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The Effect of Thiouracil on the Metabolism of L-Thyroxine

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Thiouracil not only blocks the formation of thyroxine in the thyroid gland (Franklin, Lerner & Chaikoff, 1944; Astwood, 1945), but also appears to alter its peripheral utilization. Andik, Balogh & Donhoffer (1949) found that the action of thyroxine was inhibited in thyroidectomized rats which had been given methylthiouracil for 4 weeks. Stasilli, Kroc & Edlin (1960) found that the administration of thyroxine to rats which had been given thiouracil for 6–8 weeks produced only 7% of the calorogenic action observed in untreated rats.

The decreased excretion of radioactive iodide in urine from administered L-[3'- or 5'-¹³¹I]thyroxine, which has been found in rats given propylthiouracil, indicates decreased deiodination of the phenolic ring of thyroxine. This is accompanied by an increased secretion of ¹³¹I in the bile (Van Arsdel & Williams, 1956) and excretion in faeces (Hogness, Wong & Williams, 1954; Jones & Van Middlesworth, 1960; Escobar del Rey & Morreale de Escobar, 1961).

In the metabolism of L-[3'- or 5'-¹³¹I]thyroxine in the dog partial deiodination occurs by removal of one iodine atom from the 3'- or 5'-position of the phenolic ring with the excretion of radioactive iodide in the urine and the formation of calorogenic 3,3',5-tri-iodothyronine. Small amounts of conjugated tri-iodothyronine are excreted in the bile.

Partial deiodination occurs in the dog also by removal of one iodine atom from the 3- or 5-position with the formation of non-calorogenic 3,3',5'-tri-iodothyronine (Flock, Bollman, Grindlay & Stobie, 1961).

The quantity of 3,3',5'-tri-iodothyronine glucuronide excreted in the bile or in the urine after removal of the liver is sufficiently great to indicate that this type of deiodination is an important mechanism in the inactivation of L-thyroxine. Partial deiodination proceeds further with the formation of 3,3'-di-iodothyronine which is also non-calorogenic.

A search has been made for these partially deiodinated metabolites in the rat. In addition the effect of thiouracil on the peripheral utilization of thyroxine has been studied.

A preliminary account of this work has been published (Flock & Bollman, 1961).

METHODS

Rats of the Sprague-Dawley strain, weighing 200–340 g. and maintained on Friskie dog food, were used. Some rats were given powdered Friskie dog food, to which thiouracil (0.1% or 0.3%, w/w) had been added, for 16–56 days. A biliary fistula was made in each rat, under ether anaesthesia, by cannulation of the bile duct 1 cm. below the

liver with polyethylene tubing. Cystostomy was done by insertion of a poly(vinyl chloride) tube, flared at the end, into the tip of the urinary bladder. The bladder was then ligated at the base. L-[3'- or 5'-¹³¹I]Thyroxine was administered by single intravenous injection in doses of 13–52 μ C and 0.7–2.8 μ g./100 g. body weight to rats within 1 hr. after operation in initial experiments, but in most experiments 16–26 hr. after operation. Sodium chloride (0.9%) was administered continuously at the rate of 1.25 ml./hr. through polyethylene tubing into the tail vein.

Bile and urine were collected continuously in chilled tubes for 6 hr. and then for the next 18 hr. after the injection of the L-[¹³¹I]thyroxine. Blood was collected at the end of this 24 hr. period. The radioactivity of bile, urine and plasma was measured in a well-type sodium iodide (thallium-activated) scintillation counter. Extracts of bile, urine and plasma were made with 4 vol. of methanol-acetone (1:1, v/v) (Flock, Bollman & Grindlay, 1960). Dried extracts were dissolved in 50% (v/v) ethanol and portions of this containing approx. 0.03 μ C were chromatographed on sheets (10 in. $\times$ 12 in.) of Whatman no. 3MM filter paper by development first in butanol-dioxan-2N-ammonia (4:1:5, by vol.) for 20 hr. and then at right angles in 2-methylbutan-2-ol saturated with aq. 2N-ammonia for 2 days (Flock *et al.* 1960). Radioautographs of the chromatograms were made on No-Screen X-ray film (Kodak). Markers run with the unknowns on the chromatograms included iodide (which was located by spraying with palladium chloride), thyroxine, 3,3',5-tri-iodothyronine, 3,3',5'-tri-iodothyronine, 3,3'-di-iodothyronine and tetra-iodothyroacetic acid (which were located by spraying with diazotized sulphanilic acid). The radioactive spots on the two-dimensional chromatograms were cut out, and the radioactivity was measured in the scintillation counter.

Portions of the 50% ethanolic solution of the extracted bile containing 0.6 μ C of ¹³¹I were applied as a band to strips (1.5 in. $\times$ 22 in.) of Whatman no. 3 MM filter paper. The strips were developed for 20–24 hr. in butanol-dioxan-2N-ammonia. Radioautographs were made on film (Eastman Linograph Ortho film). Radioactive bands were eluted with 0.02N-ammonia. Usually the regions selected for elution contained more than one band; thus one heavy band and two or three light bands were included in the region with R_F

approx. 0.25–0.38 which was to be subsequently incubated with β -glucuronidase. The region with R_F 0.38–0.50 included at least two light bands in addition to the iodide band and these iodine compounds were subsequently incubated with arylsulphatase (Mylase P; Nutritional Biochemicals Corp.). A portion of this region was also boiled with N-hydrochloric acid for 20 min. The hydrolytic products from all three procedures were then separated on two-dimensional chromatograms, and the percentage of the ¹³¹I in each product was determined.

RESULTS

The urinary output of ¹³¹I after injection of L-[¹³¹I]thyroxine into rats which had been given a diet containing 0.1% of thiouracil for 16–22 days was similar to that of control rats (Table 1). However, in two rats which received a dose of thiouracil amounting to 0.3% of the diet for 22 days, and in other rats which were given diets containing either 0.1% or 0.3% of thiouracil for 28–56 days, the urinary excretion of ¹³¹I was much less. Since ¹³¹I is excreted in urine chiefly as iodide, the decreased output indicates decreased deiodination from the 3' or 5'-position of L-[3'- or 5'-¹³¹I]thyroxine.

The output of ¹³¹I in the bile of rats given the diet containing 0.1% of thiouracil was greater than that in control rats. The output in the bile of rats given the diet containing 0.3% of thiouracil was generally not increased above that in the control rats in spite of the decreased urinary excretion of ¹³¹I.

Most of the radioactivity in the bile appeared in the glucuronide region of the chromatograms (Table 2), both in the control rats and in those treated with thiouracil. The composition of ¹³¹I compounds in bile excreted during the first 6 hr. and the next 18 hr. after the injection of thyroxine was similar. The bile of the thiouracil-treated rats contained about half as much ¹³¹I in the iodide

Table 1. ¹³¹I in urine, bile and plasma of rats

Results are expressed as means $\pm$ s.e. The numbers in parentheses refer to the numbers of rats where these differ from those of the first column. The plasma value was calculated on the basis that 3% of the body weight is plasma.

No. of rats	Thiouracil of diet (%)	Duration of treatment (days)	¹³¹ I (% of dose)				
			In bile		In urine		In plasma at 24 hr.
			At 0–6 hr.	At 6–24 hr.	At 0–6 hr.	At 6–24 hr.	
22 (controls)	0		10.2 $\pm$ 0.3	13.6 $\pm$ 0.6	6.8 $\pm$ 0.8 (21)	22.8 $\pm$ 1.5 (21)	4.2 $\pm$ 0.2 (21)
8	0.1	16–22	15.6 $\pm$ 0.7	16.1 $\pm$ 0.5	8.4 $\pm$ 1.0	19.1 $\pm$ 0.6	4.5 $\pm$ 0.1
2	0.3	22	8.2	13.4	4.4	4.6	4.9
3	0.1	28–30	13.7	28.6	1.1	7.6	4.1
8	0.3	28–30	6.8 $\pm$ 1.2	15.3 $\pm$ 0.4 (4)	2.4 $\pm$ 0.6 (4)	7.1 $\pm$ 1.9 (4)	6.3 $\pm$ 0.3 (4)
8	0.1	42	16.7 $\pm$ 1.9	17.8 $\pm$ 2.3	5.1 $\pm$ 1.3 (7)	9.8 $\pm$ 3.5	5.3 $\pm$ 0.4 (6)
4	0.3	42	9.3 $\pm$ 1.4	13.7 $\pm$ 0.6	2.4 $\pm$ 0.8	5.6 $\pm$ 1.2 (4)	4.9 $\pm$ 0.3 (4)
4	0.1	56	9.8 $\pm$ 2.4	17.7 $\pm$ 2.5	3.5 $\pm$ 1.2	8.1 $\pm$ 2.1	5.3 $\pm$ 0.7

region and about twice as much in the sulphate region as the bile of the control rats.

When the ^{131}I compounds in the glucuronide region were incubated with β -glucuronidase, the chief hydrolytic products were thyroxine and 3,3',5'-tri-iodothyronine. There were smaller amounts of 3,3',5-tri-iodothyronine and tetraiodothyroacetic acid (Table 3). A shift in the relative

amounts of thyroxine and 3,3',5'-tri-iodothyronine glucuronides excreted in the bile was found as early as 16–22 days after treatment with thiouracil was begun: about 50 % of the ^{131}I of the hydrolysate was in thyroxine, in contrast with 63 % in normal rats. This shift became more pronounced as the period of feeding with thiouracil was lengthened. In the bile of normal rats, about 6 % of the glucurono

Table 2. *Distribution of ^{131}I in bile of rats*

Results are expressed as means $\pm$ S.E.

No. of rats	Thiouracil of diet (%)	Duration of treatment (days)	Biliary ^{131}I (%)				
			Free thyroxine	Free tetraiodo-thyroacetic acid	Iodide	Glucuronide region	Sulphate region
			Bile (0–6 hr.)				
22 (controls)	0		4.9 $\pm$ 0.2	1.8 $\pm$ 0.1	5.8 $\pm$ 0.3	67.3 $\pm$ 0.1	6.4 $\pm$ 0.3
8	0.1	16–22	4.0 $\pm$ 0.3	2.0 $\pm$ 0.1	3.8 $\pm$ 0.2	70.0 $\pm$ 0.9	10.3 $\pm$ 1.3
2	0.3	22	4.2	2.0	3.9	68.4	10.7
3	0.1	28–30	2.8	1.1	3.5	70.7	11.3
8	0.3	28–30	3.9 $\pm$ 0.5	1.3 $\pm$ 0.3	3.3 $\pm$ 0.8	71.5 $\pm$ 1.5	10.1 $\pm$ 0.7
8	0.1	42	4.8 $\pm$ 0.6	1.6 $\pm$ 0.2	2.8 $\pm$ 0.1	68.0 $\pm$ 0.8	13.8 $\pm$ 1.0
4	0.3	42	4.4 $\pm$ 0.4	1.6 $\pm$ 0.2	3.5 $\pm$ 1.4	67.6 $\pm$ 1.1	12.9 $\pm$ 0.6
4	0.1	56	4.4 $\pm$ 0.8	2.2 $\pm$ 0.4	3.0 $\pm$ 0.7	70.1 $\pm$ 0.5	11.4 $\pm$ 2.8
Bile (6–24 hr.)							
18 (controls)	0		4.6 $\pm$ 0.2	1.8 $\pm$ 0.2	8.0 $\pm$ 0.7	67.9 $\pm$ 0.1	6.2 $\pm$ 0.8
8	0.1	16–22	4.7 $\pm$ 0.2	1.7 $\pm$ 0.2	3.2 $\pm$ 0.5	71.5 $\pm$ 0.8	8.4 $\pm$ 0.8
2	0.3	22	4.4	3.0	3.3	73.4	7.1
3	0.1	28–30	3.1	1.0	2.2	68.7	15.9
8	0.3	28–30	3.4 $\pm$ 0.5	1.3 $\pm$ 0.3	2.6 $\pm$ 0.3	73.2 $\pm$ 2.0	7.6 $\pm$ 1.2
8	0.1	42	3.6 $\pm$ 0.4	1.4 $\pm$ 0.1	3.9 $\pm$ 0.4	69.0 $\pm$ 0.4	12.2 $\pm$ 1.9
4	0.3	42	4.4 $\pm$ 0.8	1.6 $\pm$ 0.3	2.9 $\pm$ 0.4	72.3 $\pm$ 2.4	10.8 $\pm$ 3.1
4	0.1	56	5.0 $\pm$ 1.0	1.1	2.6 $\pm$ 0.2	70.1 $\pm$ 3.4	11.5 $\pm$ 3.4

Table 3. *Distribution of ^{131}I in hydrolytic products from glucuronides of bile*

Results are expressed as means $\pm$ S.E. Values for control rats for the 0–6 hr. and the 6–18 hr. collections of bile were similar and have been combined. The eluates of the glucuronide region of the bile of the second 4 rats under the 6–24 hr. collection were combined before hydrolysis and thus the values given for these animals represent the averages for these rats.

No. of rats	Thiouracil of diet (%)	Duration of treatment (days)	Glucuronide (%)			
			Of 3,3',5-tri-iodothyronine	Of tetraiodo-thyroacetic acid	Of thyroxine	Of 3,3',5'-tri-iodothyronine
			Bile (0–6 hr.)			
23 (controls)	0		8.6 $\pm$ 0.4	4.8 $\pm$ 0.5	62.9 $\pm$ 1.0	6.5 $\pm$ 0.2
8	0.1	16–22	5.0 $\pm$ 0.1	7.0 $\pm$ 0.2	49.8 $\pm$ 3.7	9.6 $\pm$ 0.4
2	0.3	22	5.8	9.2	49.8	17.6
3	0.1	28–30	2.0	5.8	32.8	37.7
7	0.1	42	4.4 $\pm$ 0.6	7.5 $\pm$ 0.5	50.9 $\pm$ 2.3	24.2 $\pm$ 2.0
4	0.3	42	4.9 $\pm$ 0.3	7.1 $\pm$ 0.6	43.1 $\pm$ 4.8	24.0 $\pm$ 5.5
4	0.1	56	4.6 $\pm$ 0.8	5.7 $\pm$ 1.0	34.6 $\pm$ 5.1	15.6 $\pm$ 5.8
Bile (6–24 hr.)						
8	0.1	16–22	4.8 $\pm$ 0.2	4.4	50.3 $\pm$ 2.5	6.9 $\pm$ 0.9
2	0.3	22	5.4	7.9	56.3	10.0
3	0.1	28–30	3.6	8.4	42.6	32.0
4	0.3	42	5.6 $\pm$ 0.4	7.7 $\pm$ 0.5	55.0 $\pm$ 2.1	10.3 $\pm$ 2.1
4	0.3	42	6.8	9.4	55.0	15.0
2	0.1	56	3.2	7.8	38.3	32.2

conjugates was found to be the glucuronide of 3,3',5'-tri-iodothyronine; in rats treated with thiouracil for 28 days, values of 10–38% were found. These values for 3,3',5'-tri-iodothyronine glucuronide in the thiouracil-treated rats must be regarded as approximations owing to difficulties in the separation of thyroxine and 3,3',5'-tri-iodothyronine on some of the chromatograms.

When the sulphate region was incubated with

buffer only, hydrolysis did not occur. When the sulphate region was incubated with arylsulphatase, less than half of the conjugates were hydrolysed. The chief product was 3,3'-di-iodothyronine, with smaller amounts of thyroxine and 3,3',5'-tri-iodothyronine (Table 4). Most of the sulphate region was hydrolysed when it was boiled with N-hydrochloric acid. The additional ^{131}I liberated by acid was found chiefly in thyroxine.

Table 4. *Distribution of ^{131}I in hydrolytic products of the sulphate region of chromatograms of bile*

Results are expressed as percentages of the ^{131}I in the sulphate region, and are means $\pm$ s.e. for 6 and 18 hr. collections of bile. The numbers in parentheses are the numbers of rats. The eluates of the sulphate region of chromatograms of bile from 4 rats were combined before hydrolysis on three occasions; those from 12 other bile specimens were hydrolysed individually. The eluates from this region in groups of 2, 3 and 4 thiouracil-treated rats were combined before hydrolysis. The values given represent the average of all groups of thiouracil-treated rats (Table 2).

Hydrolytic agent		3,3',5-Tri-iodothyronine	Thyroxine	3,3'-Di-iodothyronine	Unhydrolysed
Mylase P	No thiouracil (control)	8.8 $\pm$ 0.4 (24)	15.8 $\pm$ 1.6 (24)	8.3 $\pm$ 0.5 (24)	65.6 $\pm$ 1.8 (24)
N-HCl	No thiouracil (control)	12.7 $\pm$ 0.8 (20)	53.5 $\pm$ 1.8 (24)	11.9 $\pm$ 2.9 (20)	15.6 $\pm$ 1.8 (24)
Mylase P	Thiouracil	9.0 $\pm$ 2.2 (37)	8.3 $\pm$ 0.5 (37)	19.5 $\pm$ 2.0 (37)	57.5 $\pm$ 1.7 (37)
N-HCl	Thiouracil	11.7 $\pm$ 0.9 (37)	41.2 $\pm$ 2.9 (37)	25.7 $\pm$ 2.4 (37)	13.5 $\pm$ 1.4 (37)

Table 5. *Conjugated ^{131}I compounds in the bile of rats after the injection of thyroxine*

Average values are given for ^{131}I compounds excreted in bile during the first 6 and next 18 hr. after injection of thyroxine (Tables 2 and 3). Percentages in parentheses are the results obtained after acid hydrolysis. The increased amount of thyroxine liberated after acid hydrolysis indicates that an unidentified conjugate of thyroxine was excreted in bile in addition to the sulpho conjugate (Flock *et al.* 1961).

^{131}I compounds	Biliary ^{131}I (%)		
	Control rats	Thiouracil-treated rats	Dogs
Glucuronide region	68	70	43
Thyroxine glucuronide	43	31	22
3,3',5-Tri-iodothyronine glucuronide	6	3	3
3,3',5'-Tri-iodothyronine glucuronide	4	15	10
Tetraiodothyroacetic acid glucuronide	3	5	4
Sulphate region	6	11	18
Thyroxine sulphate	0.9 (3.2)	0.9 (4.5)	Trace
3,3',5-Tri-iodothyronine sulphate	0.5 (0.8)	1.0 (1.3)	Trace
3,3',5'-Tri-iodothyronine sulphate	—	—	1
3,3'-Di-iodothyronine sulphate	0.5 (0.7)	2.1 (2.8)	6
Unhydrolysed	4.0 (1.0)	6 (1.5)	6

Table 6. *Distribution of ^{131}I in urine and plasma of rats*

Results are expressed as means $\pm$ s.e. The numbers in parentheses refer to the numbers of rats where these differ from those of the first column.

No. of rats	Thiouracil of diet (%)	Duration of treatment (days)	Urinary ^{131}I (%)				Plasma ^{131}I (%) in 24 hr. sample	
			In 0–6 hr. collection		In 6–24 hr. collection		Iodide	Thyroxine
			Iodide	Thyroxine	Iodide	Thyroxine		
12 (controls)	None		90.8 $\pm$ 2.6	7.2 $\pm$ 1.9	97.4 $\pm$ 0.4 (5)	2.1 $\pm$ 0.7 (5)	15.3 $\pm$ 3.2 (10)	77.8 $\pm$ 3.8 (10)
8	0.1	16–22	94.4 $\pm$ 1.1	3.6 $\pm$ 0.7	96.8 $\pm$ 0.9	3.1 $\pm$ 0.9	8.4 $\pm$ 1.7	83.0 $\pm$ 2.4
2	0.3	22	87.5	7.5	87.2	6.3	13.5	72.8
3	0.1	28–30	60.0	27.3	69.7	15.1	11.1	73.0
4	0.3	28–30	85.2 $\pm$ 3.8	6.9 $\pm$ 2.7	90.8 $\pm$ 2.1	4.0 $\pm$ 1.2	1.4 $\pm$ 0.4	93.6 $\pm$ 0.6
5	0.1	42	75.4 $\pm$ 6.7	12.1 $\pm$ 2.4	80.7 $\pm$ 7.1	10.4 $\pm$ 6.0	13.3 $\pm$ 1.9	75.2 $\pm$ 3.9
4	0.3	42	81.3	10.8	78.8	13.7	9.5 $\pm$ 1.4	81.5 $\pm$ 0.4
4	0.1	56	89.6 (3)	5.7 $\pm$ 0.8	95.2	—	7.0 $\pm$ 1.6	82.8 $\pm$ 4.4

The relative amounts of the metabolites of thyroxine which are excreted in bile by the control rat, the thiouracil-treated rat and the untreated dog (Flock *et al.* 1961) are given in Table 5.

Chromatographic studies of the urine of the control rat showed that a small amount of free thyroxine was excreted during the first 6 hr. after the injection of thyroxine but that most of the ^{131}I was excreted as iodide (Table 6). The rats treated with thiouracil for 22–56 days also excreted small amounts of free thyroxine in the urine but much less radioactive iodide than the control rats (Tables 1 and 6). The major ^{131}I compound in the plasma of control and thiouracil-treated rats 24 hr. after the administration of L- ^{131}I thyroxine was free thyroxine. Small but variable amounts of iodide were also present.

DISCUSSION

A large amount of thyroxine was excreted in the bile of the normal rat after conjugation with glucuronic acid, as was shown by Taurog, Briggs & Chaikoff (1952). A small amount of 3,3',5'-triiodothyronine glucuronide was also excreted in the bile. It is not likely that this is all from preformed 3,3',5'-triiodothyronine in the commercial radioactive thyroxine, although chromatograms of this do show 4–11 % of the radioactivity in the 3,3',5'-triiodothyronine spot. However, when 3,3',5'- ^{131}I triiodothyronine is injected, a variety of metabolites are formed and only part of the 3,3',5'-triiodothyronine is excreted in bile as glucuronide. The observation that only a small amount of thyroxine sulphate is excreted in the bile of the normal rat is in agreement with observations by Roche, Michel, Closon & Michel (1959) and Closon (1959). None of the acid-labile conjugate of 3,3',5'-triiodothyronine was found in the bile of the rat. Marked differences were found in the distribution of conjugated ^{131}I compounds in the bile between the rat and the dog. Less of the biliary ^{131}I was found in the glucuronide region and more in the sulphate region in the dog than in the rat. There was more thyroxine glucuronide and less 3,3',5'-triiodothyronine glucuronide in rat bile than in dog bile. There was more thyroxine sulphate and less 3,3'-diiodothyronine sulphate in rat bile than in dog bile. Thus examination of the conjugated ^{131}I compounds in the bile of the normal rat shows that partial deiodination of thyroxine by removal of an iodine atom from the 3- or 5-position appears to be of less importance than in the dog.

The decreased output of radioactive iodide from administered [3'- or 5'- ^{131}I]thyroxine in the urine of rats given thiouracil amounting to 0.1 % of the diet for at least 28 days or to 0.3 % of the diet for 22 days or longer indicates decreased deiodination from the 3'- or 5'-position. The increased output of ^{131}I com-

pounds in the bile of rats given the diet containing 0.1 % of thiouracil reflects increased conjugation, particularly with glucuronic acid, but also with sulphuric acid and an unidentified substance. This increase was obliterated when the dosage of thiouracil was increased to 0.3 % of the diet. The increased proportion of 3,3',5'-triiodothyronine and 3,3'-diiodothyronine in the conjugates of the bile of rats given either the 0.1 % or 0.3 % dose of thiouracil indicates increased deiodination from the 3- or 5-position of thyroxine in the thiouracil-treated rats. It would be difficult to determine which of these effects of thiouracil is the primary one. These alterations in the peripheral utilization of thyroxine, and particularly the increased formation of the non-calorigenic products, 3,3',5'-triiodothyronine and 3,3'-diiodothyronine, most likely account, at least in part, for the marked reduction in calorogenic activity of administered thyroxine observed by Stasilli *et al.* (1960) in thiouracil-treated rats. They found an increased excretion of calorogenic material in the faeces of these rats: this might indicate decreased reabsorption of thyroxine after excretion as thyroxine glucuronide in the bile compared with that found in normal rats.

The administration of one or two doses of butyl hydroxydi-iodobenzoate to rats in the 24 hr. period before the administration of thyroxine produced alterations in the metabolism of this substance which were similar to, but of much greater magnitude than, those produced by thiouracil. Thus the administration of butyl hydroxydi-iodobenzoate produced a decreased output of radioactive iodide in the urine, a large increase in biliary ^{131}I (Flock & Bollman, 1960) and an increase in the proportion of 3,3',5'-triiodothyronine in the bile. It is not apparent why so much more time was required for the production of these alterations in the peripheral utilization of thyroxine by thiouracil than by butyl hydroxydi-iodobenzoate. Possibly the thiouracil effect is indirect and simply reflects the hypothyroidism that develops gradually.

No information is available concerning how thiouracil might increase deiodination from the unlabelled 3- or 5-position of thyroxine or decrease deiodination from the ^{131}I -labelled 3'- or 5'-position. Less 3,3',5'-triiodothyronine was formed by kidney slices from thyroxine when methylthiouracil was added to the incubation mixture according to Cruchaud, Vannotti, Mahaim & Deckelmann (1955), but thiouracil had no effect on deiodination of thyroxine by rat-liver extract according to MacLagan & Reid (1957). L. E. Braverman & S. H. Ingbar (unpublished work) found that degradation of thyroxine by rat-kidney slices *in vitro* was markedly decreased in propylthiouracil- or thiouracil-treated animals: the products of deiodination (iodide and iodoprotein) were decreased.

Differences have been found previously in the metabolic products of thyroxine in hepatic and extrahepatic tissues of the dog (Flock & Bollman, 1960). Thus dogs with livers readily deiodinate thyroxine in the 3'- or 5'-positions and excrete large amounts of radioactive iodide in the urine. Dogs without livers deiodinate thyroxine from the 3- and 5-positions to a greater extent than from the 3'- and 5'-positions. Conjugates of 3,3',5'-tri-iodothyronine and 3,3'-di-iodothyronine accumulate in plasma and urine of the hepatectomized dog, but only small amounts of radioactive iodide are excreted in the urine. Possibly prolonged feeding of thiouracil in the rat causes inhibition of deiodination of thyroxine by the liver, with subsequent accumulation of partially deiodinated products of thyroxine formed by extrahepatic tissues.

SUMMARY

1. The rat conjugates thyroxine chiefly with glucuronic acid and to a very small extent also with sulphuric acid and with an unidentified substance.

2. After the administration of radioactive thyroxine the rat excretes larger amounts of thyroxine glucuronide and smaller amounts of 3,3',5'-tri-iodothyronine glucuronide in the bile than the dog does.

3. Previous studies showing that deiodination of thyroxine from the 3'- or 5'-position is decreased in rats after receiving thiouracil have been confirmed.

4. Deiodination from the 3- or 5-position of thyroxine is increased in rats given a diet containing 0.1% of thiouracil for 28 days or longer. This may account in part for the marked reduction in calorogenic activity of thyroxine administered to thiouracil-treated rats.

The L-thyroxine, L-tri-iodothyronine and tetraiodothyroacetic acid used as markers on the chromatograms

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The Sodium-Stimulated Adenosine-Triphosphatase Activity and other Properties of Cerebral Microsomal Fractions and Subfractions

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The metal activation and the likely complexity of cerebral enzymes forming inorganic phosphate and adenosine diphosphate from adenosine triphosphate were examined by Gore (1952) and by Lowry, Roberts, Wu, Hixon & Crawford (1954).

Subsequently, acceleration by Na^+ ions was found to distinguish the adenosine-triphosphatase activity of cerebral microsomal fractions from that of cerebral mitochondria (Deul & McIlwain, 1961a, b); both enzymes required potassium and magnesium