INFLUENCE OF THE STATE OF THYROID ACTIVITY ON HEPATIC METABOLISM OF TRIIODOTHYRONINE

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Under certain conditions 3 : 5 : 3′-triiodothyronine (T_3) is found in thyroid and plasma together with thyroxine.[1-3] The liver is an important organ for the peripheral metabolism of these thyroid hormones; thus it selectively concentrates,[4-10] de-iodinates,[11-15] conjugates,[16-20] oxidatively deaminates,[22,23] and selectively secretes[24] them into bile.

De-iodination apparently takes place at either or both of the 5′ and 5 positions of the iodinated thyronines,[21] although free T_3 has not been unequivocally identified in bile after thyroxine (T_4) treatment. Conjugation is effected by combination with glucuronic acid or with sulphates.[22] These processes are apparently of importance for detoxication of excess hormone and also participate in controlling the level and potential activity of hormone in circulation. Thus, by conjugation the hormone is detoxicated and less readily absorbed,[25] whereas, by de-iodination of T_4 in the 5′ position, a more active substance may be formed although this reaction is questionable.[22] Similarly, by de-iodination of T_4 at the 5 position, or of T_3 at the same position, 3 : 3′ : 5′ - T_3 or 3 : 3′ diiodothyronine (T_2) are formed,[21] which have relatively less physiological activity. Excess hormone is removed by an increase in rate of metabolic breakdown, rather than by an increase in biliary excretion.[26]

At least two existing variable mechanisms are responsible for the level of circulating thyroid hormones; long-term regulation of central biosynthesis in the thyroid, and more rapid changes in the rate of peripheral metabolism. The central mechanism is controlled to a large extent by the adenohypophysis and by the thyroid hormone itself. How far the metabolic functions of the liver are controlled by the state of thyroid activity is still unknown.

The present study is an attempt to assess the rate of de-iodination (as measured by iodine release) and of conjugation (as indicated by the radioactivity in the glucuronide fraction) of T_3 by the liver during various degrees of thyroid activity.

METHODS

Twenty-four male albino rats of the Wistar strain, weighing 180 - 250 G., were used. One group (9 animals) was subjected to thyroidectomy 14 days before the experiment. A second group (6 animals) was injected intraperitoneally with 100 μg. of sodium triiodo-L-thyronine per rat per day for 5 days. The T_3 was dissolved in physiologically normal Na_2CO_3. A third (control) group (9 animals) and the thyroidectomized group received an equivalent volume (0·2 ml.) of the same solvent per day.

At the end of the period of treatment the bile ducts of the rats were cannulated under light anaesthesia by inserting a polythene tube (size 1, Sterivac) into the common bile duct. A stainless steel wire was passed from the wound between the skin and the musculature to emerge on the animal's back. One end of the wire was inserted into the tubing which was then pulled along the track of the wire to emerge on the back. A glass container[27] was strapped to the back of the animal. At this stage ^{131}I (13·4 μc./0·5 ml. physiological saline) or $^{131}T_3$ (13·3 μc./0·2 ml. propylene glycol containing 3·2 μg. T_3) were injected intravenously into the dorsal vein of the penis. The animals were allowed to recover and to move about freely while bile was aspirated from the containers at intervals up to about 30 hours (Fig. 1).

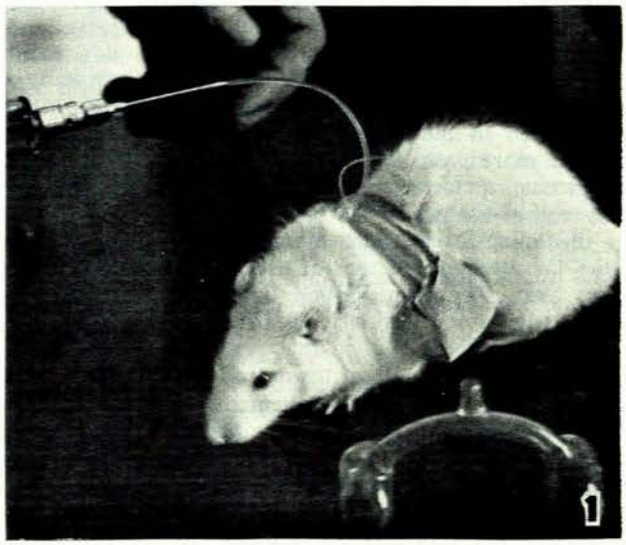

Fig. 1. Aspiration of bile from a surgically thyroidectomized rat with subsequent bile-duct cannulation. A container, similar to the one which is strapped on to the rat, is shown in the picture. At the end of each time-interval of collection the polythene tubing leading from the bile duct was pushed over into the other compartment. The width of the 'elastoplast' prevents the animal from chewing off the tubing on its back. Time for conditioning of the rat to the saddle is about 20 min.

The volume of the bile samples was measured, and 0·1 ml. of the samples was counted in an Ekco well-type scintillation counter together with an aliquot portion of the original radioactive material as standard: The rest of the bile samples for each time interval in each group of rats were pooled. Small portions of these pooled samples were applied directly to Whatman No. 1 filter paper and chromatographed one-dimensionally in redistilled collidine saturated with water (100:35·5 v/v) in an atmosphere of ammonia (CWA) or in n-butanol-dioxane (80 : 20 v/v), saturated with 2·ON NH_4OH in an atmosphere of ammonia (BDA).

For percentage distribution of radioactivity the chromatograms were either counted centimetre-wise by using a Phillips GM-tube connected to an Ekco scaler or by exposing the chromatogram to Ilford-Ilfex X-ray film. The density of the bands was scanned with a Photovolt densitometer, using a 530 mμ filter and the percentage was obtained by planimetry. Exposure time was assessed by scanning the chromatograms with a Phillips GM tube covered by a 0·5 cm. lead shield with 1 cm.^{2} slit. The band with highest activity was counted and the exposure time calculated according to the formula: exposure time (hrs.) = 100,000/maximum counts/100 sec.

$^{131}T_3$ labelled by a method of radio-isotopic exchange,[28] only in the 3′ position, was obtained from Abbott Laboratories, Oak Ridge, Tennessee, USA.

RESULTS

About 19% of the $^{131}T_3$ injected was in the form of iodide and of a substance with an R_f value close to that of T_4 in BDA (Fig. 2). This substance was previously, tentatively, identified as a radioactive decomposition compound of $^{131}T_3$.[29] Excretion products of $^{131}T_3$ in bile therefore should allow for these two artifacts.

Cumulative percentage secretion of the total radioactivity in bile of normal, thyroidectomized and T_3-treated rats after

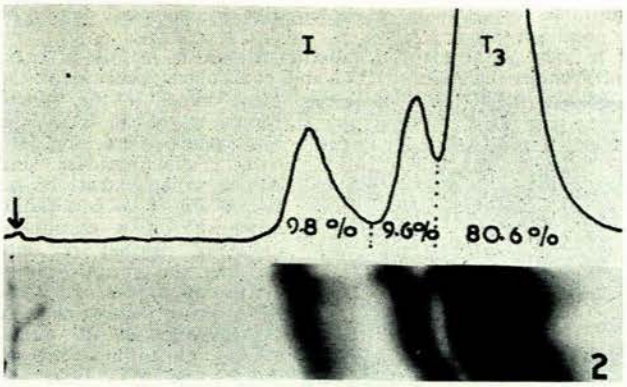

Fig. 2. A one-dimensional chromatogram of $^{131}T_3$ in BDA. The percentage distribution is indicated on the densitometer recording obtained from the radio-autograph. The arrow indicates the point of application.

^{131}I injections (13·36 μc. ^{131}I in 0·5 ml. of 0·9% saline intravenously per rat) indicate higher secretion rates in T_3-treated and normal animals as compared with those of thyroidectomized rats (Fig. 3). Similarly, the cumulative percentage secretion of total radioactivity in bile of animals which received $^{131}T_3$ (13·33 μc. $^{131}T_3$ in 0·2 ml. 0·42% Na_2CO_3 containing 3·2 μg. T_3) was also higher for T_3-treated and normal animals than for thyroidectomized rats (Fig. 4).

Comparing the secretion rates of ^{131}I and $^{131}T_3$, the rate of secretion for $^{131}T_3$ is much more rapid in bile than that for ^{131}I. Although the secretion rate is greater in animals with an increased state of thyroid activity, the concentration of radioactivity in 0·1 ml. bile samples at the same time interval after the injection did not show any obvious mean group differences, indicating that little difference exists in the concentrating power of the liver for injected ^{131}I or $^{131}T_3$ in the various groups. It also indicates that differences in secretion rates are largely a function of bile volume. Mean bile volumes as indicated in Table I are evidence of this view. However, the peak of radioactivity in 0·1 ml. bile is reached earlier (at about 1 hour) in all T_3-treated animals and appears only at about 2 hours in normal and thyroidectomized rats injected intravenously with $^{131}T_3$. Again, this observation is apparently due to differences in flow rate rather than differences in the rate of $^{131}T_3$ metabolism (Fig. 5).

TABLE I. BILE VOLUMES (ML.) SECRETED PER HOUR OVER THE FIRST 10 HOURS AFTER BILE DUCT CANNULATION

The volumes in the table are means from 9 rats per experiment

Experiment	*Normal*	*T_3-treated*	*Thyroidectomized*
1	0 494	0 625	0·484
2	0 595	0 660	0·504
Grand mean	0 563	0·672	0·499

In order to correlate the state of thyroid activity with metabolic processes in the liver, the mean percentage distributions of the various substances in bile were obtained as illustrated in Fig. 6 which is an example of the metabolic

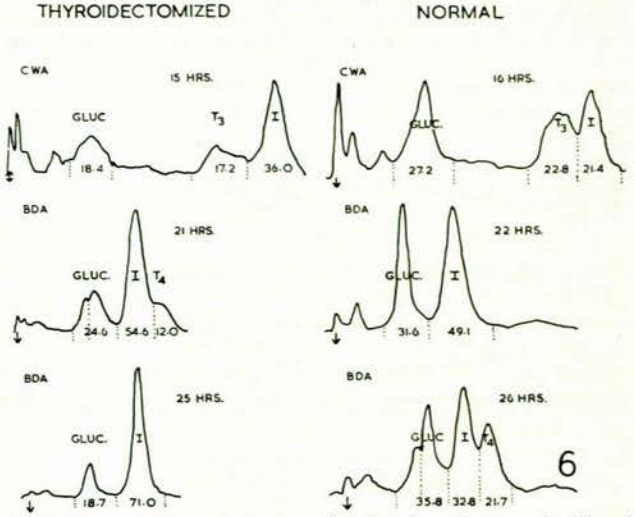

Fig. 6. The percentage distribution of radioactive substances in bile of thyroidectomized and normal rats at various intervals after $^{131}T_3$ injections. Chromatographic solvents — collidine : water : ammonia (CWA); butanol : dioxane : ammonia (BDA). Radioactive bands coincide with the Rf values of bile in rats were injected intravenously with $^{131}T_3$. (T_4). The figures below the curves represent percentage distribution of radioactivity.

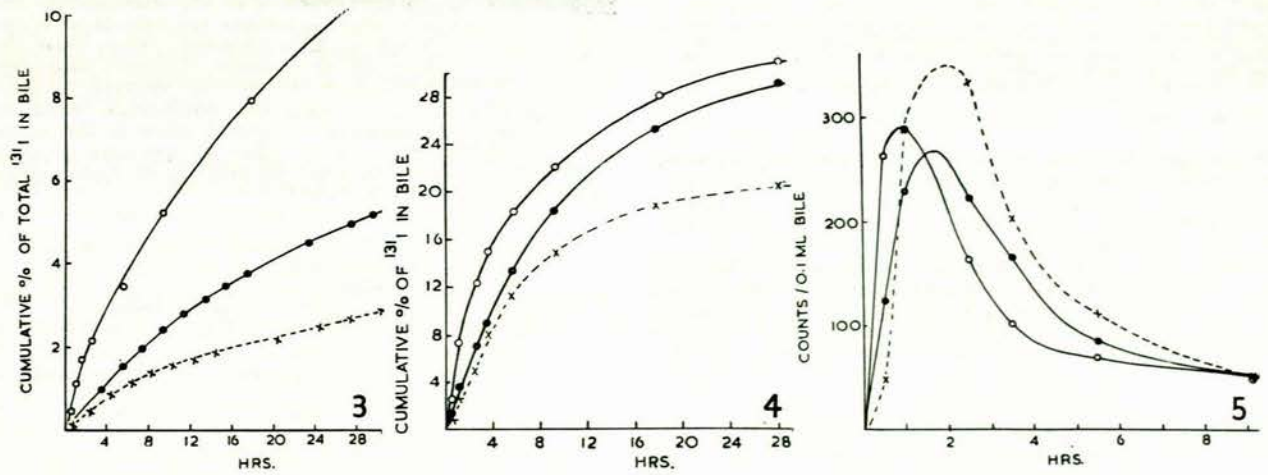

Fig. 3. Cumulative percentage secretion of total radioactivity in bile of T_3-treated, normal, and thyroidectomized rats after intravenous injection of ^{131}I. Each dot represents the mean of 2 animals per group.
— O — T_3-treated — ● — Normal --- X --- Thyroidectomized

Fig. 4. Cumulative percentage secretion of total radioactivity in bile of T_3-treated, normal and thyroidectomized rats after intravenous injection of $^{131}T_3$. Each dot represents the mean of 3 animals per group.
— O — T_3-treated — ● — Normal --- X --- Thyroidectomized

Fig 5. A demonstration of the time of maximum radioactivity in 0·1 ml. of bile in rats which were injected intravenously with $^{131}T_3$.
— O — T_3-treated — ● — Normal --- X --- Thyroidectomized

products in bile resolved by different solvents in the later stages after $^{131}T_3$ injection in Exp. I. From these figures it appears that the percentage of metabolites conjugated is greater in normal animals than in thyroidectomized rats, whereas de-iodination takes place more rapidly in thyroidectomized than in normal animals. A substance with about the same R_f value as T_4 or diiodothyronine (T_2) was obtained at approximately 21 hours after $^{131}T_3$ injection in thyroidectomized animals and at about 26 hours after $^{131}T_3$ injection in normal rats. Even though this substance seems to be the same as that in the original $^{131}T_3$ solution (Fig. 2), it is unlikely that its appearance is due to a delayed secretion, but it is conceivable that it is derived endogenously by de-iodination of T_3 or of its conjugate.

The mean percentage distribution of iodide (I) and of the glucuronide of T_3 in two different experiments in which collidine : water : ammonia was used as solvent, are plotted in Fig. 7. From this figure it is obvious that glucuronide formation precedes de-iodination; in fact the percentage glucuronide and iodide showed opposite trends in all groups of animals. Even though in Exp. 2 the initial glucuronide concentration in thyroidectomized animals exceeded that of normal animals, the maximum percentage glucuronide of about 75% in chromatograms of T_3-treated animals is far greater than that of about 50% or less in the control and thyroidectomized groups. The mean percentages of radioactivity in T_3 and I on 3 rats per group and 8 samples per group at intervals over the 18 hours after the $^{131}T_3$ injection are given in Table II. This Table also indicates that a higher percentage glucuronide formation is associated with an increased degree of thyroid activity.

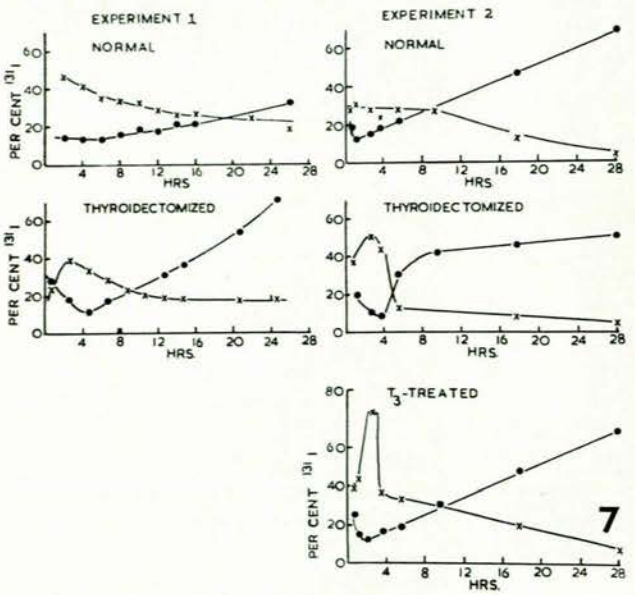

Fig. 7. Mean percentage distribution of $^{131}T_3$ metabolites in bile in normal, thyroidectomized, and T_3-treated rats in which collidine : water : ammonia was used as the chromatographic solvent. Readings for Exp. 1 were obtained by densitometry of radio-autographs and in Exp. 2 by direct countings of the chromatograms.
— X — Mean % of radioactivity in glucuronic acid conjugate of triiodothyronine.
— ● — Mean % of radioactivity in iodide.

TABLE II. MEAN PERCENTAGE DISTRIBUTION OF GLUCURONIDE AND IODIDE ON CHROMATOGRAMS OBTAINED FROM 8 BILE SAMPLES FROM 3 RATS PER GROUP OVER THE FIRST 18 HOURS AFTER $^{131}T_3$ IN TWO DIFFERENT EXPERIMENTS

Experiment	*Substance*	*Thyroid-ectomized*	*Normal*	*T_3-treated*
1	Glucuronide	25·7	34·2	—
	Iodide	24·0	17·2	—
2	Glucuronide	23·7	25·2	40·0
	Iodide	24·7	22·7	23·8

In view of the many metabolites formed in rat bile after an intravenous injection of $^{131}T_3$ and particularly because of the many bands in the 'glucuronide region' in BDA, the bile samples were incubated with β-glucuronidase and with a saline extract of rat faeces. Fig. 8 (*a*) is a radioautograph of a chromatogram of normal rat bile showing the various radioactive compounds of $^{131}T_3$ in BDA. After incubation with β-glucuronidase all bands in the 'glucuronide region' disappeared to a large extent, whereas T_3 and the fraction with the R_f value of T_4 or T_2 appeared [Fig. 8 (*b*)]. Boiling the enzyme for 3 minutes made little difference to its action.

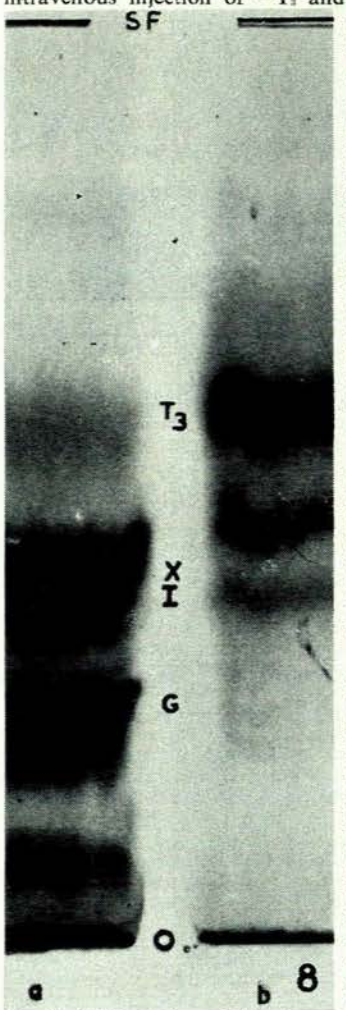

Fig. 8. (*a*) Metabolites of $^{131}T_3$ in rat bile.
(*b*) Same bile sample incubated with ox β-glucuronidase.
Solvent — BDA.

Fig. 9 shows similar release of T_3 from the glucuronide after β-glucuronidase treatment (B), whereas incubation with faecal extract containing 'chloromycetin' (C), and with faecal extract without chloromycetin (D) also released T_3.

In order to gain information about the re-utilization of ^{131}I and compounds formed in the thyroid, and to see in how far they can influence the chromatographic patterns in bile, normal and thyroidectomized rats were injected with ^{131}I and the bile samples chromatographed at intervals after the operation. From Fig. 10 it can be seen that 15 hours after the injection the first signs of glucuronide (G) appeared. This band became stronger with time and, in addition, at least one unidentified band appeared close to the origin; the rest remained as iodide (I) and no free hormone could be seen. In thyroidectomized samples, only iodide was found.

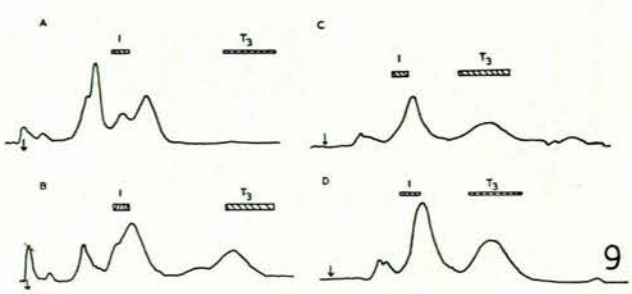

Fig. 9. A. Metabolites in rat bile after $^{131}T_3$ treatment.
B Same bile sample treated with β-glucuronidase.
C. Same bile sample treated with rat faecal extract in the presence of chloromycetin.
D. Same bile sample incubated with rat faecal extract.
Solvent — BDA.

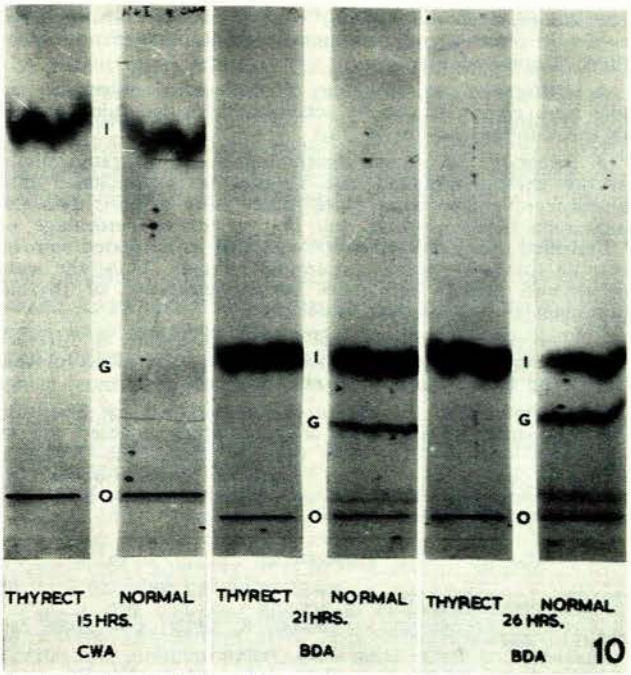

Fig. 10. Radioactive iodine substances in normal and thyroidectomized rat bile at intervals after ^{131}I injection.
CWA = collidine : water : ammonia.
BDA = butanol : dioxane : ammonia.

DISCUSSION

The reason for hepatic concentration of iodinated thyronines remains obscure. It may be related to the long latent period of thyroid hormones, in which case it could be expected that thyroid hormones with high potential activity and with a short latent period would be trapped for a relatively shorter period and that metabolic breakdown and detoxication would proceed at a faster rate. However, the rate of biliary secretion of iodinated thyronines seems to be associated to a larger extent with the number and position of the iodines on thyronine rather than with its potential activity. Thus Roche[24] found a more rapid biliary excretion of 3 : 3′ diiodothyronine than of other iodinated thyronines and little difference between the two types of triiodothyronine with dissimilar physiological activity. Nevertheless, it is clear from these results that the state of thyroid activity influences hepatic clearance of thyroid hormones as well as their metabolism.

Work on the percentage distribution of metabolic products of thyroid hormones in bile is hampered by the absence of a chromatographic solvent capable of resolving all metabolites one-dimensionally. For example, 3 : 3′-diiodothyronine cannot be distinguished from 3 : 5 : 3′ triiodothyronine by collidine : water : ammonia, and therefore this solvent cannot be used for the measurement of 3 : 5 : 3′-triiodothyronine disappearance in bile after its intravenous injection if diiodothyronine is a metabolic product of T_3. Similarly, solvents like BDA do not separate the different sulpho-conjugates of thyronines clearly. Moreover BDA does not resolve iodide clearly from the sulpho-conjugates of triiodothyronine and diiodothyronine, neither does it separate tetraiodothyroacetic acid distinctly from T_4, so that this solvent cannot be used very successfully to determine the degree of de-iodination of T_4 or of T_3. For this reason enzymes like β-glucuronidase and mylase P are extremely useful to hydrolyse the conjugates (glucuronides and sulphates) and thus dissociate them from the thyronines for identification purposes.

It is difficult to assess whether de-iodination, unrelated to oxidative de-amination or conjugation, takes place in tissues. It is believed that T_4 and T_3 are de-iodinated in many tissues, particularly in the liver. However, extraction and chromatography of $^{131}T_4$ with high specific radioactivity invariably yields ^{131}I; such de-iodination is a non-metabolic phenomenon and may result in the identification of $^{131}T_3$ in cases where $^{131}T_4$ had been labelled in more than one position, such as occurs endogenously after ^{131}I treatment. We have great difficulty in demonstrating the presence of T_3 in thyroids of rats if the ^{131}I dose is kept low. For example, when rats are injected with ^{131}I and the thyroids pooled to bring the total activity of ^{131}I up to the same level as that of a single rat given 20 μc. of ^{131}I, and the thyroid digest is applied directly to chromatography paper without extraction, no T_3 can be demonstrated if minute amounts of a reducing agent and toluene are added to the digestion mixture (unpublished). No doubt, non-metabolic de-iodination and radiation decomposition facilitated the radio-autographic identification of T_3 in thyroids and tissues in the past to such an extent that T_3 appeared sometimes as a more intense radioactive spot than T_4, even though their solubilities in the extraction medium were about the same. Radioactively labelled T_4, treated in the same way as thyroid extract, showed no $^{131}T_3$, which was adduced as evidence against non-metabolic decomposition, but no evidence was given as to the position of labelling of the radioactive T_4. Taurog *et al.*[17] remarked on the difficulty of finding a suitable developing solvent which did not decompose appreciable amounts of T_4.

Even though it is commonly believed that $^{131}T_4$, formed in the thyroid, is labelled with ^{131}I in all 4 positions, findings in our laboratory do not agree with an even distribution of radioactivity. Thus, it is difficult to re-chromatograph endogenously labelled $^{131}T_4$ without releasing ^{131}I at the same time even though $^{131}T_3$ cannot always be found. It is possible that endogenous iodination and de-iodination, and presumably even exchange, take place firstly in the 5′ position and secondly in the 5 position, and that lability of these positions persists endogenously. In the case of $^{131}T_3$ the 3′ position becomes labile giving rise to 3 : 3′ diiodothyronine. Labile positions in the thyronine nucleus are probably closely linked with the excretion and metabolic products of T_4 and T_3.

In this study $^{131}T_3$ was labelled in the 3′ position only so that the radioactive metabolic products, which could be identified by radio-autography, were expected to be of three kinds: first, those of the conjugates of T_3 (glucuronides and sulphates), second, the oxidative de-amination products of T_3 and their conjugates;[22] and third, those of ^{131}I broken off at the labelled position. It is unlikely that ^{131}I released in this way can form extrathyroidal metabolic products in thyroidectomized rats so that differences between the normal and thyroidectomized bile patterns would indicate ^{131}I re-utilization. Qualitative differences of this sort were not found in these experiments.

In view of these results and others in the literature, the hepatic metabolism of T_3 apparently proceeds somewhat as indicated in Fig. 11, but the exact identification and quantitative distribution of the numerous metabolic products awaits further investigation.

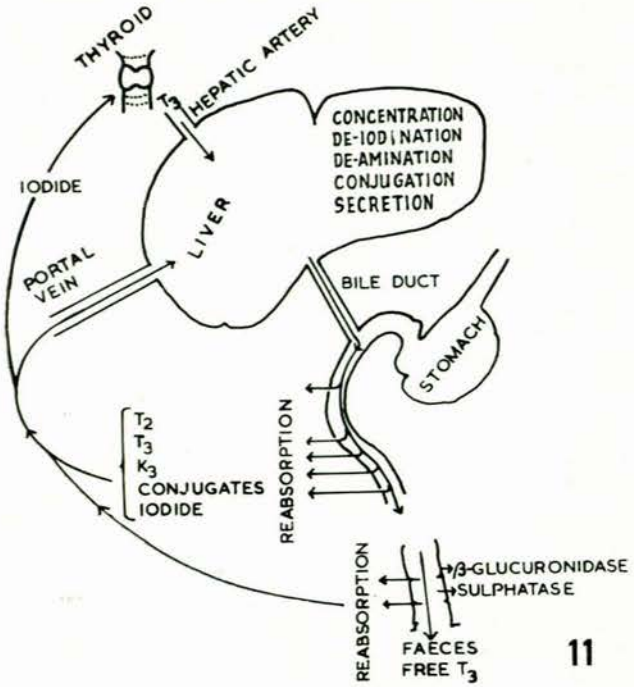

Fig. 11. A schematic diagram of some of the various metabolic processes and possible end-products of triiodothyronine in the entero-hepatic circulation.

SUMMARY

1. Bile-duct cannulations were performed on surgically thyroidectomized, normal, and triiodothyronine-treated rats which were subsequently injected intravenously with radioactive iodine or ^{131}I-labelled triiodothyronine. The secretion rates of radioactive material in bile were studied over about 30 hours while the animals moved about freely.

2. The mean total radioactivity in bile of the triiodothyronine-treated animals was greater than that for normal rats and was lowest in thyroidectomized animals after ^{131}I- and $^{131}T_3$-treatment.

3. No obvious differences were found among the different groups of animals in the mean concentration of radioactivity when counted in 0·1 ml. samples over the same time intervals, but the peak of radioactivity per volume of bile appeared earlier in triiodothyronine-treated animals than in normal or thyroidectomized rats.

4. Differences in secretion of radioactive substances in bile were mainly caused by secretion rates and bile volumes in the different groups of rats.

5. From the percentage distribution values of radioactivity in the chromatographed bile samples it is concluded that glucuroconjugation takes place much more rapidly than de-iodination in all animals, but that a greater percentage of ^{131}I-labelled triiodothyronine is conjugated in T_3-treated animals than in thyroidectomized or normal animals. Thus, the state of thyroid activity influences hepatic clearance of thyroid hormones as well as their metabolism.

6. The possible influence of lability of iodine in different positions on the thyronine nucleus and of the effect of this lability on iodine metabolism are discussed.

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REFERENCES

1. Gross, J. and Pitt-Rivers, R. (1952): Lancet, **1,** 439.
2. *Idem* (1953): Biochem. J., **53,** 645.
3. Roche, J., Lissitzky, S. and Michel, R. (1952): C.R. Acad. Sci. (Paris), **234,** 997.
4. Kendall, E. C. (1959): Endocrinology, **3,** 156.
5. Gross, J. and Leblond, C. P. (1947): J. Biol. Chem., **171,** 309.
6. *Idem* (1950): *Ibid.*, **184,** 489.
7. Albert, A. and Keating, F. R., jnr. (1952): Endocrinology, **51,** 427.
8. Myant, N. B. and Pochin, E. E. (1950): Clin. Sci., **9,** 421.
9. Gross, J. (1955): *Bookhaven Symposia in Biology,* No. 7, p. 102. New York: Upton.
10. Gross, J., Ford, D. F., Symchowicz, S. and Horton, J. H. (1957): *Ciba Foundation Colloquia on Endocrinology,* **10,** 182. London: Churchill.
11. Pitt-Rivers, R. and Tata, J. R. (1959): *The Thyroid Hormones,* p. 148. London: Pergamon Press.
12. Flock, E. V., Bollman, J. L. and Grindlay, J. H. (1960): Endocrinology, **67,** 419.
13. Sprott, W. E. and Maclagan, N. F. (1955): Biochem. J., **59,** 288.
14. Pitt-Rivers, R., Stanbury, J. B. and Rapp, B. (1955): J. Clin. Endocr., **15,** 616.
15. Hogness, J. R., Berg, M., Van Arsael, P. P., jnr. and Williams, R. H. (1955): Proc. Soc. Exp. Biol. (N.Y.), **90,** 93.
16. Taurog, A., Briggs, F. N. and Chaikoff, I. L. (1951): J. Biol. Chem., **191,** 29.
17. *Idem* (1952): *Ibid.*, **194,** 655.
18. Roche, J., Michel, R. and Tata, J. (1953): Biochim. biophys. Acta (Amst.), **11,** 543.
19. Flock, E. U. and Bollman, J. L. (1954): Fed. Proc., **13,** 209.
20. Flock, E. U., Bollman, J. L. and Grindlay, J. H. (1957): Amer. J. Physiol., **189,** 420.
21. Flock, E. U., Bollman, J., Grindlay, J. H. and Stobie, G. H. (1961): Endocrinology, **69,** 626.
22. Roche, J. and Michel, R. (1956): Recent Progr. Hormone Res., **12,** 1.
23. Roche, J., Michel, R. and Tata, J. (1954): Biochim. biophys. Acta (Amst.), **15,** 500.
24. Roche, J. (1957): *Ciba Foundation Colloquia on Endocrinology,* **10,** 223. London: Churchill.
25. Briggs, F. N., Taurog, A. and Chaikoff, I. L. (1953): Endocrinology, **52,** 559.
26. Myant, N. (1956): Clin. Sci., **15,** 227.
27. van Zyl, A. (1957): J. Endocr., **16,** 213.
28. Gleason, G. I. (1955): J. Biol. Chem., **213,** 837.
29. van Zyl, A. (1962): Clin. chim. Acta, **7,** 20.