Sequential Deiodination of Thyroxine in Rat Liver Homogenate

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Rat liver homogenate was incubated at 37°C with thyroxine, 3,3',5-tri-iodothyronine, 3,3',5'-tri-iodothyronine or 3,3'-di-iodothyronine. The degradation or accumulation of these compounds was measured by specific radioimmunoassays. (1) Production of 3,3',5-tri-iodothyronine from thyroxine was highest at pH 6.0–6.5 and was markedly stimulated by the addition of dithiothreitol and effectively inhibited in the presence of 6-propyl-2-thiouracil. (2) Accumulation of 3,3',5'-tri-iodothyronine on incubation of thyroxine with homogenate was only observed above pH 8.5. Otherwise the product was converted into 3,3'-di-iodothyronine too rapidly to allow its measurement. By measuring 3,3'-di-iodothyronine it was deduced that 5-deiodination of thyroxine was most effective at approx. pH 8.0. Dithiothreitol powerfully stimulated this reaction and 6-propyl-2-thiouracil strongly inhibited. (3) Monodeiodination of the tyrosine ring of 3,3',5-tri-iodothyronine was the slowest reaction, was optimal at pH 8.0 and was less affected by dithiothreitol and 6-propyl-2-thiouracil than the above reactions. (4) 5'-Deiodination of 3,3',5'-tri-iodothyronine was extremely rapid, with a pH optimum probably at about 6.5. Owing to the high reaction rate under the conditions used it was not possible to assess the effects of dithiothreitol and 6-propyl-2-thiouracil.

In recent years several findings have aroused new interest in the metabolism of thyroid hormones, especially the deiodinative pathways. It has become evident that under normal conditions the products of the thyroid, 3,3',5,5'-tetraiodothyronine (thyroxine) and 3,3',5-tri-iodothyronine (tri-iodothyronine), are secreted in a ratio that far exceeds the ratio of their turnover rates (Abrams & Larsen, 1973; Chopra & Solomon, 1973). This means that most of the circulating tri-iodothyronine is of extrathyroidal origin, which is in line with previous observations on plasma tri-iodothyronine concentrations in athyreotic subjects treated with thyroxine (Braverman *et al.*, 1970). Extrapolating from studies on thyroxine-supplemented individuals, Surks *et al.* (1973) calculated that in healthy humans with a sufficient iodine intake over 80% of the tri-iodothyronine turnover is derived from peripheral monodeiodination of thyroxine.

The rediscovery of 3,3',5'-tri-iodothyronine (reverse tri-iodothyronine) by Chopra (1974) has also contributed considerably to our understanding of the mechanism of regulation of thyroid-hormone activity by the peripheral tissues. It is now generally considered that the metabolism of thyroxine, at least in man, proceeds primarily by means of deiodination (for a review see Cavalieri & Rapoport, 1977). Monodeiodination yields either the principal active form of thyroid hormone, tri-iodothyronine (by deiodination of the phenolic ring), or the inactive

metabolite, reverse tri-iodothyronine (by elimination of an iodine atom from the tyrosine ring). Both products are probably subject to further degradation into lower substituted iodothyronines (Wu *et al.*, 1976; Rudolph *et al.*, 1977). It has been shown that plasma tri-iodothyronine and reverse tri-iodothyronine vary in a reciprocal fashion in several clinical situations: in starvation (Vagenakis *et al.*, 1975), systemic illness (Burger *et al.*, 1976), after surgical operation (Burr *et al.*, 1975) or on administration of the glucocorticosteroid analogue dexamethasone (Chopra *et al.*, 1975), of the β -adrenergic blocking agent propranolol (Verhoeven *et al.*, 1977) or of the goitrogenic compound 6-propyl-2-thiouracil (Westgren *et al.*, 1977). The time course of the changes during prolonged caloric restriction in the diet seems to indicate that monodeiodination of thyroxine involves separate processes (Visser *et al.*, 1978).

The development of specific radioimmunoassays for the measurement of iodothyronines provides us with valuable tools to conduct studies *in vitro* of the monodeiodinative pathways. We previously reported on the enzymic production of tri-iodothyronine from thyroxine in rat liver homogenate (Visser *et al.*, 1975a) and in the microsomal fraction (Visser *et al.*, 1976). We now demonstrate that conversion of thyroxine into reverse tri-iodothyronine also occurs in this system. This reaction, however, cannot be observed directly, owing to the very efficient

breakdown of reverse tri-iodothyronine into 3,3'-di-iodothyronine (di-iodothyronine). Similar findings have been reported by Hüfner & Grussendorf (1977). Consequently, monodeiodination of thyroxine to reverse tri-iodothyronine was determined by measuring the production of di-iodothyronine, since in this sequence the first reaction is the rate-limiting step and the contribution of the pathway via tri-iodothyronine was found to be negligible.

Experimental

Materials

L-Thyroxine, 3,3',5-tri-iodo-L-thyronine and dithiothreitol were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. 3,3',5'-Tri-iodo-L-thyronine and 3,3'-di-iodo-L-thyronine were obtained by courtesy of Dr. E. Scheffele, Henning G.m.b.H., Berlin, Germany, and 3-iodo-L-thyronine was kindly provided by Dr. P. Block, Jr., University of Toledo, Toledo, OH, U.S.A. [3',5'-¹²⁵I]Thyroxine (specific radioactivity over 200 $\mu\text{Ci}/\mu\text{g}$), tri-[3'-¹²⁵I]-iodothyronine (specific radioactivity over 1200 $\mu\text{Ci}/\mu\text{g}$) and Na¹²⁵I (approx. 14 mCi/ μg) were from The Radiochemical Centre, Amersham, Bucks., U.K. Reverse tri-[3',5'-¹²⁵I]iodothyronine (approx. 2700 $\mu\text{Ci}/\mu\text{g}$) was prepared as previously described (Visser *et al.*, 1977) and di-[3'-¹²⁵I]iodothyronine (approx. 3300 $\mu\text{Ci}/\mu\text{g}$) was prepared similarly starting from 3-iodothyronine. Goat anti-(rabbit γ -globulin) serum was from Antibodies Inc., Davis, CA, U.S.A.

Preparation of liver homogenate and conversion studies

The postnuclear supernatant of rat (Wistar) liver homogenate was prepared in 0.25M-sucrose in 5mM-Tris/HCl, pH 7.4, as previously described (Visser *et al.*, 1975a). Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard. The iodothyronines were dissolved in 0.1M-NaOH and diluted in 0.25M-sucrose in 0.025M-Tris/HCl, pH 7.4, and added in 200 μl quantities for incubation with 1800 μl of homogenate. In the experiments dealing with possible effects of dithiothreitol, EDTA and 6-propyl-2-thiouracil (propylthiouracil), 200 μl samples of homogenate were added to mixtures of 100 μl of iodothyronine solution and 700 μl of 0.25M-sucrose in 0.025M-Tris/HCl, pH 7.4, containing 2.8mM-dithiothreitol, 2.8mM-EDTA or 0.1mM-propylthiouracil. In the experiments in which the dependence of the several reactions on pH was studied, 100 μl samples of homogenate were added to mixtures composed of 100 μl of the iodothyronine solution and 800 μl of 0.15M-sodium acetate, 0.15M-sodium phosphate or 0.15M-sodium borate buffer, containing 4mM-

dithiothreitol, with a pH range of 4.0–10.0. Control experiments were conducted in which homogenate was replaced by 0.25M-sucrose in 5mM-Tris/HCl, pH 7.4. This yielded values for recovery and spontaneous deiodination. All experiments were carried out in duplicate at 37°C. After various time intervals, 250 μl portions of the reaction mixture were added to 750 μl of 95% (v/v) ethanol kept at 0°C. The precipitates were spun down (1500g, 10min) and the iodothyronine concentrations were measured in duplicate directly in 100 μl portions of the supernatant.

Measurement of thyroxine, tri-iodothyronine, reverse tri-iodothyronine and di-iodothyronine

The iodothyronines were determined by specific radioimmunoassays. Thyroxine (Visser *et al.*, 1975b), tri-iodothyronine (Visser *et al.*, 1975a) and reverse tri-iodothyronine (Visser *et al.*, 1977) were measured as previously reported. Di-iodothyronine was assayed by the method of T. J. Visser, L. M. Krieger-Quist, R. Docter & G. Hennemann (unpublished work) as briefly described in the following. Di-[¹²⁵I]iodothyronine (about 12000 c.p.m.) was made to react with appropriately diluted (1:200000) antiserum in the absence or presence of unlabelled di-iodothyronine or other materials to be tested (final volume 1 ml). All reagents were dissolved in 0.06M-sodium barbitone/0.15M-NaCl containing bovine serum albumin, pH 8.6. After incubation for 2–4 days at 8°C antibody-bound di-[¹²⁵I]iodothyronine was precipitated by addition of goat anti-(rabbit immunoglobulin G) antiserum. The lower limit of detection is 1 pg of di-iodothyronine per assay tube. The relative affinity of thyroxine for the antiserum is less than 0.004 (di-iodothyronine = 100), that of tri-iodothyronine 0.6 and that of reverse tri-iodothyronine 0.06.

The composition of the assay mixtures is shown in Table 1.

Results

Fig. 1 shows the accumulation of the various iodothyronines when rat liver homogenate was incubated with 0.15 μM -thyroxine, 0.14 μM -tri-iodothyronine or 0.013 μM -reverse tri-iodothyronine. Production of tri-iodothyronine and di-iodothyronine from thyroxine increased with time. Di-iodothyronine production from tri-iodothyronine appeared to slow down after 20 min. In contrast, formation of reverse tri-iodothyronine from thyroxine was not measurable, owing to the very high conversion rate of reverse tri-iodothyronine into di-iodothyronine. Within 3 min of incubation all reverse tri-iodothyronine added was converted into di-iodothyronine.

Table 1. *Composition of radioimmunoassay mixtures*
Standards were made in 0.06M-sodium barbitone/0.15M-NaCl, pH 8.6, containing 1% bovine serum albumin (buffer A). Antiserum was dissolved in the same buffer containing 0.1% bovine serum albumin (buffer B) and 2.5% normal rabbit serum. Extracts of reaction mixtures were made as described in the Experimental section. Extracts of homogenate for incorporation in the standard curve were prepared similarly.

	Volume for standard curve (μl)	Volume for samples (μl)
Standard	100	—
Extract of reaction mixture	—	100
Extract of homogenate	100	—
Buffer A	—	100
[¹²⁵ I]Iodothyronine in buffer A	100	100
Antiserum	100	100
Buffer B	600	600

Fig. 1. *Deiodination of several iodothyronines in rat liver homogenate at pH 7.4 as function of time of incubation*
Accumulation of tri-iodothyronine (○), reverse tri-iodothyronine (●) and di-iodothyronine (Δ) from 1.15 μM-thyroxine, of di-iodothyronine from 0.14 μM-tri-iodothyronine (▲) and of di-iodothyronine from 0.013 μM-reverse tri-iodothyronine (□) was studied. The reaction mixtures contained, in addition to the iodothyronines, 2.7 mM-dithiothreitol and 26.8 mg of protein/ml.

The stability of the iodothyronines in rat liver homogenate is shown in Fig. 2. Thyroxine and tri-iodothyronine were stable under the conditions used. Di-iodothyronine was degraded more rapidly; during 60 min of incubation its concentration decreased by over 50%. Reverse tri-iodothyronine was broken down very effectively in the homogenate;

Fig. 2. *Fate of the several iodothyronines during incubation with rat liver homogenate at pH 7.4*
Rat liver homogenate was incubated with 1.15 μM-thyroxine (○), 0.14 μM-tri-iodothyronine (●), 0.013 μM-reverse tri-iodothyronine (▲) and 0.01 μM-di-iodothyronine (Δ). After several time intervals the concentration of iodothyronine remaining was determined and expressed in terms of the concentration added. For incubation conditions see the legend to Fig. 1.

after 3 min all added reverse tri-iodothyronine had disappeared. Fig. 1 shows that this rapid disappearance reflects conversion into di-iodothyronine.

Experiments were conducted in which the effect of the addition of dithiothreitol, EDTA and propylthiouracil was studied (Table 2). The presence of 2.8 mM-dithiothreitol resulted in approx. 8-fold enhancement of the deiodination of thyroxine into tri-iodothyronine and into di-iodothyronine after incubation for 60 min. The enhancement of the conversion of tri-iodothyronine into di-iodothyronine and the degradation of added di-iodothyronine was approx. 2-fold. The effect of dithiothreitol was more pronounced after 60 min than after 5 min. Addition of EDTA further enhanced the conversion rates during incubation for 60 min. Again, tri-iodothyronine was stable; even in the presence of dithiothreitol and EDTA, loss of tri-iodothyronine was less than 20%, as with thyroxine (results not shown). Reverse tri-iodothyronine was again broken down extremely rapidly. Even in the control experiment (without any addition) only 9% of added reverse tri-iodothyronine was left after incubation for 5 min.

Propylthiouracil inhibited the conversion of thyroxine into tri-iodothyronine and di-iodothyronine markedly (80–90% inhibition). However, the inhibition of the conversion of tri-iodothyronine into di-iodothyronine was only approx. 10%. Propylthiouracil seemed to enhance the production of di-iodothyronine from reverse tri-iodothyronine.

Table 2. *Effect of addition of dithiothreitol, EDTA and propylthiouracil on the deiodination of the several iodothyronines in rat liver homogenate at pH 7.4*
Incubations were performed as outlined in the Experimental section. Liver protein concentration was 5.8 mg/ml.

Additions	Incubation time (min)	...	Iodothyronine measured (nM)					
			Tri-iodothyronine		Reverse tri-iodothyronine		Di-iodothyronine	
			5	60	5	60	5	60
0.4 μ M-Thyroxine (control)			3.3	6.4	0.0	0.0	1.1	2.0
Control+2.8 mM-dithiothreitol			6.3	57.8	0.0	0.0	2.0	14.3
Control+2.8 mM-dithiothreitol + 2.8 mM-EDTA			5.4	80.2	0.0	0.0	2.1	19.1
Control+2.8 mM-dithiothreitol + 2.8 mM-EDTA + 0.1 mM-propylthiouracil			4.3	9.2	0.0	0.0	1.8	3.8
0.1 μ M-Tri-iodothyronine (control)			100.0	99.9			0.1	0.8
Control+2.8 mM-dithiothreitol			100.0	89.6			0.1	1.6
Control+2.8 mM-dithiothreitol + 2.8 mM-EDTA			100.0	84.4			0.3	1.9
Control+2.8 mM-dithiothreitol + 2.8 mM-EDTA + 0.1 mM-propylthiouracil			100.0	87.1			0.3	1.7
0.01 μ M-Reverse tri-iodothyronine (control)					0.9	0.1	11.1	10.1
Control+2.8 mM-dithiothreitol					0.0	0.0	10.7	4.7
Control+2.8 mM-dithiothreitol + 2.8 mM-EDTA					0.0	0.0	8.7	3.4
Control+2.8 mM-dithiothreitol + 2.8 mM-EDTA + 0.1 mM-propylthiouracil					0.0	0.0	10.1	7.9
0.01 μ M-Di-iodothyronine (control)							8.6	6.2
Control+2.8 mM-dithiothreitol							9.0	3.0
Control+2.8 mM-dithiothreitol + 2.8 mM-EDTA							9.4	4.1
Control+2.8 mM-dithiothreitol + 2.8 mM-EDTA + 0.1 mM-propylthiouracil							9.5	5.1

This is probably due to the inhibitory effect of propylthiouracil on the degradation of di-iodothyronine, as shown in Table 2. All effects of propylthiouracil were, as with dithiothreitol, more pronounced during prolonged incubation.

Table 3. Effect of addition of propylthiouracil on the deiodination of the various iodothyronines in rat liver homogenate at pH 7.4 in the absence and presence of dithiothreitol

For details see legend to Table 2. Incubation time was 60 min; liver protein concentration was 6.4 mg/ml.

Reactions	Concn. of dithiothreitol (mM) ...	Inhibition by propylthiouracil (%)	
		0	2.8
Thyroxine → tri-iodothyronine		60–80	90
Thyroxine → di-iodothyronine		60–70	90
Tri-iodothyronine → di-iodothyronine		Not detectable	25
Reverse tri-iodothyronine → di-iodothyronine		20–40*	Not detectable
Di-iodothyronine → ?†		20–30	35

* Representing an approximate value, since initial reaction rates were not measured.
† The product of this reaction has not been identified.

Fig. 3. Dependence of the deiodination of thyroxine and tri-iodothyronine in rat liver homogenate on pH. The incubation mixture contained 3.2mM-dithiothreitol and 3.6mg of protein/ml. Incubation time was 60min. Accumulation of tri-iodothyronine (O), di-iodothyronine (●) and reverse tri-iodothyronine (▲) from 1.0μM-thyroxine and of di-iodothyronine (Δ) from 0.1 μM-tri-iodothyronine was studied. The buffers used were acetate (pH4–6), phosphate (6–8) and borate (8–10). For details see the Experimental section.

Next, the effects of propylthiouracil on the various reactions were studied in the absence as well as in the presence of dithiothreitol (Table 3). Owing to the slow conversion rate of tri-iodothyronine into di-iodothyronine in the absence of dithiothreitol, the inhibition by propylthiouracil of this reaction was not detectable under these conditions. Since for the generation of di-iodothyronine from reverse tri-iodothyronine initial reaction rates were too high for determination, the influence of the addition of propylthiouracil could not properly be measured. Addition of dithiothreitol did not alter the inhibitory effect of propylthiouracil. In all instances the reaction rates in the presence of both dithiothreitol and propylthiouracil were higher than without any addition.

Fig. 3 shows the pH profile of the various deiodination reactions in rat liver homogenate. The conversion of thyroxine into tri-iodothyronine was optimal at pH6.0–6.5, which has also been found by other investigators (Hüfner *et al.*, 1977; Höffken *et al.*, 1977). Production of di-iodothyronine from thyroxine or from tri-iodothyronine was optimal at pH8.0. In all circumstances less than 20% of added thyroxine and tri-iodothyronine was degraded after incubation for 60min (results not shown).

Since the pH-dependence of the conversion of reverse tri-iodothyronine into di-iodothyronine and

Fig. 4. Dependence of the deiodination of reverse tri-iodothyronine and of the degradation of di-iodothyronine in dilute rat liver homogenate on pH

Incubation mixtures contained 0.015μM-reverse tri-iodothyronine or 0.013μM-di-iodothyronine and 0.025mg of homogenate protein/ml. The concentration of dithiothreitol was 3.2μM. After incubation for 10min the production of di-iodothyronine (Δ) and the percentage of added reverse tri-iodothyronine (O) or di-iodothyronine (●) remaining were determined. The buffers used were acetate (pH4.5–5.5), phosphate (5.5–7.5) and borate (7.5–9.5). For details see the Experimental section.

of the degradation of reverse tri-iodothyronine was difficult to interpret, these reactions and the breakdown of di-iodothyronine were investigated by using dilute homogenate (Fig. 4). Both reverse tri-iodothyronine and di-iodothyronine were most rapidly degraded at pH 6.5. The pH optimum of the conversion of reverse tri-iodothyronine into di-iodothyronine could not easily be determined, because of the lability of the latter around pH 6.5. It was also noted that the extent of degradation of di-iodothyronine was not linearly dependent on the concentration of the homogenate in the reaction mixture (results not shown). This is probably the result of two counteracting processes; dilution of homogenate decreases not only the concentration of enzyme but also that of binding sites in the medium (Visser *et al.*, 1975a). Dilution of the homogenate therefore increases the availability of the substrate for interaction with the enzyme, the concentration of which has decreased concomitantly.

Discussion

The reactions in Scheme 1 were studied: reactions I and IV represent deiodination of the phenolic (or

outer) ring, whereas in reactions II and III an iodine atom is removed from the tyrosine (or inner) ring.

Under the conditions used reaction II could not be investigated directly, owing to the extreme lability of the product (reverse tri-iodothyronine). The rapid disappearance of the latter was virtually completely accounted for by reaction IV, since addition of reverse tri-iodothyronine to the homogenate resulted in a quantitative yield of di-iodothyronine. However, in the cascade $\text{thyroxine} \xrightarrow{\text{II}} \text{reverse tri-iodothyronine} \xrightarrow{\text{IV}} \text{di-iodothyronine}$, at least at pH 7.4, reaction II is apparently the rate-limiting step, and conversion of thyroxine into reverse tri-iodothyronine can be estimated by measuring the production of di-iodothyronine. By comparison the production of di-iodothyronine via the pathway $\text{thyroxine} \xrightarrow{\text{I}} \text{tri-iodothyronine} \xrightarrow{\text{III}} \text{di-iodothyronine}$ is negligible under all conditions tested.

Reaction IV is slowed down by increasing the pH to 9.0, thereby permitting the accumulation of reverse tri-iodothyronine (Fig. 3). Thus, since reaction IV is much faster than reaction II, except above pH 9.0, the pH optimum of the production of di-iodothyronine

Scheme 1. Sequential deiodination of thyroxine as observed in rat liver homogenate
R = CH₂-CH(NH₂)-CO₂H

ine actually reflects optimal conditions for the conversion of thyroxine into reverse tri-iodothyronine. This can also be deduced from the accumulation of the latter as a function of pH and the stability of this compound under these conditions (results not shown).

The pH optimum for reaction IV is probably about 6.5. This is indicated by the rate of accumulation of di-iodothyronine, taking into account the data on the stability of di-iodothyronine in the homogenate, which is least at pH 6.5 (Fig. 4). This is further substantiated by the finding that reverse tri-iodothyronine is most rapidly degraded in the homogenate at pH 6.5 (Fig. 4) and, since this compound is quantitatively converted into di-iodothyronine, this must point to a maximal rate of reaction IV at approx. pH 6.5. Thus it can be concluded that conditions for deiodination of the phenolic ring (reactions I and IV) are optimal at pH 6.0–6.5 and for deiodination of the tyrosine ring (reactions II and III) at pH 8.0. These findings are hard to interpret, since in the homogenate optimal conditions for the reactions will depend not only on the nature of the enzymic reaction but also on the availability of the substrates, since pH may be of importance in the binding of iodothyronines to cytoplasmic proteins (Höffken *et al.*, 1977). Nevertheless, conversion of thyroxine into tri-iodothyronine by rat liver microsomal fraction was also found to be maximal at pH 6.0 (Höffken *et al.*, 1977; T. J. Visser, unpublished work).

In line with our previous observations on the conversion of thyroxine into tri-iodothyronine by the microsomal fraction of rat liver homogenate (Visser *et al.*, 1976), the monodeiodination reactions studied are stimulated by the addition of dithiothreitol. This raises the question whether the enhanced reaction rates observed in the presence of thiol-group-containing compounds is due to their protection of cysteine residues of the enzymes or because these compounds act as cofactors or a combination of both effects. As discussed by Visser *et al.* (1976), if the sequential deiodination is accompanied by the formation of I^- , reactions I–IV can be regarded as reductions. In that case the reducing equivalents may be supplied by thiol-containing-compounds (RSH), such as dithiothreitol. The overall reaction may then be described by eqns. (1) and (2):

In this respect it is noteworthy that glutathione is almost as effective as dithiothreitol in supporting the conversion of thyroxine into tri-iodothyronine by rat liver microsomal fraction (T. J. Visser, unpublished work). It may therefore be postulated

that glutathione is the endogenous cofactor of the monodeiodination reactions.

All reactions are stimulated in the presence of EDTA, which has also been shown to enhance the production of tri-iodothyronine in the microsomal fraction (Visser *et al.*, 1976).

Administration of propylthiouracil to humans has been shown to result in increased reverse tri-iodothyronine and decreased tri-iodothyronine concentrations in plasma (Westgren *et al.*, 1977). This is in accordance with previous findings that administration of propylthiouracil to rats (Oppenheimer *et al.*, 1972), as well as to humans (Sabeti *et al.*, 1975; Geffner *et al.*, 1975), blocks the extrathyroidal production of tri-iodothyronine. Our data indicate that propylthiouracil decreases markedly the production from thyroxine of both tri-iodothyronine and reverse tri-iodothyronine (as indicated for the latter by the fall in the generation of di-iodothyronine).

Reaction III was much less affected by propylthiouracil, and its effect on reaction IV is impossible to assess, since initial reaction rates were not observed under the conditions used. Addition of propylthiouracil affected the reactions to a similar extent in both the absence and presence of dithiothreitol. This finding excludes the possibility that propylthiouracil interferes with the interaction of the cofactor with the enzyme or enzyme–substrate complex. It is, however, to be noted that the reaction that is least stimulated by dithiothreitol, i.e. production of di-iodothyronine from tri-iodothyronine, is also least inhibited by propylthiouracil.

Data bearing on the mechanism of deiodination of the several iodothyronines are scarce.

Deiodination of iodothyronines appears to involve an NADPH-dependent enzymic reaction (Rosenberg & Ahn, 1969). It has also been suggested that thyroxine is deiodinated by tyrosine hydroxylase (Dratman *et al.*, 1976). On the contrary, Hüfner *et al.* (1977) showed that conversion of thyroxine into tri-iodothyronine by rat liver homogenate is not influenced by α -methyltyrosine, a specific inhibitor of tyrosine hydroxylase.

For several reasons comparison of the data reported here with the mechanism of the enzymic and chemical dehalogenation of 5-bromo- and 5-iodo-2'-deoxyuridylate (Wataya & Santi, 1975, 1977) is of interest. First, the structure of 5-iodo-2'-deoxyuridylate (1) resembles not only that of iodothyronines, e.g. thyroxine (2), but also that of 5-propyl-2-thiouracil (3), a strong inhibitor of the conversion of thyroxine into tri-iodothyronine (Visser *et al.*, 1975a). Secondly, dehalogenation of 5-bromo- and 5-iodo-2'-deoxyuridylate as catalysed by thymidylate synthetase requires thiol-group-containing compounds, such as dithiothreitol (Wataya & Santi, 1975). In analogy with the

mechanism of that reaction, we suggest that for example in the 5'-deiodination of thyroxine the primary event is an attack of a nucleophile (X) of the enzyme, e.g. a thiol group, at the 2'- or the equivalent 6'-position of thyroxine (2) yielding the 2',3'-dihydro derivative (4). The removal of the iodine atom may then take place via different routes, in each of which the thiol group of the cofactor is oxidized (Wataya & Santi, 1975).

Two more observations appear to fit remarkably well with this model.

(1) The pH optima of the conversion of thyroxine into tri-iodothyronine and of the production of di-iodothyronine from reverse tri-iodothyronine were shown to be somewhat below the pK of the phenolic hydroxyl group, which is approx. 6.6 (Handwerger *et al.*, 1975). Dissociation of the phenol would render the primary attack of a nucleophile an unlikely event.

(2) Deiodination of the phenolic ring is much more rapid when reverse tri-iodothyronine is the substrate than with thyroxine. This may indicate that substitution of both the 3- and 5-positions hinders the approach of the nucleophile to position 2'. It remains, however, to be investigated whether both reactions I and IV are catalysed by the same enzyme. The resemblance of 5-propyl-2-thiouracil to the substrates of the deiodination reactions suggests that this compound may act as a competitive inhibitor. The resemblance to 6-propyl-2-thiouracil is, however, less apparent. Nevertheless this compound is also an effective inhibitor of the several monodeiodination reactions in the homogenate.

We have found that iodothyronines are not deiodinated by thymidylate synthetase of bacterial origin (amethopterin-resistant strain of *Lactobacillus casei*) (T. J. Visser, unpublished work). We suggest, even so, that removal of iodine atoms from iodothyronines involves a mechanism that is similar to that described for the deiodination of 5-iodo-2'-deoxyuridylate by thymidylate synthetase (Wataya & Santi, 1975) and is mediated by enzymes with similar specificities. This can only be tested when the reactions are investigated with purified enzyme preparations. Such studies will also reveal whether reactions I and IV and reactions II and III are catalysed by the same enzymes.

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