DEPENDENCE OF $^{131}$I-LABELLED THYroxINE BIOSYNTHE$^{131}$I UPTAKE AND $^{131}$I-LABELLED DI-IODOTYROSINE FORMATION IN VIVO

ROSALIND PITT-RIVERS

National Institute for Medical Research, Mill Hill, London, N.W.7

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SUMMARY

(1) $^{131}$I uptakes and the distribution of $^{131}$I in the iodoamino acids of the thyroid have been measured in rats fed a low iodine diet and very small doses of antithyroid agents. (2) 16 hr. and 4 hr. after the dose the labelled monoiodotyrosine content was inversely proportional, and the labelled di-iodotyrosine content directly proportional to the $^{131}$I uptake. (3) The labelled thyroxine content was directly proportional to the $^{131}$I uptake and labelled di-iodotyrosine content. (4) No obvious correlation between labelled tri-iodothyronine content and $^{131}$I uptake or labelled iodotyrosine content was apparent.

INTRODUCTION

Experiments were undertaken in an attempt to obtain rat thyroid glands containing labelled iodotyrosines in the absence of labelled iodothyronines by the administration of very small amounts of antithyroid agents according to Richards & Ingbar (1959). The object was to obtain glands containing labelled iodotyrosines only, so that the coupling reaction leading to thyroid hormone formation might be studied in vitro.

MATERIALS AND METHODS

Male hooded rats (180–200 g.) were maintained on a low iodine diet for 1–4 weeks. In some animals the diet was supplemented with 0·001% thiouracil or 0·001% propylthiouracil in the drinking water 2–4 days before injection of $^{131}$I. By this means a wide variety of $^{131}$I uptakes was obtained, but in no instance was complete suppression of iodothyronine formation observed.

The rats were injected intraperitoneally with 20–35 $\mu$C carrier-free $^{131}$I obtained from the Radiochemical Centre, Amersham, and in the first experiment were killed 16 hr. later. The thyroid glands were immediately removed and the $^{131}$I uptake was measured. The glands were then hydrolysed with Pronase in 0·2 M-phosphate buffer, pH 7·5, for 4–4½ hr. and chromatographed on paper in $n$-butanol equilibrated with 2 $n$-acetic acid and in $n$-butanol:ethanol:0·5 $n$-ammonia (5:1:2). The distribution of radioactivity on the chromatograms was determined in a recording strip counter.
RESULTS

The results are shown in Figs. 1–3. In Figs. 1 and 2, the labelled iodoamino acids have been expressed as a percentage of organic $^{131}$I rather than the total, since in some of the more blocked glands values of 20–40% $^{131}$I$^-$ were obtained.

Figure 1 shows that the labelled di-iodotyrosine (DIT) content is directly proportional to the $^{131}$I uptake, whereas the labelled monoiodotyrosine (MIT) content is inversely proportional to the uptake (see Broadhead, Pearson & Wilson, 1965). Similarly in Fig. 2, the labelled thyroxine (T$_4$) content depends on $^{131}$I uptake;

![Graph showing the distribution of $[^{131}I]$monoiodotyrosine (▲) and $[^{131}I]$di-iodotyrosine (△) as a function of thyroidal $^{131}$I uptake.](image-url)

with tri-iodothyronine (T$_3$) this dependence is probably only seen at very low uptakes. Figure 3 shows the dependence of labelled T$_4$ formation on the labelled DIT content of the gland, and also shows the lack of dependence of labelled T$_3$ formation on the DIT content. When labelled T$_3$ is plotted against labelled MIT, a similar lack of correlation is observed.

In a second experiment, rats were maintained on the same regimen, but were killed 4 hr. after intraperitoneal injection of $^{131}$I, in order to determine whether any dependence of labelled T$_3$ formation on labelled iodothyrosine content could be observed at a time before the T$_3$ curve had flattened out (Pitt-Rivers, 1962). The results obtained in this experiment were very similar to those described above, and have not been plotted. The only difference was that the maximum levels of labelled hormones were somewhat lower than in the first experiment.
In only two thyroids was the amount of labelled T₃ found to equal the labelled T₄. This occurred when the uptake was low (< 5%) and when the labelled DIT content was less than 15%. In no instance did the labelled T₃ exceed significantly the labelled T₄ (see Lachiver & Leloup, 1955; Querido, Schut & Terpstra, 1957; Studer & Greer, 1965).

DISCUSSION

The failure of labelled T₃ to exceed labelled T₄ in any of these experiments, compared with the findings of Lachiver & Leloup (1955), cannot be explained. These authors found labelled T₃:T₄ ratios of 2:1 in the thyroids of iodine-deficient rats when the MIT:DIT ratio approached 3:1.

One question arises: why does the labelled T₃ not exceed a maximum level of 5% of the thyroidal ¹³¹I when the labelled T₄ has reached 3 to 4 times that value and when there appear to be adequate amounts of both its precursors? A possible explanation is that the rate of formation of T₄ is somewhat greater than that of T₃, thereby favouring the incorporation of their common precursor, DIT, into T₄. Another possibility is that the rate of hydrolysis of thyroglobulin, with release and subsequent deiodination of the iodotyrosines occurs faster than the biosynthesis of T₃ (but not of T₄), thereby preventing the accumulation of more than a limited amount of T₃. A third possibility is that the spatial distribution of DIT in thyroglobulin favours its coupling with itself rather than with MIT. These suggestions can only be considered as tentative, since the mechanism of the coupling reaction in vivo is at present unknown.

One point seems clear from these results: neither of the iodothyronines appears to be the precursor of the other, both of which hypotheses have been suggested (see...
Pitt-Rivers & Cavalieri, 1964). If T₄ were the precursor of T₃, then the levels of the latter would be expected to increase with increasing levels of the former. If T₃ were the precursor of T₄, then the rapid levelling off of T₃ should be accompanied by a plateau in the T₄ curve; but these results were not obtained.

From these results it appears that the biosynthesis of T₄ depends only upon an adequate supply of DIT. The situation with regard to T₃ is more difficult to interpret. It seems that, given a minimum of DIT and MIT in thyroglobulin, T₃ will be formed in an amount which is constant within certain limits. Moreover, above this minimum, T₃ formation appears to be independent of the level of either of its precursors. Its synthesis is only seriously impaired when the level of inorganic ¹³¹I⁻ reaches significant proportions (30% or more of thyroidal ¹³¹I), and reaches a maximum when T₄ synthesis has reached only about one-fifth of its maximum value.

REFERENCES


