

Triiodothyronine in Feces of Rats Fed Low Iodine Diet (35514)

L. VAN MIDDLESWORTH, B. JASANI,¹ AND K. N. SINHA

*Department of Physiology and Biophysics, University of Tennessee,
Memphis, Tennessee 38103*

Heninger and Albright (1) have shown that the ratio T_3/T_4 is greater in the fecal excretion than in the thyroid or serum of rats fed a normal diet or a low iodide diet. Other authors (2, 3) have shown that T_3 is absorbed from the intestine more completely than T_4 , therefore differential absorption could not account for the high fecal T_3 excretion described by Heninger and Albright. In view of the recent (4) resurgence of interest in the importance of T_3 as a primary thyroid hormone and the possible conversion of T_4 to T_3 *in vivo*, we have confirmed and extended the results of Heninger and Albright. In addition we have studied the availability of T_3 and T_4 from bile and we have studied the T_3/T_4 in feces after acute injection of ^{125}I into rats maintained on a low iodide diet.

Methods. Four Long-Evans² male rats were maintained on Remington diet³ and distilled water for 1 year and they were fed ^{125}I -labeled Remington diet (initially 0.015 $\mu\text{Ci/g}$) for the final 100 days; they approached isotopic equilibrium between ^{125}I and natural ^{127}I (5).

A second group of female Wistar⁴ rats was maintained similar to the animals fed low iodine diet but these were fed a supplement of 0.14 μg of iodine/g of Remington diet, which resulted in 10 times more iodine intake than occurred on the low iodine diet alone.

¹ Present address, University of Birmingham, Birmingham, England.

² Long-Evans hooded rats obtained from Rockland Farms, Inc., Gilbertsville, Pennsylvania.

³ Remington diet pellets obtained from General Biochemicals Corp., Chagrin Falls, Ohio; powder (used for chronic label) obtained from Nutritional Biochemical Co., Cleveland, Ohio. The powder contained 0.014 to 0.03 μg of iodine/g.

⁴ Wistar albino rats obtained from Harland Industries, Inc., Cumberland, Indiana.

This diet was also maintained continuously labeled with ^{125}I (initially 0.015 $\mu\text{Ci/g}$) for 100 days before the fecal collections were obtained.

Two additional Long-Evans² male rats were maintained on low iodine diet for 1 year to develop severe iodine depletion. They were then injected intraperitoneally with a single dose of 50 μCi of ^{125}I and killed after 12 hr. These rats had intensely labeled iodide pools in the thyroid, plasma, bile, and feces.

Fresh fecal pellets (100 to 200 mg) were obtained for analysis, the total radioactivity was determined and the homogenized pellets were extracted with 0.5 to 1.0 ml of butanol-acetic acid-water (15:2:3, v/v/v) containing 20 mg of PTU/ml in a glass homogenizer. The first butanol extract contained 50% of the radioactivity and this was analyzed by paper chromatography. An additional 40% of the radioactivity was extracted by repeated washings with the butanol solvent but this residue was not analyzed by chromatography. Ten percent of the fecal iodine was unextractable from the solid feces.

Ascending paper chromatography was performed using 3MM Whatman filter paper in the following solvents (6, 7): (A) butanol-ethanol-0.5 *N* ammonia (5:1:2), (B) butanol-acetic acid-water (15:2:3); and a descending system of (C) tertiary amyl alcohol and 2 *N* ammonia (1:1). After 16 to 20 hr of chromatography, the filter paper was cut into 30 to 35 strips 1 cm wide and these were counted in the scintillation well counter. Chemical standards were tested separately on the paper chromatograms and stained with ceric arsenite solution as described previously (5). In the first experiment only solvents A and B were used and all the thyronines traveled together in solvent B; solvent C was most satisfactory for separation of T_4 and T_3 as well as the acetic acid analogs.

TABLE I. Body and Thyroid Weight of Rats Fed Low Iodine Diet for 1 Year.^a

Rat no.	Dietary iodine (μg of I/g of diet)	Body wt (g)	Thyroid wt (mg)
1	0.015	606	273
2	0.015	600	466
3	0.015	560	487
4	0.015	526	330
5	0.15	304	30
6	0.15	525	33
7	0.15	326	31
8	0.02	440	363
9	0.02	400	170

^a Animals 1-4, 8, 9 were Long-Evans males, animals 5, 6, 7 were Wistar females.

After the larger dose of radioiodide, serum, bile, intestinal contents, and feces were tested for the composition of the ¹²⁵I-labeled compounds. To determine the thyroid hormones available from conjugated hormones in bile, 0.5 ml of the bile from the rats given one dose of ¹²⁵I was incubated with 50 mg of fresh feces from nonradioactive rats fed the low iodine diet. The mixture of bile and fecal material was incubated at 37° for 3 hr, centrifuged, and the supernatant solution was extracted with acid butanol and fractionated by paper chromatography using solvents A and C.

Results. The data of Table I confirm that large goiters developed in the male rats fed Remington diet for 1 year, but when 0.13 μg of iodide was added/g of diet, the thyroids were of normal size (in the female rats).

Fecal samples from rats fed low iodine diet had 90% of the fecal iodine extracted by butanol-acetic acid solvent. Table II shows that the predominant iodinated compound (58.5 to 68.3% of the extracted ¹²⁵I) traveled as triiodothyronine in solvent A, while 13.2 to 18.5% traveled as thyroxine and less than 5% as iodine. The data from rat nos. 5, 6, 7 in Table II show that a small iodide supplement to the diet resulted in thyroxine-like material being the major iodinated component of the feces.

Table II also shows that the serum of rats maintained on a low iodine diet contained only 4.4 to 13.6% of its iodine as triiodothyronine (T₃) and 25.6 to 43.3% as thyroxine (T₄) while 33 to 50% was present as origin material [possibly iodinated plasma proteins (8)]. In rats with the lowest iodine intake, the ratio of the concentration of T₃ to T₄ in the serum was the inverse of that in the fecal excretion. The rats which were fed the iodide supplemented diet (nos. 5, 6, 7) had relatively more triiodothyronine in the feces than they had in the serum but thyroxine was the major component of both serum and feces in the animals without goiter.

TABLE II.^a Iodinated Compounds in Feces and Plasma of Rats Fed ¹²⁵I-Labeled Diets for 100 Days (percentage of total fecal or plasma ¹²⁵I).^b

Rat no.	Feces			Serum			
	I	T ₄ (+ Tetrac)	T ₃ (+ Triac)	Origin	I	T ₄	T ₃
Low iodine diet							
1	3.3	13.6	62.4	48	4.9	32.2	7.5
2	4.3	18.5	58.5	39	4.5	43.3	8.8
3	1.9	13.2	68.3	51	4.4	38.5	4.4
4	2.0	13.3	67.9	33	6.6	25.6	13.6
Iodine supplemented diet							
5	4.7	65	20	1.8	13.1	75.2	0.7
6	4.5	63	20	3.2	17.4	60.1	5.7
7	6.5	59.7	17.4	2.6	11.8	71.4	5.6

^a Chromatographic solvent A.

^b T₄ = thyroxine; Tetrac = tetraiodothyroacetic acid; T₃ = triiodothyronine; and Triac = triiodothyroacetic acid.

TABLE III.^a Radioiodinated Compounds in Feces and Thyroids of 2 Rats Fed Low Iodine Diet 1 Year and Injected with ¹²⁵I 12 Hr (percentage of total fecal ¹²⁵I).

Rat no.	I	T ₄	Tetrac	T ₃	Triac	SF ^b
Feces						
8	2.5	6.2	3.3	59.7	2.8	3.0
9	6.6	3.5	2.0	55.2	5.6	14.0
Thyroids						
8	4.3	8.8		13.9		
9	5.7	4.5		10.2		

^a Chromatographic solvent C.^b SF = solvent front.TABLE IV.^a Radioiodinated Compounds in Bile Incubated with Feces and in Serum of Iodine-Depleted Rats, 12 hr After ¹²⁵I (percentage of total fecal or serum ¹²⁵I).

Rat no.	Bile after incubation with normal feces				Serum			
	Origin	I	T ₄	T ₃	Origin	I	T ₄	T ₃
8	2.9	2.8	12.0	71	13.6	5.7	75	12.6
9	4.6	4.4	9.0	76.9	20.0	14.2	46.8	12.8

^a Chromatographic solvent C.

Greater fractionation of iodinated compounds was achieved by the use of tertiary amyl alcohol-ammonia chromatographic system. Rat nos. 8 and 9 were studied with 200 times more radioactivity than animals 1 through 7 and this large dose of ¹²⁵I was given as a single injection. Tetraiodothyroacetic acid (Tetrac) and triiodothyroacetic acid (Triac) were identified in the fecal extracts of animals 8 and 9 (Table III). It is, therefore, likely that the triiodothyronine and thyroxine in Table II may have contained some quantity of Tetrac and Triac but the samples from animals 1 through 7 were not tested in solvent C.

Discussion. Rats equilibrated to ¹²⁵I-labeled diet, similar to those reported here, excreted (5) 0.2 μg of iodine/day, 75% of which was in the feces. Their thyroidal T₃/T₄ ratios were less than 2 but the present study shows the T₃/T₄ ratios in the feces were greater than 4.

The rats maintained on low iodine diet and injected with a single dose of ¹²⁵I showed T₃/T₄ ratios that increased from 1.8 in the thyroids to 7 in the bile (Table IV) and to 10.8 in the feces (although the serum had a T₃/T₄ ratio of only 0.2).

It is suggested that the progressive increase of T₃/T₄ that has been observed between storage in the thyroid and excretion in the feces may be due to a rapid conversion of T₄ to T₃ in the body or to T₃ synthesis in stimulated thyroids, independent of T₄ synthesis.

Summary. The present data, and those of Heninger and Albright (1), suggest that in rats fed a severely low iodine diet, T₃ may be released from the thyroid faster than T₄. The thyroids contained less than 2 times more T₃ than T₄ but the bile contained an average of 7 times more T₃ than T₄ and the feces averaged 4 to 11 times more T₃ than T₄. These data suggest that there may be a rapid conversion of T₄ to T₃ in the body or there may be T₃ synthesis in stimulated thyroids, independent of T₄ synthesis.

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