that about one third (28-36 per cent) of the iodine of the thyroid of the turtle, the alligator, and the dog appeared in the thyroxine fraction. With the same method, Michel and Lafon obtained similar values for pork and beef thyroids. The results obtained in both of these investigations are in close agreement with the findings presented here. According to Dey *et al.* (1945), the ratio of thyroxine to total iodine is the same in shark and cattle thyroids; but since Harington and Randall's method was used, their ratios are higher than ours. Parkes (1946) has also emphasized the close relation between acid-insoluble iodine (thyroxine) and total iodine in dried preparations of various mammalian thyroids.

TABLE 2. THE EFFECT OF THYROTROPIC HORMONE ON IODINE FRACTIONS OF RAT THYROIDS
(Each animal received two daily subcutaneous injection of 40 units* of thyrotropic hormone)

Treatment	No. of rats	Mean body wt.	Mean thyroid wt.	Mean thyroxine iodine	Mean total iodine	Mean ratio and S.E. †
		gm.	mg.	mg. per cent	mg. per cent	per cent
Controls	5	222	21	16	53	30.2 ± 0.7
Thyrotropic Hormone (7 days)	5	210	68	1.0	5.7	17.5 ± 0.6

* We are indebted to Dr. D. A. McGinty of Parke, Davis and Co., Detroit, for the supply of thyrotropic hormone. A unit is defined as the total dose in mg. which when injected subcutaneously over a period of 4 days into 180-200 gm. guinea pigs produces on the fifth day minimal but definite hyperplasia of the thyroid in all of 6 animals.

† S.E. = Standard Error of the Mean.

The ratio of thyroxine iodine to total iodine thus remains relatively constant from species to species and from animal to animal within in a given species. Although no explanation can be offered at present for this constancy, it is difficult to visualize how it could be so widespread unless the mechanism involved in the synthesis of thyroxine were quite similar throughout the vertebrates.

The normal relation between thyroxine and diiodotyrosine is upset during the prolonged injection of thyrotropic hormone. In rats that were injected daily for 7 days with thyrotropic hormone, the ratio of thyroxine iodine to total iodine was reduced to 17.5 ± 0.6 (Table 2). This finding in the rat is in agreement with previous observations of Gutman *et al.* (1932) on thyroid tissue excised from patients suffering from hyperthyroidism. These workers regard this lowered ratio as evidence for a change in the composition of the thyroglobin formed by the hyperactive thyroid.

SUMMARY

The fraction of total iodine present as thyroxine in the thyroid gland was found to be constant in 11 vertebrates: 1 fish, 1 reptile, 2 birds, and 7 mammals.

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