THE INHIBITORY ACTION OF EXCESSIVE IODIDE UPON THE SYNTHESIS OF DIIODOTYROSINE AND OF THYROXINE IN THE THYROID GLAND OF THE NORMAL RAT[1]

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BOTH CHEMICAL (Harington, 1944, 1945; von Mutzenbecher, 1939; Block, 1940, Reineke and Turner, 1942) and biochemical evidence (Perlman *et al.*, 1941; Morton *et al.*, 1943; Taurog *et al.*, 1947; Leblond, 1942) has shown that at least 2 steps are involved in the synthesis of thyroxine: 1) iodination of tyrosine to form diiodotyrosine and 2) coupling of 2 diiodotyrosine molecules to form thyroxine. While the exact nature of step 1 is not well understood, it is apparent that the iodide reaching the thyroid gland from the blood must be oxidized either to I_2 or to HIO before it can be incorporated into the phenolic group of tyrosine. The subsequent coupling of 2 diiodotyrosine molecules is believed by both Harington (1945) and Johnson and Tewkesbury (1942) to be an oxidative reaction in which the oxidized form of iodide (I_2 or HIO) plays a significant role.

In a recent communication (Wolff and Chaikoff, 1948a, 1948b) it was demonstrated that the administration of relatively large doses of inorganic iodide to *normal* rats blocked the formation of organic iodine in the thyroid gland. This block was related to the level of plasma iodine. So long as the level of plasma iodine remained above 20–35 gamma per cent, no appreciable conversion of inorganic iodide to organic forms occurred in the gland (Wolff and Chaikoff, 1948c). In keeping with the considerations presented above, excessive iodide could thus exert its inhibitory effect by blocking 1) the iodination of tyrosine *and/or* 2) the coupling mechanism involved in the formation of thyroxine from diiodotyrosine. These possibilities are examined in the present communication.

EXPERIMENTAL

Male rats of the Long-Evans strain, weighing 170–240 gms., were used throughout. Their mean body and thyroid weights are recorded

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in Table 1. They were fed a diet containing 0.3γ iodine per gm. This diet consisted of 68.5 per cent wheat, 5 per cent casein, 10 per cent fish meal, 10 per cent alfalfa, 1.5 per cent sodium chloride, and 5 per cent sardilene (a fish oil that supplied vitamins A and D).

Amounts of iodide as KI labeled with I^{131} varying from 5 to 100γ were injected intraperitoneally (Tables 2 and 3). The rats of each experiment were sacrificed at single interval. In experiments 2, 5, and 6, this interval was 4 hours; in experiment 1, it was 4.6 hours.

TABLE 1. BODY WEIGHTS AND THYROID WEIGHTS OF RATS

Experiment	Number of rats	Body weights	Thyroid weights
		gm.	mg.
1	9	214 ± 19*	21 ± 0.3*
2	10	188 ± 9	17 ± 0.2
5	10	174 ± 8	17 ± 0.2
6	13	177 ± 7	22 ± 0.3

* Standard devation.

The animals were anesthetized with sodium pentobarbital (20. mg. per rat) and then exsanguinated. The thyroid glands were rapidly excised, weighed, and immediately thereafter transferred to 1 cc. of cold 10 per cent trichloroacetic acid (T.C.A.). The inorganic (T.C.A.-soluble) fraction was separated as previously described (Wolff and Chaikoff, 1948b), and discarded. The organic fraction (T.C.A.-insoluble) was separated into diiodotyrosine-like and thyroxine-like fractions by the method described elsewhere (Taurog and Chaikoff, 1946a).

RESULTS AND DISCUSSION

In each experiment (Table 2) the amounts of I^{127} injected were chosen so that for the duration of the experiment (i.e., 4 or 4.6 hours) the concentration of plasma iodine remained well above or well below the critical level previously shown to produce inhibition of organic binding of iodine in the gland (Wolff and Chaikoff, 1948b). The non-inhibitory doses were either 5γ (experiments 1 and 2) or 10γ of I^{127} (experiments 5 and 6), whereas 50 or 100γ were injected to obtain inhibition. The diiodotyrosine and thyroxine (I^{127}) contents of the thyroid gland, as well as the percentages of the injected I^{131} recovered in these 2 fractions, are recorded in Table 2.

1. Calculation of Newly Synthesized Diiodotyrosine

Since both plasma and thyroid glands of the rats fed our low-iodine diet contained negligible amounts of inorganic iodide before the injections were made (Taurog and Chaikoff, 1946b, 1947), and furthermore since the amounts of I^- injected were large with respect

TABLE 2. DIIODOTYROSINE-LIKE, AND THYROXINE-LIKE FRACTIONS FOUND IN THYROIDS OF RATS INJECTED WITH VARIOUS AMOUNTS OF LABELED IODIDE

Expt.	Iodide injected	I^{127} in thyroid as		Per cent of injected I^{131} recovered as	
		Diiodotyrosine	Thyroxine	Diiodotyrosine	Thyroxine
1*	γ	γ	γ		
		7.2	2.8	14.9	7.3
		6.8	2.9	12.8	4.9
	5	7.3	3.0	14.1	8.2
		6.8	2.7	8.7	3.2
		5.5	2.7	8.9	4.5
		5.1	2.7	0.16	0.019
		8.5	3.1	0.11	0.018
	100	6.8	2.7	0.18	0.029
		5.5	2.0	0.12	0.011
2†		5.4	2.4	7.1	2.7
		3.9	1.7	10.5	2.2
	5	6.6	2.7	11.1	2.6
		5.6	2.4	8.1	2.1
		5.9	2.7	7.5	2.5
		3.3	1.3	0.095	0.013
		3.2	1.3	0.045	0.007
	100	3.8	1.5	0.057	0.009
		7.0	2.9	0.048	0.008
		4.8	2.4	0.085	0.014
5†		2.8	1.0	5.4	2.2
		4.3	2.0	8.1	3.3
	10	5.4	2.8	11.4	4.5
		3.9	1.8	6.7	2.0
		6.6	2.4	11.8	4.0
		3.7	1.5	0.15	0.027
		3.2	1.1	0.061	0.013
	100	4.3	1.5	0.054	0.010
		3.5	1.8	0.041	0.007
		3.4	1.6	0.033	0.006
6†		3.0	1.5	8.5	3.2
		4.9	1.8	11.7	4.5
	10	7.1	2.8	15.7	5.8
		2.8	1.5	7.7	2.6
		5.6	2.5	11.3	4.4
		3.2	2.0	6.2	1.6
		3.4	1.8	2.5	0.48
	50	4.0	1.7	1.2	0.28
		3.1	1.5	0.82	0.24
		2.6	1.2	0.14	0.020
		4.9	2.4	0.088	0.014
	100	2.8	1.3	0.13	0.019
		3.6	1.5	0.096	0.012

* The rats of these experiments were sacrificed at 4.6 hours after the injection of labeled iodide.

† The rats of these experiments were sacrificed 4 hours after the injection of labeled iodide.

to the amount of I^- turned over during the 4-hour period of observation,[2] we are safe in assuming that the specific activity of the plasma *inorganic iodide* throughout the 4-hour period of observation remained the same as that of the injected sample. We have therefore calculated the absolute amounts of iodide converted to diiodotyrosine in the gland during the 4 hours by multiplying the fraction of the injected

TABLE 3. AVERAGE VALUES* FOR 1) THE AMOUNT OF INJECTED I^{127} CONVERTED TO DIIODOTYROSINE AND 2) THE SPECIFIC ACTIVITIES OF DIIODOTYROSINE AND THYROXINE

Expt.	Iodide injected	Injected I^{127} converted to diiodotyrosine	S.A.† of diiodotyrosine iodine	S.A. of thyroxine iodine	S.A. thyroxine I / S.A. diiodotyrosine I
	γ	γ			
1	5	0.60	1.8	2.1	1.2
	100	0.14	0.022	0.007	0.32
2	5	0.45	1.7	1.0	0.59
	100	0.062	0.017	0.003	0.18
5	10	0.87	1.9	1.7	0.90
	100	0.068	0.019	0.008	0.42
6	10	1.1	2.4	2.1	0.88
	50	0.77	0.80	0.35	0.44
	100	0.10	0.036	0.011	0.33

* Each value is the average of 4 or 5 results obtained from as many rats (see Table 2)
† Specific activity refers to the percentage of the injected I^{131} per unit weight of chemically measured iodine (I^{127}).

radioactivity recovered in the diiodotyrosine-like fraction of the gland by the gamma of I^{127} injected. For example, in experiment 2 (Tables 2 and 3), 9 per cent of the injected I^{131} was recovered in the diiodotyrosine fraction of the gland of the rats that were injected with 5γ of I^{127}; hence 0.09×5 or 0.45γ of the injected I^{127} had been incorporated into diiodotyrosine in the gland.

In Table 3 are recorded the average values obtained as described above for the diiodotyrosine synthesized by the thyroid glands of rats that were injected with either 5, 10, 50, or 100γ of I^{127} as KI. It is clear that in each experiment the gland synthesized far more diiodotyrosine when 5 or 10γ of I^{127} were injected than when 10 to 20 times these amounts of I^{127} were administered. These results therefore provide convincing evidence that excessive iodide exerts its inhibitory effects upon the reaction leading to the iodination of the phenolic group of tyrosine. This is in agreement with the *in vitro* observations of Morton *et al.* (1944) on surviving sheep-thyroid slices and with

[2] Since the diet contained 0.3γ of iodine per gram, only negligible amounts of iodine could have been absorbed during the period of study. The rats were not fed during this period.

those of Li (1942), who showed from kinetic considerations that inorganic iodide inhibits the iodination of tyrosine in the test tube.

2. The Effect of Various Amounts of Injected Iodide Upon the Conversion of Diiodotyrosine to Thyroxine in the Thyroid Gland

The calculation made above for the injected I^{131} converted to diiodotyrosine during the 4-hour period of observation is valid only because the injected inorganic iodide is not appreciably diluted by the amounts of inorganic iodide present in the gland and plasma before the injection. The calculation for the amount of thyroxine synthesized is complicated by the fact that the newly formed diiodotyrosine mixes with a large amount of diiodotyrosine already present in the gland. For example, in experiment 1 the 0.6γ of newly formed diiodotyrosine (i.e., during the 4 hours after the injection of labeled iodide) was diluted appreciably by the diiodotyrosine (Table 2). Hence a calculation similar to that described in the preceding section for the amount of *injected* labeled I^{127} converted to thyroxine in the gland would be meaningless as a measure of the *total amount* of thyroxine formed from diiodotyrosine during the interval studied. In other words, a calculation for the amount of injected I^{127} converted to thyroxine would provide a minimal value for the amount of diiodotyrosine converted to thyroxine, because it does not take into account the dilution of the newly formed diiodotyrosine by the diiodotyrosine already present in the gland.

Hevesy (1938) has pointed out that the ratio,

$$\frac{\text{specific activity of compound A}}{\text{specific activity of the precursor of compound A}}$$

can be used to compare the turnover of compound A in a single tissue under 2 different conditions. Many factors may influence the specific activity of the compound or that of its precursor, but the ratio of their specific activities is independent of changes in permeability, distribution of the labeling agent, etc., and varies only with the turnover rate of compound A. A recent application of the use of relative specific activities as an index of turnover was made by Zilversmit *et al.* (1948).

We have made use of relative specific activities

$$\left(\text{in this case, } \frac{\text{specific activity of thyroxine}}{\text{specific activity of diiodotyrosine}}\right)$$

to compare the conversion of diiodotyrosine to thyroxine under the 2 experimental conditions studied here, namely high- and low-iodide injections. The values for the ratios are recorded in Table 3. If the rate of conversion of diiodotyrosine to thyroxine had remained the same under both conditions, then this ratio would also have remained the same in the presence of low- and high-iodine injections. Table 3 shows, however, that a 2-to-4-fold decrease in the ratio occurred when

the amount of I^{127} was increased from 5 or 10γ to 100γ. This indicates that the conversion of diiodotyrosine to thyroxine was considerably slower after the injection of the high amounts of iodine. Whether this is due to a specific action of the excess iodide on this step or whether the interference in the conversion of diiodotyrosine to thyroxine results simply from the smaller amounts of diiodotyrosine formed can not be stated with certainty.

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SUMMARY

The effects of the introduction of excessive amounts of inorganic iodide upon 1) the incorporation of iodide into diiodotyrosine and 2) the conversion of diiodotyrosine to thyroxine in the thyroid gland were investigated with the aid of I^{131}.

Since the amounts of inorganic iodide injected were large with respect to those present in the gland and plasma before the injection, it was possible to calculate the amounts of newly formed diiodotyrosine in the gland from the proportions of the injected I^{131} incorporated into this fraction. Far more diiodotyrosine was synthesized by the rat thyroid when 5 or 10γ of iodide were injected than when 10 or 20 times these amounts were administered.

In order to compare the conversion of diiodotyrosine to thyroxine in rats that received high- and low-iodide injections, use was made of the ratio of the specific activity of thyroxine to that of its precursor, namely diiodotyrosine. A 2-to-4-fold decrease in this ratio was observed when the amount of I^- injected was increased from 5 or 10γ to 100γ. This indicates that the conversion of diiodotyrosine to thyroxine was considerably slower when the higher amounts of iodine were injected.

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