Intrathyroidal Iodine Metabolism in the Rat

THE INFLUENCE OF DIET AND THE ADMINISTRATION OF THYROID-STIMULATING HORMONE

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1. Ratios of mono[^{131}I]iodotyrosine and di[^{131}I]iodotyrosine (R values) and the incorporation of ^{131}I into iodothyronines have been estimated in rat thyroid glands from 30 min. to 38 hr. after the administration of [^{131}I]iodide. 2. In rats receiving a powdered low-iodine diet the R values were close to unity and did not change with time after the administration of [^{131}I]iodide. In rats receiving a commercial pellet diet the R values fell from a mean of 0.8 at 30 min. after [^{131}I]iodide administration to 0.49 at 38 hr. 3. Administration of 0.5–2.0 i.u. of thyroid-stimulating hormone before giving the injection of [^{131}I]iodide caused a small diminution in the R value when the time between injecting [^{131}I]iodide and killing the animal was 16 hr. or more. 4. Iodothyronines represented a greater percentage of the total thyroid-gland radioactivity in the iodine-deficient animals than in animals fed on the pellet diet. Thyroid-stimulating hormone had little effect, if any, on the iodothyronine contents.

There have been several reports that after the administration of [^{131}I]iodide to the rat the ratio, R , of mono[^{131}I]iodotyrosine to di[^{131}I]iodotyrosine is considerably greater than unity 30 min. after the dose, but gradually diminishes, approaching 0.33 at 24 hr. (Michel, 1956; Taurog, Tong & Chaikoff, 1958; Pitt-Rivers & Tata, 1959; Pitt-Rivers, 1962). This finding has been interpreted to mean that the specific radioactivity of monoiodotyrosine is initially higher than that of di-iodotyrosine, a circumstance that is consistent with a precursor-product relationship between mono- and di-iodotyrosine.

In a previous paper from this Laboratory (Plaskett, Barnaby & Lloyd, 1963b) an attempt was made to use this difference in specific radioactivity to find out whether mono- and di-iodotyrosine are the direct precursors of tri-iodothyronine. If tri-iodothyronine is formed in a coupling reaction between one molecule of monoiodotyrosine and one molecule of di-iodotyrosine in a manner analogous with thyroxine biosynthesis (Harington, 1944), then each iodothyronine must contribute one ring of the tri-iodothyronine: consequently, in a situation in which the specific radioactivities of the two iodothyronines are different the specific radioactivity of the iodine on the two rings of tri-iodothyronine must also be different. If monoiodotyrosine

is the more heavily labelled, then the β -ring of tri-iodothyronine must become more heavily labelled than the α -ring.

Experiments were therefore carried out in which tri-iodothyronine was isolated from rat thyroid glands at various time-intervals after the administration of [^{131}I]iodide, and was degraded to determine the distribution of ^{131}I between the rings. However, these experiments did not lead to a clear conclusion because in our experiments the R values did not change with time, indicating that the specific radioactivities of mono- and di-iodotyrosine did not differ. This result has already been reported (Plaskett *et al.* 1963a,b) and found to be unaffected by using a different strain of rats, giving sodium iodide in the drinking water or administering anti-thyroid drugs. It has also been found (C. F. Barnaby & L. G. Plaskett, unpublished work) that alteration of environmental temperature within the range 11–22° is ineffective in inducing the R values to change with time.

The present paper attempts to overcome these problems. It reports the effect on the R values of (i) the administration of thyroid-stimulating hormone, and (ii) a change of diet. It describes conditions in which the R values change with time from 0.8 at 30 min. after the injection of [^{131}I]iodide to less than 0.5 at 38 hr. Hence it opens the

possibility of determining the biosynthetic pathway of tri-iodothyronine unequivocally by isotope-position experiments.

METHODS

Animals. Male Wistar albino rats (200–250 g.) were used. They were given either a low-iodine diet (Boyd & Oliver, 1960) for 2 weeks before the experiments or a commercial pellet diet manufactured by McGregor and Co., Quayside Mills, Leith (diet no. 1).

Administration of $[^{131}\text{I}]$ iodide. A solution of $[^{131}\text{I}]$ iodide (10–20 μC) was injected subcutaneously.

Administration of thyroid-stimulating hormone. A solution of thyroid-stimulating hormone (0.5–2.0 i.u.) was injected subcutaneously several hours before the administration of $[^{131}\text{I}]$ iodide. A range of injection times was used initially to find out whether it was possible to demonstrate a time-course of the hormonal effect. It was found that no effect of thyroid-stimulating hormone was observed when the interval between administering the hormone and killing the animal was less than 24 hr. Therefore in most of the work this interval was 24–40 hr.

Preparation of thyroid-gland hydrolysates. The thyroid gland was dissected out rapidly immediately after the death of the animal, homogenized and hydrolysed enzymically as described by Plaskett *et al.* (1963a).

Paper chromatography. Samples of the thyroid-gland hydrolysate were applied as a 1.25 in. band on Whatman no. 3MM paper and developed by the descending method in the solvent system butan-1-ol–2N-acetic acid (1:1, v/v).

Assay of radioactivity. After development the chromatograms were cut transversely into segments 1 cm. in width. The radioactivity of each segment was determined separately by placing the source between two mica end-window Geiger–Müller tubes (20th Century Electronics type Ew2C), having an end-window thickness of 1.5 mg./cm.². This counting technique gives high efficiency in counting ^{131}I (about 40% with Whatman no. 3MM paper) and was used with an anticoincidence shield to reduce the background.

RESULTS

Uptake of ^{131}I . Fig. 1 illustrates the uptake of $[^{131}\text{I}]$ iodide during the period 0–6 hr. after the injection in rats receiving each of the two types of diet. In accord with numerous other studies, it was found that the iodine-deficient gland is much the more avid for iodide.

Chromatographic separations. Chromatography of thyroid-gland hydrolysates in the butan-1-ol–acetic acid system separates four main components: (i) I^- ion, (ii) monoiodotyrosine, (iii) di-iodotyrosine and (iv) iodothyronines, the spots of thyroxine and tri-iodothyronine being superimposed. This result is illustrated in Fig. 3(a). As in previous work, an unidentified minor component was found (R_f 0.13). Its chromatographic behaviour in the butan-1-ol–acetic acid system resembles that of monoiodo-histidine, as described by Roche, Lissitzky & Michel (1954). In addition some radioactivity was

Fig. 1. Uptake of ^{131}I by the thyroid glands of rats 0–6 hr. after the administration of $[^{131}\text{I}]$ iodide. $\circ$, Rats fed on the powdered low-iodine diet; $\square$, rats fed on the commercial rat pellets.

Fig. 2. Relation between $[^{131}\text{I}]$ iodothyronine content of rat thyroid-gland hydrolysates and the quantity of radioactive material remaining at the origin of the chromatograms.

always present in a minor peak at the origin, never amounting to more than 2.5% of the total, and consisting of incompletely hydrolysed iodoprotein.

Origin material. The percentage of the radioactivity of the hydrolysate that was present in the origin peak increased with time after the administration of [^{131}I]iodide. This is in agreement with a similar finding by Rosenberg, Goldman, LaRoche & Dimick (1964): these authors attributed the effect to differing labilities of the ^{131}I -labelled tyrosyl residues at different times after the dose. In the present work, however, the radioactivity of the origin could be related to the content of [^{131}I]iodothyronines in the gland (Fig. 2). The effect may therefore be explained by supposing that the origin material is iodothyronine-rich; since the specific radioactivity of the iodothyronines increases only slowly after a dose of [^{131}I]iodide, iodothyronines represent initially only a small percentage of the radioactivity after a dose of [^{131}I]iodide, gradually increasing as isotopic equilibrium is approached (Plaskett *et al.* 1963a). Clearly, an iodothyronine-rich origin material would follow a similar pattern of labelling. The suggestion that origin material is iodothyronine-rich is in accord with the finding of Tong & Chaikoff (1958) that iodothyronines are less readily released from iodoproteins than are iodotyrosines during enzymic digestion.

Iodothyronines. The radioactivity of the iodothyronine peak was determined in rats that were being given each of the two diets with and without stimulation by thyroid-stimulating hormone. The results are presented in Table 1. Under all conditions tested the iodothyronine fraction, expressed as a percentage of the total radioactivity of the hydrolysate, increased with time after the administration

of [^{131}I]iodide. In rats fed on the pellet diet the maximum reached was 12.6%, whereas rats given the low-iodine diet attained 17.5%. In neither case were these results markedly affected by the administration of thyroid-stimulating hormone. The only observed effect of thyroid-stimulating hormone was a very small fall in the iodothyronine content of the iodine-deficient glands.

R values. Monoiodotyrosine and di-iodotyrosine together usually constituted 60–80% of the total radioactivity of the hydrolysate, but the experimental conditions markedly affected the ratio between them (Table 2). In rats fed on the low-iodine diet and given no thyroid-stimulating hormone the *R* values were close to unity and did not change with time after the administration of [^{131}I]iodide. This confirms the finding of Plaskett *et al.* (1963a). However, in rats fed on the pellet diet the *R* values were all well below unity and showed a significant decrease with time after the administration of [^{131}I]iodide. In neither case were the *R* values markedly affected by the administration of thyroid-stimulating hormone. The only effect of thyroid-stimulating hormone observed was a small diminution of the *R* values in the late time-intervals (16–38 hr.) after the injection of [^{131}I]iodide.

Occasional additional component of thyroid-gland hydrolysates. A fifth major component of the thyroid-gland hydrolysate was found in about 5% of the animals used for this work. In the butan-1-ol-acetic acid solvent system it runs between di-iodotyrosine and the iodothyronines. When present, it varied from very low amounts up to 31% of the total radioactivity. A curious feature was that the radioactivity of the unknown component appeared

Table 1. *Incorporation of ^{131}I into the iodothyronine fraction of rat thyroid gland after a single dose of [^{131}I]iodide*

The results were obtained with rats fed on different diets with and without acute dosage with thyroid-stimulating hormone (TSH). The results are given as means $\pm$ s.e.m. with the numbers of determinations in parentheses.

Time after administration of [^{131}I]iodide (hr.)	Iodothyronines (% of total thyroid-gland radioactivity)					
	Rats fed on commercial rat pellets	Rats fed on commercial rat pellets and receiving TSH	Rats fed on low-iodine diet	Rats fed on low-iodine diet and receiving various doses of TSH		
				0.5 i.u.	1.0 i.u.	2.0 i.u.
0.5	3.1 $\pm$ 0.16 (12)	—	3.3 $\pm$ 0.18 (5)	—	—	—
1	—	—	5.7 $\pm$ 0.19 (6)	—	—	—
2	5.9 $\pm$ 0.19 (16)	6.1 $\pm$ 0.35 (10)	6.7 $\pm$ 0.21 (6)	4.5 $\pm$ 0.33 (6)	5.6 $\pm$ 0.40 (9)	5.3 $\pm$ 0.19 (11)
4	—	—	9.0 $\pm$ 1.1 (8)	—	—	—
6	9.1 $\pm$ 0.61 (16)	10.6 $\pm$ 0.46 (10)	—	—	—	—
8	—	—	14.8 $\pm$ 1.0 (6)	—	—	—
16	8.7 $\pm$ 0.17 (10)	10.0 $\pm$ 0.65 (10)	—	15.0 $\pm$ 1.1 (9)	13.8 $\pm$ 0.92 (9)	12.8 $\pm$ 1.0 (6)
24	11.1 $\pm$ 0.40 (10)	11.6 $\pm$ 0.25 (10)	17.5 $\pm$ 1.2 (5)	—	—	—
38	12.6 $\pm$ 0.52 (10)	10.7 $\pm$ 0.49 (10)	—	—	—	—

Table 2. *Ratio of mono[¹³¹I]iodotyrosine to di[¹³¹I]iodotyrosine (*R* values) in rat thyroid glands after a single dose of [¹³¹I]iodide*

The results were obtained with rats fed on different diets with and without acute dosage with thyroid-stimulating hormone (TSH). The results are given as means $\pm$ s.e.m. with the numbers of determinations in parentheses.

Time after administration of [¹³¹ I]iodide (hr.)	<i>R</i> value					
	Rats fed on commercial rat pellets	Rats fed on commercial rat pellets and receiving TSH	Rats fed on low-iodine diet	Rats fed on low-iodine diet and receiving TSH		
				0.5 i.u.	1.0 i.u.	2.0 i.u.
0.5	0.80 $\pm$ 0.01 (6)	—	1.06 $\pm$ 0.03 (5)	—	—	—
1	—	—	1.09 $\pm$ 0.03 (5)	—	—	—
2	0.73 $\pm$ 0.005 (18)	0.76 $\pm$ 0.01 (12)	0.98 $\pm$ 0.03 (6)	1.07 $\pm$ 0.03 (6)	1.08 $\pm$ 0.03 (9)	1.05 $\pm$ 0.02 (11)
4	—	—	1.02 $\pm$ 0.03 (6)	—	—	—
6	0.66 $\pm$ 0.005 (18)	0.66 $\pm$ 0.01 (12)	—	—	—	—
8	—	—	1.01 $\pm$ 0.02 (6)	—	—	—
16	0.61 $\pm$ 0.01 (12)	0.51 $\pm$ 0.01 (12)	—	0.94 $\pm$ 0.02 (9)	0.95 $\pm$ 0.02 (6)	0.87 $\pm$ 0.02 (3)
24	0.50 $\pm$ 0.01 (12)	0.45 $\pm$ 0.01 (12)	1.06 $\pm$ 0.03 (7)	—	—	—
38	0.49 $\pm$ 0.01 (12)	0.46 $\pm$ 0.01 (12)	—	—	—	—

Table 3. *Analytical results on rat thyroid-gland hydrolysates showing the presence of a fifth, unidentified, major component*

Values for mono[¹³¹I]iodotyrosine, di[¹³¹I]iodotyrosine and the ¹³¹I-labelled additional component are each expressed as a percentage of total thyroid-gland radioactivity. MIT, Monoiodotyrosine; DIT, di-iodotyrosine; U, unidentified component.

MIT	DIT	U	MIT+U	MIT/DIT ratio	MIT+U/DIT ratio
Low-iodine diet; mean value for other rats given this diet = 1.01					
27.1	35.3	9.2	36.3	0.77	1.03
28.7	33.3	6.2	34.9	0.86	1.05
30.2	33.4	1.3	31.5	0.90	0.94
19.7	38.9	26.9	46.6	0.51	1.19
6.8	34.2	31.0	37.8	0.20	1.11
22.9	37.5	8.3	31.2	0.61	0.83
36.1	39.8	2.2	38.3	0.91	0.96
27.5	38.5	9.1	36.6	0.72	0.95
23.8	31.6	8.3	32.1	0.75	1.02
Commercial pellet diet					
(a) 24 hr. after administration of [¹³¹ I]iodide and after thyroid-stimulating hormone. Mean <i>R</i> value for other rats under these conditions = 0.45. Duplicate determinations on one hydrolysate					
7.6	46.0	17.1	24.7	0.16	0.54
8.1	47.1	16.5	24.6	0.17	0.52
(b) 38 hr. after administration of [¹³¹ I]iodide and after thyroid-stimulating hormone. Mean <i>R</i> value for other rats under these conditions = 0.46. Duplicate determinations on one hydrolysate					
13.1	45.8	7.6	20.7	0.29	0.45
13.9	45.7	7.0	20.9	0.30	0.46

to replace a similar amount of radioactivity that was normally present as monoiodotyrosine. In all cases where the additional component was present the *R* value was abnormal because the monoiodotyrosine content was diminished compared with other rats investigated under the same conditions, but the value of the ratio of monoiodotyrosine plus

the additional component to di-iodotyrosine was similar to the normal *R* value. It therefore seems likely that the additional component may be derived from monoiodotyrosine, either by a metabolic reaction *in vivo* or as an artifact during the analysis.

Table 3 shows analytical results obtained with

Fig. 3. Chromatogram scans showing results of analysing rat thyroid-gland hydrolysates in the solvent system butan-1-ol-2*N*-acetic acid (1:1, v/v). (a) An example of the usual result obtained in rats fed on the low-iodine diet; (b) an example of a hydrolysate containing an unknown additional component (see also Table 3); this rat was also given the low-iodine diet. O, Origin; SF, solvent front; I⁻, I⁻ ion; MIT, moniodotyrosine; DIT, di-iodotyrosine; T₄, thyroxine; T₃, 3,3',5-tri-iodothyronine; U, unidentified component.

11 hydrolysates that contained the additional component. Because the *R* values are abnormal these results have been omitted during the compilation of Tables 1 and 2.

The chromatographic separation of the additional component is illustrated in Fig. 3(b), and may be compared with the normal separation in Fig. 3(a).

DISCUSSION

Effect of diet on the R values. The observation reported above that iodine deficiency increases the ratio of mono[¹³¹I]iodotyrosine to di[¹³¹I]iodotyrosine in the thyroid gland confirms previous findings to this effect by many workers (Leloup & Lachiver, 1955; Querido, Schut & Terpstra, 1957; Bois & Larsson, 1958a).

However, it is a new finding that the kinetics of

labelling of the iodotyrosine pools can be markedly affected by diet. Rosenberg *et al.* (1964), the only authors who have hitherto published investigations in this field, found that diet profoundly affected the equilibrium percentages for mono- and di-iodotyrosine, but their data show no very marked effect of diet on the way in which these pools become labelled.

The high *R* values observed in rats given the low-iodine diet are constant from shortly after the administration of [¹³¹I]iodide up to 24 hr. afterwards. This indicates that, if the pool sizes of the two iodotyrosines remain constant during the period of the experiment, then the ratio of their specific radioactivities does not change with time. Since isotopic equilibrium, at which these specific radioactivities are, by definition, equal, is almost reached in 24 hr. (Pitt-Rivers, 1962), one must conclude that the specific radioactivities of the two iodotyrosines are equal throughout the experiment. This is illustrated in Fig. 4(a), in which the relative specific radioactivity [calculated as described by Pitt-Rivers (1962) from the data in Table 2] has been plotted against time for rats on the low-iodine diet.

Fig. 4(b) illustrates the result of the same calculation for rats on the pellet diet. The change in the *R* value with time in rats on this diet clearly reflects a change in the ratio of the specific radioactivities of the two iodotyrosines. For comparison with Figs. 4(a) and 4(b), Fig. 4(c) shows corresponding curves drawn from the data of Pitt-Rivers (1962). These show a much greater change in the ratio of the specific radioactivities than Fig. 4(b), the *R* values at the shortest time-intervals being particularly high.

These results are compatible with the view that the difference between the labelling kinetics described by Pitt-Rivers (1962) and those described by Plaskett *et al.* (1963a) may be due to a dietary difference, the pellet diet used in the present work producing labelling kinetics intermediate between these two extremes. The dietary factor or factors responsible for the differences have yet to be identified. The simplest explanation would be that the dietary iodide intake is responsible. The low-iodine diet used in the present work and used by Plaskett *et al.* (1963a,b) is made up in this Laboratory from spray-process skimmed milk powder (25%), dried yeast (5%) and wholemeal flour (70%). By contrast, the commercial pellet diet contains 5% of the iodine-rich component, fish meal. If iodide is the responsible factor the effect could be explained readily on the basis of the two-compartment mechanism of thyroid-hormone formation put forward by Plaskett *et al.* (1963a,b). As suggested by these authors, iodide could exert such an effect by altering differentially the pool

Fig. 4. Relationship between relative specific radioactivity and time after the injection of $[^{131}\text{I}]$ iodide. Values for relative specific radioactivity have been calculated from experimental data as described by Pitt-Rivers (1962). (a) Rats fed on the powdered low-iodine diet; (b) rats fed on a commercial pellet diet; (c) reconstructed from the data of Pitt-Rivers (1962), the results obtained by that author being with a commercial pellet diet. $\square$, Monoiodotyrosine; $\circ$, di-iodotyrosine.

size and turnover times of two metabolically distinct fractions of thyroglobulin.

However, the present authors consider that further work is necessary before the influence of dietary history on labelling kinetics can be ascribed with certainty to iodide because the diets differ in numerous respects other than iodide content. Moreover, Plaskett *et al.* (1963a) found that the administration of sodium iodide to rats on the low-iodine diet merely diminished the R values

without inducing them to change markedly with time.

Effect of thyroid-stimulating hormone on the R values. Previous work on the effect of thyroid-stimulating hormone on the R values has been extensive, and the evidence available indicates that the R values at isotopic equilibrium are diminished by thyroid-stimulating hormone. Taurag *et al.* (1958) showed that in hypophysectomized rats the R values are very high and are restored to

normal by the administration of thyroid-stimulating hormone. A smaller effect of hypophysectomy on the R values, but in the same direction, is apparent from the data of Rosenberg, Dimick & LaRoche (1963). Two studies have reported R values lower than normal resulting from over-stimulating with endogenous thyroid-stimulating hormone (Cukier & Triantaphyllidis, 1959; Grinberg, Volpert & Werner, 1962). Thyroid-stimulating hormone thus promotes the di-iodination of tyrosine.

Measurement of R values after the administration of exogenous thyroid-stimulating hormone to normal animals has given less conclusive results. For example, Grinberg *et al.* (1962), in support of the thesis that the hormone promotes di-iodination of tyrosine, found that in mice given 0.5 i.u. the R value 24 hr. after the administration of [^{131}I]iodide was below control levels. However, Bois & Larsson (1958b) found that the R value was increased (they reported that the di-iodotyrosine/moniodotyrosine ratio decreased) in rats after the administration of 2.0 i.u. of thyroid-stimulating hormone. The literature has become confused on this point since Grinberg *et al.* (1962) incorrectly quoted Bois & Larsson (1958b) as having been in agreement with their own findings.

In the present work thyroid-stimulating hormone was found to cause a very small diminution of the R value when the interval between the administration of [^{131}I]iodide and death of the animal was 16 hr. or more. For example, the mean of the 35 determinations of the R value in animals receiving the low-iodine diet and no thyroid-stimulating hormone was 1.035 ± 0.014 ; with thyroid-stimulating hormone in addition, 16 hr. after the administration of [^{131}I]iodide, the mean R value fell to 0.931 ± 0.016 . Similarly, in rats fed on the pellet diet the R value observed after the administration of thyroid-stimulating hormone was lower than that of the control animals at 16, 24 and 38 hr. after the administration of [^{131}I]iodide (Table 2). Therefore the report of Grinberg *et al.* (1962) that thyroid-stimulating hormone promotes di-iodination of tyrosine is substantiated; however, it is also clear, again in agreement with Grinberg *et al.* (1962), that this effect is rather weak in normal animals already receiving stimulation by thyroid-stimulating hormone from their own pituitaries.

In the present work the effect of thyroid-stimulating hormone was investigated because it seemed likely that it would induce altered labelling kinetics because of the control that it exerts on key steps in hormone synthesis. It is clear from Table 2 that thyroid-stimulating hormone was not very effective in this respect. The rather weak effect that thyroid-stimulating hormone did exert on the R values was apparent only at late times after the administration of [^{131}I]iodide; it therefore probably

reflects a small change in the ratio of the mono- [^{127}I]iodotyrosine and di [^{127}I]iodotyrosine pools rather than a change in the way in which these pools become labelled.

Effect of diet on the iodothyronine content of the gland. Iodothyronines represented a greater percentage of the total thyroid-gland radioactivity in the iodine-deficient animals than in animals fed on the pellet diet. Apparently the rat thyroid gland compensates for iodine deficiency, not only by an increased avidity for plasma iodide, and, as is well known, a faster turnover of iodine, but also by converting a greater proportion of its iodine content into iodothyronines (Table 1).

Pathway of tri-iodothyronine biosynthesis. The finding that the R values change with time in rats given the pellet diet opens the way for a further investigation of the problem of tri-iodothyronine biosynthesis. If tri-iodothyronine arises by direct coupling of mono- and di-iodotyrosine then the tri-iodothyronine isolated from the thyroid glands of these rats 30 min. after the administration of [^{131}I]iodide would be expected to contain iodine of different specific radioactivity on the α - and the β -rings.

Up to the time of original submission of this paper no workers had succeeded in measuring the specific radioactivity of iodine on the two rings of tri-iodothyronine at sufficiently short time-intervals after administering [^{131}I]iodide. This has now been achieved by Lissitzky, Simon & Benabdeljlil (1964) and Plaskett & Barnaby (1964). Although the results of the two groups of workers are at variance in some respects, both have found that the tri-iodothyronine rings are unequally labelled and have concluded that tri-iodothyronine does not arise in the thyroid from thyroxine.

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