- Maschmann, E. (1938c). *Biochem. Z.* **300**, 89.
 Merrill, A. T. & Clark, W. M. (1928). *J. Bact.* **15**, 267.
 Michaelis, L. (1931). *Biochem. Z.* **234**, 139.
 Mueller, J. H. (1935). *J. Bact.* **29**, 383.
 Oakley, C. L., Warrack, G. H. & van Heyningen, W. E. (1946). *J. Path. Bact.* **58**, 229.
 Oakley, C. L., Warrack, G. H. & Warren, M. E. (1948). *J. Path. Bact.* **60**, 498.
 Pope, C. G. (1938). *Brit. J. exp. Path.* **19**, 245.
 Pope, C. G. (1939a). *Brit. J. exp. Path.* **20**, 132.
 Pope, C. G. (1939b). *Brit. J. exp. Path.* **20**, 201.
 van Heyningen, W. E. & Bidwell, E. (1948). *Biochem. J.* **42**, 130.
 Walbum, L. E. & Reymann, G. C. (1934). *J. Path. Bact.* **39**, 669.
 Weil, L. & Kocholaty, W. (1937). *Biochem. J.* **31**, 1255.
 Weil, L., Kocholaty, W. & Smith, L. D. (1939). *Biochem. J.* **33**, 893.

Absorption and Excretion of the Mono-sodium Salt of Thyroxine Labelled with Radioactive Iodine

By J. C. CLAYTON, AUDREY A. FREE, J. E. PAGE, G. F. SOMERS AND E. A. WOOLLETT
Research Division, Glaxo Laboratories Ltd., Greenford, Middlesex

(Received 7 December 1949)

There has been doubt in the past as to the efficacy of thyroxine administration by the oral route; some workers have even considered that thyroxine is inactive by mouth. A preference has been shown for the administration of dried glandular extracts, in which thyroxine is bound to protein or polypeptide. It was believed that thyroxine, being a relatively insoluble substance, was poorly absorbed from the gastro-intestinal tract (Harrington, 1933). However, Schittenhelm & Eisler (1932) and Barnes & Bueno (1933), using Thiry-Vella fistulas in dogs, were able to show that thyroxine was absorbed from the closed intestinal loops. There is some evidence that the form in which thyroxine is administered has a bearing on its activity after oral administration, and Thompson, Thompson & Dickie (1933) found that thyroxine by this route was most active as the sodium salt and that it can be used successfully in the treatment of myxoedema (cf. also Hart & MacLagan, 1950). Bailey, Bartlett & Folley (1949) have recently shown that sodium L-thyroxine by mouth also stimulates milk secretion in lactating cows.

Following the development of an improved synthesis of thyroxine (Borrows, Clayton & Hems, 1949; Clayton & Hems, 1950) it became of interest to obtain data on its absorption after oral dosage. We have prepared and administered the mono-sodium salt of DL-thyroxine, labelled with radioactive ^{131}I in the 3'- and 5'-positions, to rats and cats in small doses with a view to determining its distribution and excretion.

The earlier work on the fate of thyroxine in the body has been reviewed by Elmer (1938) and Leblond (1948). In most of the earlier experiments the distribution of thyroxine was studied either by chemical estimation of iodine or by biological assays which, in view of low tissue concentrations and lack of

specificity, were far from satisfactory. Such studies have been considerably facilitated by the advent of labelled thyroxine, which was first prepared by Joliot, Courier, Horeau & Süe (1944a; see also Horeau & Süe, 1945). These investigators carried out a few experiments on rabbits (Joliot *et al.* 1944a, b; Joliot, 1945). The first detailed studies are due, however, to Gross & Leblond (1947), who gave relatively large intravenous injections of labelled thyroxine to rats.

We have administered physiological doses by the oral, subcutaneous and intravenous routes to both rats and cats. A preliminary account of our results has been published (Clayton, Free, Page, Somers & Woollett, 1949).

EXPERIMENTAL

Preparation of labelled thyroxine

The labelled thyroxine was prepared from 3:5-diiodothyronine by a modification of the method of Clayton & Hems (1950). To a solution of ^{131}I (1 mc.) as iodide in buffered bisulphite solution (pH 7; approx. 1 ml.), 2.3 mg. of NaI were added. The solution, in a centrifuge tube, was overlaid with 1 ml. of ether, and one drop of N-HCl and excess 0.3% H_2O_2 solution (approx. 0.5 ml.) were added. After occasional shaking for 2 hr., the lower iodine-free layer was removed by means of a pipette. DL-3:5-Diiodothyronine (2 mg.) in 0.06 ml. of 33% (w/v) aqueous ethylamine solution was added and the mixture shaken carefully when the iodine colour was discharged. The ether layer was removed by blowing a stream of air over the surface, and the aqueous layer was diluted with 1 ml. of water and adjusted to between pH 4 and 5 by the addition of glacial acetic acid. The precipitated thyroxine was spun down in a centrifuge and washed with successive amounts (1 ml.) of distilled water until the washings contained less than 5% of non-thyroxine ^{131}I as demonstrated by *n*-butanol extraction of the aqueous washings (Blau, 1935). About 14 washings were

required. The thyroxine (about 2 mg.) was taken up in 10 ml. of 0.02N- Na_2CO_3 solution and assayed for thyroxine polarographically (cf. Borrows, Hems & Page, 1949). A 1 ml. portion of the solution was added to a mixture of 0.125 ml. of 2.0N- Na_2CO_3 , 1.0 ml. of redistilled isopropanol and 0.5 ml. of 10% (w/v) tetramethylammonium bromide. The mixture was made up to 5.0 ml. with distilled water and transferred to the cell of a recording polarograph (Cambridge Instrument Co.). After passing N_2 for 10 min., the sample was examined over the potential range -0.6 to -1.8 V. The heights of the first step and of the total steps were measured. Typical polarograms for thyroxine and diiodothyronine in a 20% (v/v) isopropanolic solution containing 1.0% (w/v) of tetramethylammonium bromide and 2.65% (w/v) Na_2CO_3 are shown in Fig. 1. Thyroxine gives three polarographic steps, the

Fig. 1. Polarograms for 6.0×10^{-4} M-thyroxine (A) and 6.0×10^{-4} M-diiodothyronine (B) in 20% (v/v) isopropanolic solution containing 1.0% (w/v) of tetramethylammonium bromide and 2.65% (w/v) of Na_2CO_3 . The dropping mercury electrode at a pressure of 55.5 cm. of mercury and on open circuit in 0.1N-KCl at 25° had a drop time of 3.13 sec.; the weight of Hg dropping per sec. was 1.82 mg.

first step being at -1.12 V. (measured against the saturated calomel electrode); and diiodothyronine two steps, the first step being at -1.18 V. It is thus not possible to determine thyroxine directly in the presence of diiodothyronine. However, the total height of the three thyroxine steps is 3.4 times that of the first thyroxine step, whereas the total height of the two diiodothyronine steps is only twice that of the first diiodothyronine step. A value of 3.4 for the ratio of the total step height to that of the first step will therefore indicate that the labelled sample contains at least 90% of thyroxine; any impurity is due to unchanged and unlabelled diiodothyronine. In these circumstances the height of the first step gives a measure of the amount of labelled thyroxine in the solution.

The specific activity of the labelled thyroxine was about 100 $\mu\text{C}/\text{mg}$.

In order to reduce the possibility of exchange between ^{131}I and ^{127}I , all labelled thyroxine solutions and extracts were kept at alkaline pH. Miller, Anderson, Madison & Salley (1944), and Frieden, Lipsett & Winzler (1948), have shown, and we have confirmed this observation, that exchange between iodide and either 3:5-diiodotyrosine or thyroxine proceeds rapidly at pH 5.0. Exchange was negligible at alkaline pH.

Biological activity of the labelled thyroxine

The biological activity of the material by the oral route was compared with a standard reference sample of pure sodium DL-thyroxine, using the method of Smith, Emmens & Parkes (1947) for determining thyroid activity. The method depends on the lowering of survival times of mice enclosed in jars after administration of thyroxine. Mice weighing approximately 20 g. were divided into two groups of 20. In one group every animal received 20 μg . of ordinary Na DL-thyroxine, in the other 20 μg . of labelled Na DL-thyroxine. The thyroxine was dissolved in 0.02N- Na_2CO_3 and given orally on alternate days for 1 week, so that each animal received in all 80 μg . A control group of 20 mice received Na_2CO_3 solution alone. The mean survival times are shown in Table 1. The results show that both thyroxine solutions were biologically active and that the activity of labelled thyroxine was not significantly different from that of the control.

Table 1. Survival times of mice after treatment with labelled or normal thyroxine

Group	Mean survival time (min.)	Standard error (min.)
Controls	140	± 6.8
Non-labelled monosodium thyroxine	96.6	± 3.9
Labelled monosodium thyroxine	104.5	± 3.5

Technique of animal experiments

Rat experiments. In our initial experiments, we examined the excretion and breakdown of labelled thyroxine by rats. Wistar strain rats of the same age and weight (70–100 g.) were placed in metabolism cages in which the urine and faeces could be collected separately. Throughout the experiments, the rats received a stock diet consisting of a paste prepared from whole-meal flour, dried milk and dried yeast, mixed with water. Paired rats in each cage were given single oral or subcutaneous doses of 1 mg. of labelled thyroxine/kg. of body weight, either as a solution of the monosodium salt in 0.02N- Na_2CO_3 or as a suspension. The thyroxine suspensions were prepared by the careful addition of 0.1N-HCl to a Na_2CO_3 solution, the pH being adjusted to 4.5. The urine and faeces were collected every 24 hr. for a period of 4 days and examined for total radioactive iodine, labelled thyroxine, labelled 3:5-diiodotyrosine and labelled iodide. At the end of each collection period, the metabolism cages, funnels and separators were washed down with a 'carrier' solution consisting of 0.4N-NaOH containing 0.01% of DL-thyroxine, 0.01% of DL-3:5-diiodotyrosine and 0.01% of NaI. The final washings were carried out with distilled water. All the washings were added to the urine samples, the total volumes being recorded. At the end of the experiment the rats were killed and the thyroids, liver, kidneys and carcasses examined for radioactive iodine. The experiment was repeated on a second group of rats.

Cat experiments. A series of cat experiments were undertaken to determine the proportion of ^{131}I appearing in the bile. Since it was necessary to use anaesthetized cats the duration of these experiments was comparatively short. After anaesthetization with ether, followed by tracheotomy, the femoral vein was exposed and cannulated. A chloralose

solution (0.08 g./kg. of body weight) was then introduced intravenously during the remainder of the anaesthesia. For collection of the urine, the abdomen was opened and the urethra cannulated, thus enabling the bladder to be completely emptied at each collection and so giving representative samples. A continuous collection of bile was obtained by cannulation of the common bile duct, the gall bladder being isolated by ligation of the cystic duct. Samples of blood were obtained at hourly intervals from the carotid artery.

Labelled thyroxine in 0.02N-Na₂CO₃ was administered to the cats intraduodenally, subcutaneously or intravenously, the doses being from 34 to 100 µg. of thyroxine/kg. of body weight. The solutions were injected directly into the duodenal lumen, since preliminary experiments had shown that there was a considerable delay in the emptying of the stomach in anaesthetized animals, most of the dose being still present in the stomach at the end of the experiment. Three cats were treated by each route. Samples of blood, urine and bile were collected every hour for a period of 6 hr. and examined for total radioactive iodine, labelled thyroxine, labelled 3:5-diiodotyrosine and labelled iodide. At the end of the experiments the cats were killed and the thyroids, liver, stomach, kidneys and the small and large intestines removed and examined for radioactive iodine.

Analytical technique

Urine and blood. Samples of cat blood (10 ml.) and urine (diluted to 10 ml.) containing radioactive iodine were digested on a steam bath for 4 hr. with 2 ml. of 2N-NaOH containing as 'carrier' 0.01 % thyroxine, 0.01 % 3:5-diiodotyrosine, and 0.01 % NaI. The pH of the digest was adjusted with 35 % (v/v) nitric acid to about 9, and a 10 ml. portion transferred to a jacketed Geiger-Müller counter (cf. Veall, 1948). The number of disintegrations per minute was measured on a Dynatron scaling unit. The background count was about 10/min.

Faeces. Samples of faeces (5 g.) were digested on a steam bath for 4 hr. with 20 ml. of 2N-NaOH containing as 'carrier' 0.01 % of thyroxine, 0.01 % of 3:5-diiodotyrosine and 0.01 % of NaI. The residual solid matter was removed by centrifuging and digested for 4 hr. with a further 10 ml. of 2N-NaOH. A third extraction was performed and the pH of the combined alkaline extracts adjusted with 35 % (v/v) nitric acid to 9.0. A 10 ml. portion was examined in the jacketed Geiger-Müller counter.

Stomach, intestines, liver, thyroid and other organs. In our earlier experiments the organs were digested with 2N-NaOH; as there were difficulties due to gel formation, Howarth's (1949) technique was subsequently adopted. The organ was minced and a sample portion (5 g.) refluxed for 4 hr. with 10 ml. 2N-LiOH in 20 % (v/v) ethanol, containing as 'carrier' 0.01 % thyroxine, 0.01 % 3:5-diiodotyrosine and 0.01 % NaI. The residual solid matter was centrifuged off and the supernatant liquid, after adjustment to pH 9, examined in the counter.

Labelled thyroxine and iodide determinations. The labelled thyroxine in the extracts was isolated by the method of Blau (1935). The sample was acidified (pH 3.5) and extracted successively with an equal volume and a half volume of *n*-butanol. The combined butanol extracts were then washed successively with an equal and a half volume of 4N-NaOH containing 5 g. Na₂CO₃/100 ml. The butanol extract was examined; after due allowance for the specific gravity of

butanol (cf. Veall, 1948) the count gave a measure of the labelled thyroxine in the sample.

The radioactivity in the combined aqueous layers was identified as iodide by the method of Gross & Leblond (1947). Inactive NaI (100 mg.) was added to the aqueous layer, which was then neutralized with conc. HNO₃, the iodide being precipitated by a solution of 10 % (w/v) AgNO₃ in 2 % (v/v) HNO₃. The precipitate was centrifuged off, redissolved in 10 ml. of hot 25 % (w/v) Na₂S₂O₃ and examined. The supernatant liquid from the AgNO₃ precipitation gave a measure of the labelled 3:5-diiodotyrosine in the sample.

Radio autographs. Several samples of urine and faeces containing large quantities of radioactive iodine were examined by the radio autograph technique of Fink, Dent & Fink (1947). A sample of a neutral extract was run on a phenol one-dimensional paper chromatogram, and a radio autograph obtained by placing the paper chromatogram in contact with a Kodak 'Industrex' type D X-ray film for up to 7 days. After development of the film, exposed areas appeared, owing to the presence on the chromatogram of 'spots' containing radioactive iodine. It was confirmed that in urine the radioactive iodine was present as iodide and in faeces as a mixture of thyroxine and iodide.

RESULTS

Rat experiments

In our initial experiments we followed the excretion of radioactive iodine by rats for a period of 4 days after a single oral or subcutaneous doses of either a solution or a suspension of labelled thyroxine. The subsequent excretion and final distribution of ¹³¹I are shown in Tables 2 and 3. The results are the mean values obtained from two separate experiments and indicate that the solution and the suspension are metabolized in the same way. The amounts of labelled diiodotyrosine found were negligible and have hence been omitted from the Tables. The results show clearly that after oral administration, absorption occurred from the intestinal tract. There was a higher percentage (65 and 57 %) of ¹³¹I in the faeces of the orally treated rats than in those of the injected rats (44 and 39 %). By both routes from 50 to 80 % of the ¹³¹I administered had been excreted in the first 24 hr. and, allowing for experimental errors, almost all the dose had been excreted by the fourth day. The ¹³¹I was present in the urine almost, if not entirely, as iodide, but in the faeces nearly three-quarters of the ¹³¹I present was butanol-soluble (Tables 2 and 3). This was confirmed by the radio autographs. Analysis of the individual organs and the carcasses of the rats at the end of the experiments showed very little residual ¹³¹I in the animals. Only a very small amount of ¹³¹I was present in the liver and kidneys; by far the highest concentrations were found in the thyroid glands (1.4-4.8 % of the dose). Only a small proportion of the ¹³¹I in the thyroids was butanol-soluble (Table 3).

Table 2. *Excretion of ^{131}I by groups of two rats after dosage with 1.0 mg. of labelled thyroxine per kg. of body weight*

		^{131}I as percentage of ^{131}I in dose							
Sample	Time of collection (hr.)	Group A, oral dose of solution containing 192 $\mu\text{g.}$ of labelled thyroxine		Group B, subcutaneous injection of solution containing 187 $\mu\text{g.}$ of labelled thyroxine		Group C, oral dose of suspension containing 180 $\mu\text{g.}$ of labelled thyroxine		Group D, subcutaneous injection of suspension containing 173 $\mu\text{g.}$ of labelled thyroxine	
		Total ^{131}I	Thyroxine ^{131}I	Total ^{131}I	Thyroxine ^{131}I	Total ^{131}I	Thyroxine ^{131}I	Total ^{131}I	Thyroxine ^{131}I
Urine	0-24	26.8	0.6	36.2	0.2	26.2	0.5	34.1	0.3
	24-48	5.1	0.2	9.1	0.1	5.7	0.2	6.8	0.2
	48-72	2.1	0.1	2.1	0.1	1.2	0.1	3.1	0.1
	72-96	0.6	0.1	0.9	0.1	0.5	0	1.2	0.1
Faeces	0-24	55.8	35.0	24.8	15.0	48.2	36.5	19.1	13.2
	24-48	8.1	5.2	15.3	13.2	6.9	5.1	14.2	10.2
	48-72	1.4	1.1	3.2	2.6	1.5	1.4	4.2	4.6
	72-96	0.2	0.1	1.1	0.6	0.1	0.1	0.8	0.8

Table 3. *Distribution of ^{131}I in groups of two rats four days after dosage with 1.0 mg. of labelled thyroxine per kg. body weight*

(The total ^{131}I content of the liver, kidney and 'rest of body' samples was too low to permit an accurate thyroxine ^{131}I determination.)

		^{131}I as percentage of ^{131}I in dose							
Organ		Group A, oral dose of solution containing 192 $\mu\text{g.}$ of labelled thyroxine		Group B, subcutaneous injection of solution containing 187 $\mu\text{g.}$ of labelled thyroxine		Group C, oral dose of suspension containing 180 $\mu\text{g.}$ of labelled thyroxine		Group D, subcutaneous injection of suspension containing 173 $\mu\text{g.}$ of labelled thyroxine	
		Total ^{131}I	Thyroxine ^{131}I	Total ^{131}I	Thyroxine ^{131}I	Total ^{131}I	Thyroxine ^{131}I	Total ^{131}I	Thyroxine ^{131}I
Thyroid		3.9	0.6	3.7	0.5	1.4	0.2	4.8	0.9
Liver		<0.03	—	<0.03	—	0.06	—	0.05	—
Kidney		0.006	—	<0.003	—	<0.003	—	<0.003	—
Rest of body		0.8	—	1.5	—	0.5	—	0.55	—
Urine: total		35	1.0	48	0.4	34	0.8	45	0.7
Faeces: total		65	41	44	31	57	43	39	29

Cat experiments

The previous experiments showed that following the oral administration of thyroxine to rats absorption occurred. However, interpretation of the results is complicated by the secretion of thyroxine into the intestinal lumen. It is known that thyroxine is secreted in the bile (Gross & Leblond, 1947). It was therefore of interest to carry out experiments on anaesthetized cats in which the common bile duct can be cannulated, thus enabling us to determine the rate and form of the ^{131}I excretion. Re-absorption of ^{131}I from the intestinal lumen would also be prevented. Anaesthetized cats were therefore injected with labelled thyroxine subcutaneously, intravenously or directly into the duodenal lumen. Because of the large number of extractions involved, it was only possible to study three cats by each route. In general, the dose administered was equivalent to 34 $\mu\text{g.}$ of thyroxine/kg. of body weight, but occasionally with less active material it

was necessary to employ 100 $\mu\text{g.}/\text{kg.}$ The experimental results, when calculated as a percentage of the dose administered, were of the same order at both dose levels; we have therefore given the mean results of three experiments by each route in Tables 4 and 5. The percentage of the dose present in the blood was calculated on the assumption that the blood volume in millilitres was equivalent to 6.5 % of the body weight in grams (cf. Dukas, 1947).

Fifteen minutes after injecting labelled thyroxine into the intestinal lumen, radioactive iodine was detected in the blood, indicating that some absorption had occurred. Radioactive iodine was found in the bile after all three methods of administration, and there appeared to be a direct relationship between the bile and blood concentrations of ^{131}I . Of the total ^{131}I in the bile (cf. Table 6) about 50 % was present as unchanged thyroxine, except after intravenous administration, when the proportion of thyroxine ^{131}I showed a tendency to fall to about 30 % of the total after the third hour.

Table 4. *Metabolism of ^{131}I by cats after dosage with labelled thyroxine*

Method of administration and quantity administered	Time of collection (hr.)	Percentage of ^{131}I in dose in blood*	Percentage of ^{131}I in dose secreted in bile	Percentage of ^{131}I in dose excreted in urine
Intraduodenal injection (34 $\mu\text{g.}$ of labelled thyroxine/kg.)	0-1	1.4	0.05	0.05
	1-2	1.6	0.16	0.07
	2-3	2.7	0.19	0.16
	3-4	2.8	0.18	0.24
	4-5	3.1	0.20	0.34
	5-6	2.9	0.22	0.80
Subcutaneous injection (100 $\mu\text{g.}$ of labelled thyroxine/kg.)	0-1	3.1	0.23	0.23
	1-2	3.3	0.47	0.26
	2-3	3.6	0.66	0.40
	3-4	4.4	0.49	0.48
	4-5	4.6	0.45	0.97
	5-6	4.9	0.31	0.86
Intravenous injection (34 $\mu\text{g.}$ of labelled thyroxine/kg.)	0-1	18.7	2.25	0.80
	1-2	13.5	1.82	0.54
	2-3	12.0	1.25	0.83
	3-4	9.2	0.70	1.2
	4-5	7.8	0.46	1.3
	5-6	5.8	0.50	2.0

* Calculated on the assumption that the blood volume in ml. was equivalent to 6.5 % of the animal's body weight in g.

Table 5. *Distribution of ^{131}I in organs of a cat 6 hr. after dosage with labelled thyroxine*

(— means 'not determined')

Organ or fluid	^{131}I found in organ or fluid as percentage of ^{131}I administered		
	Intraduodenal injection of 34 $\mu\text{g.}$ of labelled thyroxine/kg.	Subcutaneous injection of 100 $\mu\text{g.}$ of labelled thyroxine/kg.	Intravenous injection of 34 $\mu\text{g.}$ of labelled thyroxine/kg.
Total urine (0-6 hr.)	1.7	3.2	6.7
Total bile (0-6 hr.)	1.0	2.6	7.0
Blood	2.9	4.9	5.8
Stomach	4.2	2.8	8.8
Small intestine and contents	74	1.7	4.6
Large intestine and contents	0.57	0.36	0.57
Thyroid gland	0.05	0.002	0.10
Liver	1.8	4.3	6.0
Kidney	0.72	1.75	3.3
Adrenal gland	<0.003	—	0.024

Table 6. *Distribution of labelled thyroxine in bile of a cat which received a single dose of labelled thyroxine*

(— means 'not determined')

Time after dosage (hr.)	^{131}I present as thyroxine expressed as percentage of total ^{131}I in bile		
	Intraduodenal injection of 34 $\mu\text{g.}$ of labelled thyroxine/kg.	Subcutaneous injection of 100 $\mu\text{g.}$ of labelled thyroxine/kg.	Intravenous injection of 34 $\mu\text{g.}$ of labelled thyroxine/kg.
0-1	—	—	40
1-2	50	54	45
2-3	48	47	48
3-4	48	39	28
4-5	—	37	34
5-6	51	—	40

The ^{131}I excreted in the urine was almost entirely present as iodide. It was of interest to observe that the urine concentrations had hardly reached a maximum by the sixth hour. The blood levels indicate that, following intravenous injection, excess thyroxine is quickly removed from the circulation, the ^{131}I content rapidly falling from its initial high level. By the subcutaneous and intraduodenal routes, after an initial sharp rise during the first 1-2 hr., the ^{131}I concentration levels off and is maintained more or less constant during the remaining period of the experiment. Probably after administration by these two routes an equilibrium becomes established between absorption and excretion.

At the end of the experiments the ^{131}I remaining in the body was chiefly concentrated in the liver,

kidneys and gastro-intestinal tract. A high concentration was also found in the stomach, even in the injected animals. In one experiment 11.6 % of the original intravenous dose was recovered from the stomach, the ^{131}I being present mainly as iodide. After intraduodenal administration about 75 % of the initial dose was still unabsorbed at the end of 6 hr., indicating that the rate of absorption was not so rapid as the rat experiments would suggest, though it is possible that the anaesthetic had some influence. Very little radioactivity was found in the thyroids of these animals, except in one experiment, the results of which were discarded because the original labelled thyroxine solution had deteriorated and contained some labelled iodide.

DISCUSSION

The presence of radioactivity in the blood and of labelled thyroxine in the bile after oral administration confirmed that absorption of the monosodium salt does occur. However, the rapid disappearance of thyroxine from the blood stream precludes the use of blood levels for measuring the efficiency of the absorption. Labelled thyroxine was found in the faeces of the injected rats, as has been reported by Gross & Leblond (1947). It was confirmed in our experiments, using cats with cannulated bile ducts, that the faecal excretion of thyroxine was due to its elimination in the bile. Therefore, thyroxine present in the faeces of the orally treated rats could not be entirely due to failure of absorption; interpretation of the results is further complicated by the fact that some at least of the thyroxine and its metabolites excreted in the bile would be re-absorbed from the intestinal lumen (cf. Schittenhelm & Eisler, 1932; Gross & Leblond, 1947). Our results indicate that about 70 % of an oral dose of sodium DL-thyroxine is absorbed by rats. Frieden, Tuckich & Winzler (1949) found by the goitre-prevention test that sodium DL-thyroxine exhibited half of its parenteral activity when administered orally to rats. These results are in contrast with the suggestions of Bailey *et al.* (1949) that only 6.3 % of an oral dose of sodium L-thyroxine was absorbed by cows and of Turner & Reineke (1946) that 12.5 % of an oral dose of sodium DL-thyroxine was absorbed by sheep, but they compare favourably with the observation of Monroe & Turner (1949) who found that 45 % of an oral dose of sodium DL-thyroxine was absorbed by the domestic fowl. The variations may well be due to species differences.

In general, our results on the excretion and distribution of thyroxine are in close accord with those of Gross & Leblond (1947), in spite of the fact that these workers used higher intravenous doses. It would appear that the administration of physiological doses of thyroxine raises the concentration of the circulating hormone above normal levels, and

that the body then inactivates the excess by direct excretory and destructive mechanisms.

Previous authors have reported that thyroxine tends to concentrate in the liver (Kraye, 1928; Gross & Leblond, 1947), where it is partially broken down and then eliminated through the bile (Kendall, 1919, Asimoff & Estrin, 1931; Schittenhelm & Eisler, 1932; Barnes, 1933; Barnes & Chang, 1933; Elmer & Luczynski, 1933, 1934). Our experiments with cats (cf. Table 6) showed that about 50 % of the thyroxine in the bile had been broken down with formation of inorganic iodide, observations that are in reasonable agreement with those of Elmer & Luczynski (1933, 1934), who injected larger doses (2-6 mg.) of thyroxine intravenously and into the stomach of rabbits. About 5.0-6.5 % of the iodine from the intravenous dose and 0.8-1.4 % from the stomach dose appeared in the bile during the first 12 hr. After the intravenous dose, Elmer & Luczynski found that about 50 % of the iodine in the bile was present as thyroxine and after the stomach dose about 20 %. The results listed in Table 4 suggest that there is a relationship between the blood and bile concentrations of radioactive iodine.

Very little of the administered thyroxine was retained in the body, the rats excreting between 50 and 80 % of the dose within the first 24 hr. and practically all by the end of the fourth day. It is difficult to explain these results in view of the known delay in the onset of maximal metabolic effects.

Thyroxine itself was never found in significant quantities in the urine of cats or rats. Urinary excretion of ^{131}I was entirely as iodide (cf. Asimov & Estrin, 1931; Elmer & Scheps, 1933, 1934; Gross & Leblond, 1947); the kidney does not appear to be permeable to thyroxine itself. Further evidence that the kidney deals only with thyroxine decomposition products is indicated by the delayed excretion of ^{131}I shown in the cat experiments.

Studies on the final distribution of ^{131}I in the body showed differences depending on the duration of the experiments. In the short-term cat experiments the highest proportions of radioactive iodine were found in the liver, kidneys and gastro-intestinal tract, with very little in the thyroid glands. At the conclusion of the rat experiments, which extended over 4 days, practically all residual radioactive iodine was concentrated in the thyroid glands and about 85 % was present as iodide. It is therefore fairly certain that the thyroid gland is incapable of absorbing and storing its own hormone. The gland probably absorbs the inorganic iodide liberated from the decomposed thyroxine and incorporates it in new thyroxine. Taurog, Tong & Chaikoff (1949) have shown that 24 hr. after an injection of labelled iodine only 15 % of the labelled iodine in the thyroid was present as thyroxine.

After this paper had been prepared for publication, we received a copy of Leblond's (1949) article on the distribution of ^{131}I after intravenous administration of physiological doses of labelled thyroxine for rats. The labelled thyroxine had been prepared by the butanol extraction of the thyroxine fractions from the thyroids of rats previously treated with radioactive iodine. The distribution of radioactive iodine in two rats 2 hr. after dosage was studied. Our results are in general agreement with those of Leblond (1949).

SUMMARY

1. Single doses of the monosodium salt of DL-thyroxine labelled with radioactive iodine in the 3'- and 5'-positions were administered either in solution or as a suspension, orally or subcutaneously, to rats; the subsequent excretion in urine and faeces was determined over a 4-day period. The final distribution of radioactive iodine in the carcass was studied.

2. It was shown that the thyroxine in solution and as a suspension is metabolized in the same way and that it is absorbed from the gastro-intestinal tract. The radioactive iodine in the urine was present almost entirely as iodide, whereas that in the

faeces was present in both organic and inorganic forms.

3. Single physiological doses of labelled monosodium DL-thyroxine were administered intraduodenally, subcutaneously, or intravenously to anaesthetized cats; the subsequent radioactive iodine blood levels and urinary and biliary excretion rates were measured over a 6 hr. period. The distribution of labelled thyroxine and its breakdown products was studied.

4. For all routes of administration, the amount of radioactive iodine in the bile was related to the blood concentrations; that in the bile was present in approximately equal proportions as thyroxine and iodide. Appreciable amounts of iodine were secreted into the stomach.

5. Rat thyroids, in contrast to cat thyroids, contained appreciable quantities of radioactive iodine; very little of the radioactive iodine in the rat thyroids was present as thyroxine.

We are indebted to the Medical Research Council for supplying the radioactive iodine, part of which had been made available by the United States Atomic Energy Commission and part by the British Atomic Energy Research Establishment. We wish to thank Mr J. K. S. Bell and Mr M. Robson for technical assistance.

REFERENCES

- Asimov, G. & Estrin, E. (1931). *Z. ges. exp. Med.* **76**, 399.
 Bailey, G. L., Bartlett, S. & Folley, S. J. (1949). *Nature, Lond.*, **163**, 800.
 Barnes, B. O. (1933). *Amer. J. Physiol.* **103**, 699.
 Barnes, B. O. & Bueno, J. G. (1933). *Amer. J. Physiol.* **103**, 570.
 Barnes, B. O. & Chang, T. H. (1933). *Amer. J. Physiol.* **105**, 3.
 Blau, N. F. (1935). *J. biol. Chem.* **110**, 351.
 Borrows, E. T., Clayton, J. C. & Hems, B. A. (1949). *J. chem. Soc.* p. S199.
 Borrows, E. T., Hems, B. A. & Page, J. E. (1949). *J. chem. Soc.* p. S204.
 Clayton, J. C., Free, A. A., Page, J. E., Somers, G. F. & Woollett, E. A. (1949). *Biochem. J.* **45**, xx.
 Clayton, J. C. & Hems, B. A. (1950). *J. chem. Soc.* p. 840.
 Dukes, H. H. (1947). *Physiology of Domestic Animals*, 6th ed, p. 61. New York: Comstock Publishing Co. Inc.
 Elmer, A. W. (1938). *Iodine Metabolism and Thyroid Function*. London: Oxford University Press.
 Elmer, A. W. & Luczynski, Z. (1933). *C.R. Soc. Biol., Paris*, **114**, 351.
 Elmer, A. W. & Luczynski, Z. (1934). *C.R. Soc. Biol., Paris*, **115**, 647.
 Elmer, A. W. & Scheps, M. (1933). *C.R. Soc. Biol., Paris*, **114**, 350.
 Elmer, A. W. & Scheps, M. (1934). *Acta med. scand.* **82**, 126.
 Fink, R. M., Dent, C. E. & Fink, K. (1947). *Nature, Lond.*, **160**, 801.
 Frieden, E. Lipsett, M. B. & Winzler, R. J. (1948). *Science*, **107**, 353.
 Frieden, E., Tuckich, E. B. & Winzler, R. J. (1949). *Endocrinology*, **45**, 82.
 Gross, J. & Leblond, C. P. (1947). *J. biol. Chem.* **171**, 309.
 Harington, C. R. (1933). *The Thyroid Gland*, 1st ed., p. 154. London: Oxford University Press.
 Hart, F. D. & MacLagan, N. F. (1950). *Brit. med. J.* **1**, 512.
 Horeau, A. & Süe, P. (1945). *Bull. Soc. Chem. biol., Paris*, **27**, 483.
 Howarth, F. (1949). *Nature, Lond.*, **163**, 249.
 Joliot, F. (1945). *Proc. roy. Soc. A*, **184**, 1.
 Joliot, F., Courrier, R., Horeau, A. & Süe, P. (1944a). *C.R. Acad. Sci., Paris*, **218**, 769.
 Joliot, F., Courrier, R., Horeau, A. & Süe, P. (1944b). *C.R. Soc. Biol., Paris*, **138**, 325.
 Kendall, E. C. (1919). *Endocrinology*, **3**, 156.
 Kraymer, O. (1928). *Arch. exp. Path. Pharmacol.* **128**, 116.
 Leblond, C. P. (1948). *Advanc. biol. med. Physics*, **1**, 353.
 Leblond, C. P. (1949). *Ann. N.Y. Acad. Sci.*, **50**, 444.
 Miller, W. H., Anderson, G. W., Madison, R. K. & Salley, D. J. (1944). *Science*, **100**, 340.
 Monroe, R. A. & Turner, C. W. (1949). *Amer. J. Physiol.* **156**, 381.
 Schittenhelm, A. & Eisler, B. (1932). *Z. ges. exp. Med.* **80**, 569.
 Smith, A. U., Emmens, C. W. & Parkes, A. S. (1947). *J. Endocrinol.* **5**, 186.
 Taurog, A., Tong, W. & Chaikoff, I. L. (1949). *Nature, Lond.* **164**, 182.
 Thompson, W. O., Thompson, P. K. & Dickie, L. F. N. (1933). *Arch. intern. Med.* **52**, 576.
 Turner, C. W. & Reineke, E. P. (1946). *Bull. Mo. agric. Exp. Sta.* **397**.
 Veall, N. (1948). *Brit. J. Radiol.* **21**, 347.